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Sistemas poliméricos de entrega de substâncias: do aumento da eficácia do processo de cicatrização a aplicações oncológicas

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“The saddest aspect of life right now is that science gathers knowledge faster than society gathers wisdom.”

Isaac Asimov

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LISTA DE ABREVIATURAS

BCNU: 1,3-bis(2-cloroetil)-1-nitrosouréia, também conhecido como carmustina

BHE: barreira hematoencefálica

DDR: respostas a danos no DNA, do inglês, *DNA damage response*

DDS: sistemas de entrega de fármacos, do inglês, *drug delivery systems*

FDA: Agência federal regulatória norte-americana para alimentos e medicamentos, do inglês, *Food and Drug Administration*

F/T: congelamento/descongelamento, do inglês, *freeze-thaw*

GB: glioblastomas

INCA: Instituto Nacional do Câncer

Nek1: quinase 1 relacionada ao NIMA, do inglês, *NIMA-related kinase 1*

OMS: Organização Mundial da Saúde

PCL: poli(caprolactona)

PEG: poli(etilenoglicol)

PLA: poli(ácido láctico)

PLGA: poli(L-ácido láctico-*co*-ácido glicólico)

PVA: poli(álcool vinílico)

PVP: poli(vinilpirrolidona)

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RESUMO

Os sistemas de entrega de fármacos são uma área de estudo relativamente nova, mas em rápido desenvolvimento, que descreve como materiais biocompatíveis são empregados para servir como meio de ferramenta de diagnóstico e/ou para fornecer agentes terapêuticos a tecidos de maneira controlada. Essa tecnologia oferece múltiplos benefícios no tratamento de doenças humanas crônicas como a entrega específica e controlada de fármacos ao tecido alvo. Neste contexto, esta tese apresenta uma série de capítulos de livro sobre os avanços em sistemas poliméricos de entrega de substâncias em que formulações, tanto provenientes de fontes sintéticas como naturais, foram aplicadas para o tratamento do câncer ou para proteger feridas e/ou acelerar o processo de cicatrização. Ainda, formulações contendo o poli(álcool vinílico) foram produzidas pela técnica de eletrofiação, caracterizadas em relação ao tamanho, morfologia, estabilidade e liberação de fármacos, e testadas *in vitro* e *in vivo*, incluindo (i) formulações contendo um extrato padronizado de *Plantago australis,* as quais demonstraram significante potencial terapêutico acelerando o processo de cicatrização, e (ii) microfibras poliméricas contendo um inibidor da proteína Nek1 e temozolomida para o tratamento *in situ* de glioblastomas, um tipo de câncer cerebral muito agressivo. As microfibras contendo o co-tratamento foram capazes de melhorar significantivamente a eficácia do tratamento em modelo animal pré-clínico, aumentando a sobrevida e diminuindo o volume tumoral de quando comparado à quimioterapia tradicional. Ademais, as oportunidades e os desafios de sistemas de entrega de substâncias utilizadas no tratamento e diagnóstico concomitante de doenças complexas foram revisados e discutidos em um capítulo de livro. Foi possível concluir que sistemas de entrega de substâncias são ferramentas promissoras para aprimorar a eficácia de terapias.

Palavras-chave: Sistemas de entrega de fármacos, nanomateriais, poli(álcool vinílico), direcionamento de fármacos, produtos naturais, cicatrização, câncer, glioblastoma.

ABSTRACT

Drug delivery systems are a relatively new but rapidly developing science in which biocompatible materials are used to serve as diagnostic tools and/or to deliver therapeutic agents to tissues in a controlled manner. This technology offers multiple benefits in the treatment of chronic human diseases such as specific and controlled delivery of drugs to target tissue. In this context, this thesis presents a series of book chapters on advances in polymeric drug delivery systems in which formulations, both from synthetic and natural sources, have been applied to treat cancer or to protect wounds and/or accelerate the wound healing process. Additionally, formulations containing polyvinyl alcohol were produced using electrospinning, characterised in terms of size, morphology, stability and drug release, and tested *in vitro* and *in vivo*. including (i) formulations containing a standardised extract of *Plantago australis*, which demonstrated significant wound healing potential, and (ii) polymeric microfibers cointaining an inhibitor of Nek1 protein and temozolomide for *in situ* treatment of glioblastomas, a very aggressive type of brain cancer. The microfibers loaded with the co-treatment were able to significantly improve the effectiveness of glioblastoma treatment in a pre-clinical animal model, increasing survival and decreasing the tumour volume when compared to standard of care chemotherapy. Furthermore, the opportunities and challenges of drug delivery systems used in concurrent treatment and diagnosis of complex diseases were reviewed and discussed in a book chapter. It was possible to conclude that drug delivery systems are promising tools to improve the effectiveness of therapies.

Keywords: Drug delivery systems, nanomaterials, polyvinyl alcohol, drug targeting, natural products, wound healing, cancer, glioblastoma.

1. INTRODUÇÃO

Os sistemas de entrega de fármacos ou *drug delivery systems* (DDS) são usados principalmente para obter controle espacial e temporal durante administração de fármacos e/ou agentes biológicos (1). Essencialmente, os DDS permitem que fármacos sejam entregues por um longo período de tempo e em um local de ação específico. Estes sistemas são projetados para aumentar a segurança e eficácia de fármacos e para melhorar a adesão do paciente ao tratamento. Ainda, o uso destes sistemas visa manter níveis terapêuticos de fármacos, reduzir efeitos colaterais, diminuir a quantidade necessária de fármacos para atingir uma resposta terapêutica e a frequência de dosagem, e facilitar entrega de fármacos com meia-vida curta (2).

Os DDS funcionam permitindo que as propriedades farmacocinéticas inerentes às moléculas e fármacos sejam manipuladas pelas propriedades da matriz do sistema, incluindo a natureza da matriz e as características de sua superfície (3). Além disso, os DDS permitem que fármacos potentes, porém instáveis sejam administradas com flutuações mínimas de doses, assegurando uma concentração plasmática uniforme do fármaco durante um período de tempo prolongado e, potencialmente, com uma incidência mais baixa de toxicidade (4).

Idealmente, um DDS deve atravessar barreiras biológicas e oferecer uma lenta absorção de fármacos e apresentar uma liberação sustentada de moléculas contrastando com as apresentações farmacêuticas convencionais como comprimidos e cápsulas, os quais fornecem uma liberação rápida e transitória, também conhecida como *burst release*, em que uma resposta farmacológica só é observada se a quantidade de um fármaco está acima da concentração mínima efetiva (3). Formulações com liberação prolongada, porém, reduzem o *burst release*, mantendo as concentrações plasmáticas a um nível eficaz e diminuindo a taxa de absorção devido a uma taxa de liberação mais lenta dos fármacos, o que pode ser necessário para melhorar o tratamento de alguns tipos de doenças como o câncer.

Visando uma aplicação eficaz de um sistema, uma compreensão das características do fármaco e da matriz de escolha é essencial. Por exemplo, alterando as propriedades químicas ou físicas de um polímero, a taxa de liberação de uma molécula pode ser controlada. Além do mais, o tecido alvo em que um DDS deve funcionar afeta a escolha de matrizes, fármacos e sistemas. Uma abordagem

multifacetada é, portanto, crucial para a entrega bem-sucedida de fármacos a partir de um DDS.

Nas últimas décadas, as estratégias de DDS tiveram papel fundamental em converter fármacos promissores em terapias de sucesso (5-7). À medida que o cenário terapêutico evoluiu, as estratégias e tecnologias de entrega se adaptaram rapidamente para refletir as mudanças nas necessidades de entrega de fármacos (8). Atualmente, existe uma série de materiais disponíveis que podem ser utilizados no desenvolvimento de sistemas, incluindo lipídeos, fosfolipídeos, óxidos, metais e principalmente, polímeros, os quais são o foco deste trabalho e serão discutidos na seção a seguir.

1.1 SISTEMAS POLIMÉRICOS NA ENTREGA DE FÁRMACOS

Um grande número de polímeros pode ser usado na produção de DDS para acomodar a vasta gama de designs, interações medicamentosas e perfis de liberação que são requeridos em diferentes aplicações (Figura 1).

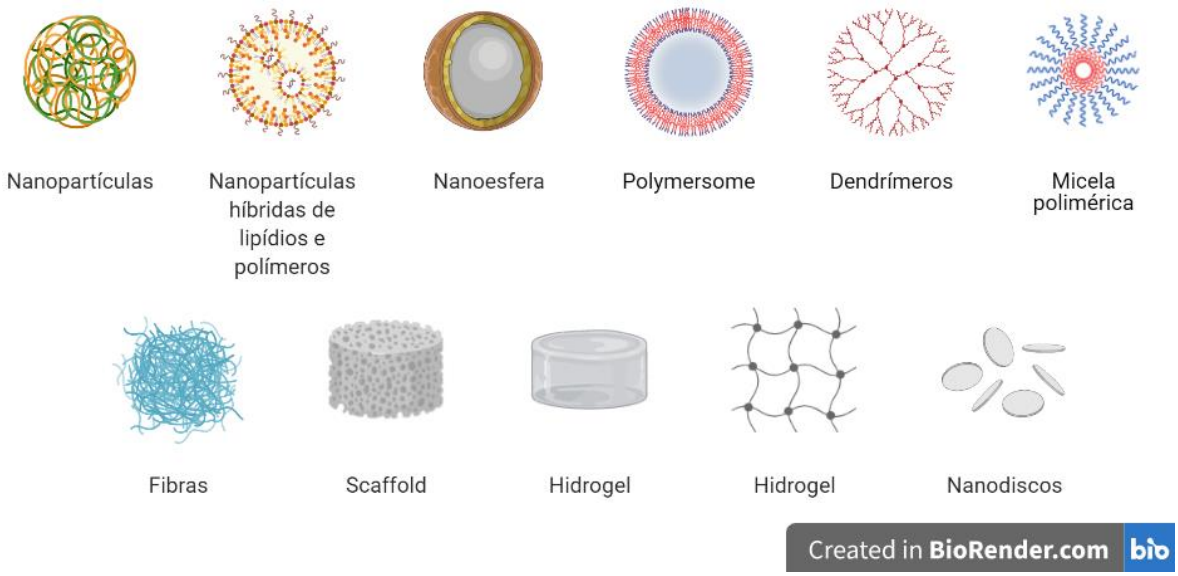

Figura 1. Tipos de sistemas poliméricos de entrega de fármacos. Figura elaborada pela autora utilizando a ferramenta BioRender.

Os polímeros biodegradáveis têm sido significativamente utilizados nos últimos anos em diferentes aplicações nas áreas relacionadas à produção de materiais biodegradáveis, devido às suas características físicas e químicas. Estes polímeros

podem ser sintéticos e naturais. Polímeros sintéticos são amplamente utilizados em implantes e dispositivos biomédicos porque podem ser moldados em diferentes formas, por outro lado, polímeros naturais são biocompatíveis e normalmente não causam efeitos adversos (1, 9-14) (Tabela 1).

Tabela 1. Classificação de polímeros biodegradáveis.

Polímeros biodegradáveis			
Naturais		**Sintéticos**	
Fontes renováveis	Síntese Microbiana	Biotecnológicos	Petroquímicos
Polissacarídeos, proteínas	Polihidroxialcanoatos	Poliácidos	Policaprolactona, poliesteraminas, co-poliésteres

1.1.1 POLÍMEROS NATURAIS

Polímeros naturais são derivados de uma ampla variedade de fontes, plantas, animais e microorganismos e têm sido utilizados em uma variedade de aplicações biomédicas, como produtos farmacêuticos, estruturas para regeneração de tecidos, agentes de entrega de fármacos e sistemas de imagem para diagnóstico de doenças (15). Polímeros naturais são altamente disponíveis, biodegradáveis, biocompatíveis e apresentam baixa toxicidade (14, 15). Esses incluem polímeros como albumina, gelatina, soja, colágeno, e polissacarídeos, como quitosana, agarose, dextrano, ácido hialurônico, alginato e carragenina (14). Estruturalmente, os colágenos, por exemplo, são compostos de três cadeias polipeptídicas torcidas que formam uma tripla-hélice e são a principal proteína da matriz extracelular de mamíferos. Da mesma forma, o ácido hialurônico, um polissacarídeo glicosaminoglicano aniônico, também é encontrado em quase todos os tecidos de animais adultos. Alternativamente, o alginato, a agarose e a quitosana são polissacarídeos hidrofílicos, lineares e derivados de fontes de algas marinhas (alginato e agarose) ou crustáceos (quitosana). Os DDS visando aplicações em engenharia de tecidos como hidrogéis podem ser produzidos com polímeros naturais como colágeno, quitosana, ácido hialurônico, alginato e agarose, tendo em vista que estes polímeros apresentam

propriedades macromoleculares semelhantes à matriz extracelular e, portanto, são prontamente aceitos pelo corpo. Além disso, esses polímeros são biodegradáveis pois sofrem degradação enzimática e hidrolítica no ambiente biológico (9).

No tratamento de feridas, os polímeros naturais são amplamente usados como curativos para feridas agudas ou crônicas e como modelos de regeneração (15). Devido a suas propriedades. tais como semelhança com a matriz extracelular, flexibilidade, alta biocompatibilidade e alta capacidade de retenção de água, os *scaffolds,* a base de polímeros naturais, são vistos como promissores para fins de reparo e regeneração da pele. Porém, a seleção e modificação do material apropriado para a produção de *scaffolds* que possuam propriedades específicas para o tipo de ferida alvo são um dos obstáculos do uso de polímeros naturais (15).

Apesar de muitos méritos como biomateriais, os polímeros naturais possuem várias desvantagens, incluindo variação nas propriedades do material com base na fonte, ampla distribuições de peso molecular, contaminação microbiana, absorção descontrolada de água, baixa resistência mecânica e padrão de degradação imprevisível (9). Essas inconsistências limitaram o uso de polímeros naturais em aplicações biomédicas, fazendo com que o uso destes polímeros como carreadores seja considerado um desafio.

Portanto, vários polímeros sintéticos com propriedades mecânicas e de degradação bem definidas foram desenvolvidos para atender às necessidades tecnológicas de aplicações biomédicas. No entanto, esses polímeros, do ponto de vista biológico, carecem de bioatividade e biocompatibilidade quando comparados com polímeros naturais, podendo causar toxicidade. Para obter propriedades intermediárias, dois ou mais polímeros podem ser misturados e/ou ligados quimicamente (copolimerizados) (10). Desse modo, é possível modificar as propriedades físico-químicas de polímeros naturais como polissacarídeos alterando seus grupos funcionais como hidroxilas e aminas modificando sinteticamente sua estrutura. Por exemplo, enxertar monômeros sintéticos nas cadeias de polissacarídeos oferece uma maneira fácil de controlar a solubilidade, absorção de água e degradação destes polímeros, melhorando suas características e propriedades físico-químicas quando comparados com polímeros naturais e sintéticos (9).

1.1.2 POLÍMEROS SINTÉTICOS

Polímeros sintéticos são normalmente usados na construção de DDS, uma vez que é possível controlar suas propriedades inerentes. Em geral, os polímeros sintéticos apresentam grandes vantagens em relação aos polímeros naturais para aplicação em engenharia de tecidos e medicina regenerativa, pois podem ser adaptados gerando uma ampla gama de possibilidades para diferentes tipos de produtos. Os biomateriais podem ser facilmente sintetizados com qualidade e pureza reprodutíveis e fabricados em várias formas com propriedades desejadas. As vantagens específicas incluem a capacidade de adaptação das propriedades mecânicas e da cinética de degradação desses materiais para atender a várias aplicações. Produtos derivados de polímeros sintéticos são muito utilizados nas áreas de DDS devido ao controle que cientistas têm sobre a estrutura, biodegradação, resistência mecânica e resposta química e biológica a estímulos.

Exemplos de polímeros sintéticos biodegradáveis amplamente utilizados na produção de DDS são poli(hidroxiácidos), como poli(L-ácido láctico-*co*-ácido glicólico) (PLGA), e poli(ácido láctico) (PLA) (Tabela 2). Estes polímeros ganharam popularidade devido ao seu processamento, consistência, propriedades mecânicas adequadas e biodegradabilidade, além disso, são aprovados pelo *Food and Drug Administration* (FDA) para uso humano em uma variedade de aplicações, incluindo suturas, curativos e como carreadores de fármacos (9). Outros polímeros como o poli(etilenoglicol) (PEG) e o poli(álcool vinílico) (PVA) podem ser produzidos de forma reprodutível, tendo em vista seus pesos moleculares específicos assim como reprodutibilidade nas taxas de degradação e propriedades mecânicas.

Em relação à biocompatibilidade, a ligação éster nestes polímeros é degradada por hidrólise e os produtos de degradação são eliminados do corpo na forma de dióxido de carbono e água. Ainda, a taxa de degradação destes polímeros pode ser controlada pela alteração dos seus pesos moleculares e modificação das regiões cristalinas em suas estruturas. Uma vez que esses polímeros são termoplásticos, eles podem ser moldados em estruturas tridimensionais com micro ou nanoarquitetura, forma e dimensão desejadas (9, 11).

Apesar dos polímeros sintéticos possuírem estruturas bem definidas e versáteis para modificações subsequentes, garantindo taxas de degradabilidade e funcionalidade adequadas (9), eles geralmente carecem de atividades biológicas

intrínsecas e propriedades bioativas inerentes, que os produtos feitos de polímeros naturais possuem. Além disso, em alguns casos, os produtos de degradação de polímeros modificados podem causar efeitos adversos ou alterar o microambiente do tecido alvo. Ainda, a hidrofobicidade da superfície de alguns polímeros sintéticos pode mediar a desnaturação de proteínas e induzir efeitos adversos (9, 11).

Tabela 2. Características de polímeros sintéticos e biodegradáveis (16).

Polímero	Propriedades mecânicas*	Propriedades físico-químicas	Observações
PLGA	↑	Hidrofóbico	Baixa toxicidade. Aplicação em nanotecnologia.
PLA	↑	Hidrofóbico	Baixa toxicidade. Aplicação em nanotecnologia.
PCL	↑	Hidrofóbico	Aplicação em dispositivos de longo prazo (implantes).
PVP	↑	Hidrofílico	Baixa toxicidade. Aplicação em filmes e hidrogéis.
PVA	↑	Hidrofílico	Baixa toxicidade e boa solubilidade em água. Aplicação em hidrogéis.
PEG	↓	Hidrofílico	Boa solubilidade em água.

PLGA: poli (L-ácido láctico-co-ácido glicólico); PLA: poliácido láctico; PCL: policaprolactona; PVP: polivinilpirrolidona; PVA: poli(álcool vinílico); PEG: polietilenoglicol.
*resistência, rigidez e dureza em relação a outros polímeros sintéticos.

Entre vários bio-polímeros, nosso grupo de pesquisa tem focado no estudo do PVA, o qual tem um excelente histórico em aplicações biomédicas (17-21). O PVA é um polímero não-iônico e semicristalino, fácil de processar e é uma opção muito viável na produção de DDS, uma vez que esse polímero é biocompatível e não-tóxico (22) (Tabela 2). Devido às suas características mecânicas e e capacidade de intumescimento, hidrogéis de PVA produzidos com a técnica de congelamento/descongelamento, ou *freeze-thaw*, (F/T) podem ser produzidos com potencial significativo para uso como DDS. Esses produtos podem ser produzidos pela exposição de soluções de PVA/água a ciclos de F/T; esse processo resulta na formação de géis estáveis pela presença de regiões cristalinas (23). Além disso, o

PVA é uma opção interessante para projetar nanoprodutos, uma vez que apresenta baixa imunotoxicidade e é biodegradável (22).

Recentemente, nosso grupo de pesquisa produziu nanofibras de PVA pela técnica de eletrofiação contendo o quimioterápico dacarbazina, para o uso no tratamento de glioblastomas (GB). As nanofibras demonstraram uma liberação prolongada do fármaco por cinco dias e a eficácia do sistema foi avaliada *in vitro,* demonstrando um aumento significativo na citotoxicidade e genotoxicidade induzida pelo fármaco (22). Portanto, tendo em vista as vantagens do uso do PVA em aplicações em engenharia de materiais e compatibilidade biológica, os Capítulos 2 e 6 desta tese irão apresentar formulações produzidos com PVA pela técnica de eletrofiação para o uso no tratamento de feridas e do GB, respectivamente.

Como pode ser observado, uma grande variedade de polímeros pode ser usada como veículos na produção de DDS e as propriedades de liberação de fármacos podem ser adaptadas manipulando as características físico-químicas dos polímeros. As vantagens e desvantagens de polímeros naturais e sintéticos foram resumidas na Tabela 3 (24).

Tabela 3. Vantagens e desvantagens de polímeros naturais e sintéticos.

	Polímeros naturais	**Polímeros sintéticos**
Vantagens	Baixa toxicidade; Biocompatibilidade; Biodegradabilidade; Baixa imunogenicidade; Altamente disponível.	Biocompatibilidade; Estabilidade; Reprodutibilidade; Propriedades mecânicas e de conjugação; Biodegradabilidade.
Desvantagens	Alto nível de variabilidade; Complexidade; Contaminação de microorganismos ou metais pesados; Processo de extração pode ser complicado e caro.	Toxicidade; Degradabilidade; Processo de fabricação pode ser complicado e caro.

É importante ressaltar que as propriedades do polímero escolhido para a produção de um DDS precisam complementar a aplicação escolhida, permitindo que a interação com o fármaco esteja funcionando efetivamente no ambiente em que o sistema de entrega será destinado. Neste contexto, esta tese irá abordar aspectos teóricos e práticos da construção de DDS.

1.2 SISTEMAS POLIMÉRICOS PARA USO NA CICATRIZAÇÃO

A pele, o maior órgão do corpo humano, fornece uma barreira protetora contra danos físicos, patógenos, perda de líquidos e possui funções imuno-neuroendócrinas que contribuem para a manutenção da homeostase corporal. Entretanto, após uma lesão em que há comprometimento da integridade da pele, a mesma deve ser prontamente restaurada para manter suas funções (25). Curativos modernos estão em desenvolvimento há décadas, e embora exista uma grande variedade de curativos, pomadas e dispositivos médicos para uso clínico, o processo de recuperação de feridas é restrito principalmente à proteção de lesões (26). O principal problema da regeneração da pele é a restauração da estrutura e da função do órgão lesionado, incluindo os capilares sanguíneos. Recentemente, os DDS mudaram o foco da proteção da lesão para a qualidade da regeneração da pele em termos de função, redução de cicatrizes e estética aprimorada para cirurgias de reconstrução e queimaduras (26).

Para enfrentar os desafios dos tratamentos de feridas agudas e crônicas, como perda de pele em grandes áreas, queimaduras, úlceras, trauma e feridas especialmente infectadas, os DDS devem ser produzindos focando na entrega precisa de fármacos e na manutenção dos tecidos. Um curativo ideal deve seguir algumas características como: (i) controlar a umidade ao redor da ferida, (ii) possibilitar a oxigenação, (iii) eliminar o excesso de exsudato, (iv) proteger a ferida de infecções e microorganismos, (v) diminuir a necrose da superfície da ferida, (vi) possibilitar uma proteção mecânica, (vii) serem facilmente trocados e removidos, (viii) biocompatíveis, biodegradáveis e elásticos, (ix) aliviar a dor da ferida, e (x) possuirem baixo custo (26, 27).

Vários materiais biocompatíveis têm sido amplamente investigados na entrega de fármacos na forma de curativos. Neste contexto, esforços significativos têm sido realizados no uso de polissacarídeos como DDS e este tópico será apresentado em

um capítulo de livro, no item 3.1 desta tese (Capítulo 1). Porém, a facilidade do uso de polímeros sintéticos permite uma maior reprodutibilidade. Ainda, os DDS para o uso na cicatrização que liberam fármacos antimicrobianos e anti-inflamatórios representam uma grande oportunidade de prevenir infecções, aumentar e eficácia de fármacos comercialmente disponíveis e possibilitar o uso de novos compostos, incluindo produtos naturais.

Desde os tempos antigos, os seres humanos têm utilizado produtos naturais como fármacos contra doenças. Atualmente, os medicamentos modernos são derivados principalmente de ervas com base nos conhecimentos e práticas tradicionais. Quase 25% dos principais compostos farmacêuticos e seus derivados disponíveis hoje em dia são obtidos de recursos naturais (28). Produtos naturais exibem características notáveis como extraordinária diversidade química, atividades biológicas e baixa toxicidade (29). Portanto, compostos naturais com diferentes origens moleculares apresentam uma base para a descoberta de novos medicamentos.

No entanto, preocupações associadas à biocompatibilidade, biodisponibilidade, e problemas com entrega específica dos compostos naturais apresentam um desafio na aplicação dos mesmos (28). Portanto, o uso de novos sistemas de administração que direcionam fármacos mesmos para partes específicas do corpo podem ser uma opção para resolver estas questões críticas. De tal modo, curativos e outras formulações podem ser produzidos com o auxílio da nanotecnologia, proporcionando a liberação controlada de compostos naturais.

Deste modo, nosso grupo de pesquisa vem trabalhando com uma planta chamada *Plantago australis* Lam. (Plantaginaceae), popularmente conhecida como tansagem e amplamente encontrada no América Latina, principalmente no Brasil (20, 30, 31). A *P. australis* é vastamente usada como anti-inflamatório e bactericida, e, com este foco, nosso grupo de pesquisa produziu um extrato padronizado utilizando as folhas de *P. australis,* que demonstrou atividade anti-inflamatória e cicatrizante *in vivo* (31). Porém, a biodisponibilidade do extrato a partir da aplicação por via oral não é ideal, por isso, o objetivo do artigo de dados, apresentado no item 3.2 desta tese (Capítulo 2) foi a produção e caracterização de curativos contendo o extrato padronizado de *P. australis*, os quais, possibilitaram a aceleração do processo de cicatrização e proporcionaram um ambiente ideal para a regeneração do tecido.

1.3 SISTEMAS POLIMÉRICOS TRATAMENTO DO CÂNCER

O câncer inclui uma série de doenças que surgem como resultado do crescimento desregulado de células malignas que têm o potencial de invadir ou se espalhar para outras partes do corpo. Com mais de 10 milhões de novos casos a cada ano, as mortes relacionadas ao câncer devem aumentar nos próximos anos, com uma estimativa da Organização Mundial da Saúde (OMS) de aproximadamente 13,1 milhões de mortes relacionadas ao câncer até 2030 (32). Para o Brasil, a estimativa do Instituto Nacional do Câncer (INCA) para cada ano do triênio 2020-2022 foi de 625 mil casos novos de câncer (33). No entanto, a taxa de mortalidade diminuiu nos últimos 5 anos devido a uma melhor compreensão da biologia dos tumores e a melhores modalidades de tratamento e diagnóstico. As opções atuais de tratamento do câncer incluem intervenção cirúrgica, quimioterapia e radioterapia ou uma combinação dessas modalidades (34).

A quimioterapia convencional funciona principalmente por interferir na síntese de DNA ou por induzir lesões na molécula de DNA, ativando as vias de morte celular. Porém, os agentes quimioterápicos não são seletivos e podem danificar tecidos normais, causando graves efeitos colaterais. Embora a quimioterapia convencional tenha sido bem-sucedida, até certo ponto, as suas principais desvantagens são a baixa biodisponibilidade, altas doses que causam efeitos colaterais sistêmicos, baixos índices terapêuticos, desenvolvimento de resistência múltipla a medicamentos e direcionamento inespecífico. Portanto, é desejável desenvolver quimioterápicos que possam atingir passivamente ou ativamente apenas as células cancerígenas, reduzindo assim os efeitos adversos, enquanto melhoram a eficácia terapêutica. Nos últimos anos, uma maior disponibilidade de materiais versáteis, incluindo polímeros, lipídios, e carreadores inorgânicos levaram ao desenvolvimento de sistemas que podem administrar fármacos em tumores com melhor eficácia terapêutica (8, 28, 35, 36).

O principal objetivo no desenvolvimento de DDS neste contexto é abordar com sucesso os problemas relacionados ao fornecimento e transporte de fármacos para os locais desejados, reduzindo os efeitos adversos. Nesta tese, os diferentes tipos de polímeros usados como veículos de entrega para agentes quimioterápicos, suas características estruturais e os recentes avanços científicos na área de quimioterapia serão descritos em capítulos de livros.

No apêndice A é abordado o uso de DDS baseados em polissacarídeos para a entrega de quimioterápicos, ácidos nucleicos, peptídeos e proteínas durante o tratamento de diversos tipos de câncer, ressaltando que apesar desta abordagem ser promissora, atualmente existem poucos ensaios clínicos que utilizam DDS baseados em polissacarídeos.

O uso de sistemas nanopoliméricos para o tratamento de cânceres cerebrais é apresentado no item 3.3, Capítulo 3, desta tese, destacando os aspectos moleculares dos GB, os tratamentos convencionais utilizados e mencionando a dificuldade imposta pela barreira hematoencefálica (BHE) no tratamento de GB, uma das considerações fundamentais para entender a importância da nanomedicina neste contexto. Ainda, este capítulo discute a técnica de eletrofiação na produção de DDS, o uso de nanopartículas e de nanofibras no tratamento, levando em consideração os efeitos adversos destas abordagens, e como é possível predizer a interação droga-matriz polimérica utilizando métodos computacionais garantindo o sucesso durante a produção de DDS.

Seguindo a mesma linha de raciocínio, o Capítulo 4, item 3.4 desta tese, aborda o uso de sistemas formados de biopolímeros com duas fases, os bionanocompósitos, em que uma das fases é obrigatoriamente na escala nano, como DDS no tratamento *in situ* do câncer. Este capítulo descreve o uso de DDS no tratamento do câncer, mais especificadamente o uso de bionanocompósitos, e como escolher o polímero certo para determinadas aplicações levando em consideração as propriedades físico-químicas dos compostos e as interações droga-matriz. Além disso, este capítulo discute em detalhe as conquistas atuais destes DDS no tratamento local do câncer, mais especificamente hidrogéis injetáveis, e nanofibras e *scaffolds* produzidos pela técnica de eletrofiação. Já o Capítulo 5 da tese (item 3.5) discute o uso de nanocompósitos poliméricos na terapia alvo do câncer, como esses sistemas podem contornar as desvantagens da quimioterapia convencional e como simulações moleculares em multiescala podem orientar o desenvolvimento de nanocompósitos.

Nexte contexto, o surgimento da nanotecnologia teve um profundo impacto na terapêutica clínica em geral nas últimas duas décadas. Comparados aos agentes quimioterápicos convencionais, os portadores de fármacos em nanoescala demonstraram o potencial de enfrentar alguns desses desafios, melhorando a eficácia do tratamento e evitando a toxicidade em células normais (12). Atualmente, vários

quimioterápicos baseados em nanopartículas estão sendo clinicamente aprovados e muitos outros estão em diferentes estágios de desenvolvimento clínico ou pré-clínico (8, 28, 36).

Embora os nano DDS ofereçam muitas vantagens como sistemas transportadores de fármacos, a biodegradabilidade, baixa biodisponibilidade, instabilidade na circulação, distribuição inadequada de tecido e potencial toxicidade levantam preocupações sobre sua segurança, especialmente para administração a longo prazo. Por esse motivo, são necessários novos DDS com melhor capacidade de direcionamento para a prevenção do câncer, a supressão de efeitos colaterais adversos e o gerenciamento da dor associados à quimioterapia do câncer.

Visando a melhora da efectividade de fármacos quimioterápicos e diminuição dos efeitos colaterais sistêmicos como toxicidades hematológicas (37-40), DDS *in situ* têm sido amplamente produzidos para adminitração local do tratamento previnindo a recorrência tumoral (19, 41-43). Nos últimos anos, novos sistemas de entrega, como implantes, impactaram dramaticamente o tratamento do câncer. Muitos medicamentos contra o câncer têm vida curta, por isso DDS *in situ* oferecem proteção adequada para permitir que fármacos sejam administrados a uma taxa e duração controladas (19, 35, 44-49). A fim de diminuir a toxicidade sistêmica induzida por fármacos, a capacidade de um sistema em entregar localmente o tratamento pode melhorar tanto a segurança quanto a eficácia de quimioterapia para câncer.

Uma limitação que é frequentemente vista com implantes é uma resposta inflamatória elevada (50). Na tentativa de minimizar isso, o implante deve ser extremamente biocompatível, ter uma superfície mínima e lisa e ter uma estrutura semelhante ao tecido que está sendo implantado. Outro fator que também precisa ser considerado com implantes é a perda de propriedades mecânicas que ocorrerá à medida que o polímero degrada. Quando ocorre a clivagem da cadeia polimérica, a resistência do implante diminui e a integridade do implante pode ser comprometida. Logo, o implante pode não ter um desempenho ideal.

Consequentemente, novos métodos e DDS que viseam a produção de implantes que produzam uma liberação mais linear de um medicamento para o tratamento local especialmente para cânceres difícies de tratar, como GB, são necessários. O GB é o tumor cerebral mais frequente e maligno e apesar de todas as estratégias de tratamento disponíveis, a sobrevida média para esta doença é de aproximadamente 15 meses (34).

Pesquisas pré-clínicas e clínicas demostraram que o processo de resistência relacionado em GB são relacionados às vias de reparo de danos ao DNA, células-tronco tumorais, seletividade da BHE e toxicidade devido a altas dose do tratamento sistêmico (Revisado em (36) – apêndice B). Nesse contexto, o advento de DDS para o tratamento *in situ* é uma alternativa promissora e versátil para superar os desafios das atuais abordagens de tratamento. Ainda, visando contornar os mecanismos de resistência do tumor, terapias combinatórias mais eficazes devem ser identificadas, como o uso de quimioterápicos que ativam as vias de reparo de DNA combinados com a inibição de alvos relacionados à resposta ao dano do DNA (DDR; do inglês, *DNA damage response*). Neste contexto, proteínas relacionadas ao reparo de DNA induzido por fármacos, como a temozolomida, são alvos terapêuticos interessantes (36). Por isso, o Capítulo 6, item 3.6 da tese, descreve a produção de um micro-sistema polimérico contendo o fármaco temozolomida e um inibidor da cinase Nek1 (do inglês, *NIMA-related kinase 1*), uma proteína envolvida na sinalização e reparo de DNA, como uma estratégia de co-terapia para o tratamento *in situ* de GB. Estes implantes cerebrais foram capazes de melhorar significativamente o tratamento, tanto *in vitro* quanto *in vivo,* quando comparado com o tratamento convencional, indicando a importância desta abordagem para superar o limitante cenário clínico relacionado ao GB. É importante ressaltar que este é o primeiro trabalho demonstrando que o co-tratamento com o inibidor de Nek1 apresenta uma opção promissora para superar a resistência tumoral relacionada ao reparo de lesões no DNA induzidos por quimioterápicos.

1.4 TERANÓSTICA: FUTURO PROMISOR PARA O TRATAMENTO DE DOENÇAS COMPLEXAS

O desenvolvimento de DDS é um campo científico em constante evolução e, consequentemente, novas tecnologias e novos aprimoramentos de tecnologias existentes estão surgindo continuamente. Um grande avanço nesta área de pesquisa é o desenvolvimento de modalidades terapêuticas que podem tratar e diagnosticar doenças ao mesmo tempo. Para isso, uma das opções mais viáveis e promissoras é o desenvolvimento de DDS biofuncionalizados capazes de carrear fármacos e sondas ou partículas usadas em diagnóstico e monitoramento da resposta ao tratamento em um mesmo momento (51-54). Este conceito é chamado de teranóstica (do inglês,

theranostics) e envolve a integração de compostos com propriedade diagnóstica e com propriedade terapêutica em um único DDS.

Dentre as opções de DDS para aplicações teranósticas, os nanocompósitos que associam nanopartículas biofuncionalizadas e fotossensibilizadores têm sido significativamente estudados nos últimos anos. No entanto, apesar dos resultados disponíveis serem promissores, os efeitos adversos relacionados ao uso desses sistemas são desconhecidos. Portanto, o objetivo do apêndice C da tese foi revisar sistemas nanocompósitos usados em aplicações teranósticas para diversas doenças.

2. OBJETIVOS

2.1 Objetivo Geral

O objetivo geral deste projeto é investigar e desenvolver sistemas poliméricos de entrega de fármacos para o uso no tratamento de cicatrizes e no tratamento do câncer, em especial do GB.

2.2 Objetivos Específicos

Meta 1: Investigar o uso de sistemas poliméricos no tratamento de cicatrizes durante a elaboração de um capítulo de livro.

Meta 2: Desenvolver formulações contendo um extrato hidroetanólico de *Plantago australis* e testar suas eficácias *in vitro* e *in vivo* no tratamento de feridas.

Meta 3: Investigar o uso de sistemas poliméricos na entrega de fármacos quimioterápicas no tratamento do câncer durante a elaboração de capítulos de livro.

Meta 4: Desenvolver microfibras poliméricas contendo temozolomida e um inibidor da proteína Nek1 e testar sua eficácia *in vitro* e *in vivo* no tratamento de GB.

3. CAPÍTULOS DE LIVRO E ARTIGOS CIENTÍFICOS

3.1 Capítulo 1: Modified polysaccharides in wound healing

Capítulo de livro publicado no livro Tailor-Made Polysaccharides in Biomedical Applications.

Chapter 10

Modified polysaccharides in wound healing

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10.1 Wound healing process

The wound healing process comprises an essential feature of the human body for its survival. By definition, it is a repairing response of the organism as a result of external damage on the skin barrier, which can be in different levels of severity in terms of wound depth [1]. It constitutes the movement of the mononuclear phagocytic system cells, platelets, growth factors, fibroblasts, collagen, chemotaxins, and adhesion factors for a unique intent: to protect the organism against external exposition by cleaning the local damage and reepithelializing it. Due to its interrelated mechanistic aspects, some authors categorized the phases of wound healing into four different degrees/stages, such as hemostasis, inflammatory, granulation, and remodeling; or even three stages, if the hemostasis phase is considered within the inflammatory phase [1–3].

10.1.1 Hemostasis

Straight after the damage to the skin barrier, there is local vasoconstriction unchained by thromboxane A2 and prostaglandins released from the plasmatic membrane of local cells. This vasoconstriction reduces the outflow of blood from the lesion and approximates the blood cells to the damaged skin. Initially, the platelets establish the clot, together with blood constituents, weakly damming the wound. It is a primary aggregation caused by platelet activation, unchained by adenosine diphosphate (ADP) discharges from other platelets, in which part, was in behalf of epithelial collagen exposition. When activated, the platelets can also perform degranulation of its granules, dividing alpha-granules

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such as transformation growth factor beta (TGF-β), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF); and dense granules such as vasoactive amines, and ADP [2].

The coagulation cascade can be completed with fibrinogen being cleaved into fibrin. The fibrin framework provides support for the skin by having an immediate barrier against external microorganisms and preventing blood outflow in a more stabilized structure [2]. This process occurs about 3 h after the damage. The clot leads to hemostasis, and with dehydration, it forms a scab. Each factor has its role within the phases of the wound healing, for example, the EGF stimulates the epithelialization of the wound site. Vasoactive amines such as histamine and serotonin are also released from the platelets, which promote fluid extravasation from the circulation to the tissue so the repairing cells can reach the site. Chemotaxis, derived from the action of local chemotaxins, promotes the peripheral attraction of white cells (inflammatory cells) from the circulatory system [4,5].

10.1.2 Inflammatory phase

Eicosanoids and other products derived from cell injury act directly on the start and potentiation of inflammatory phase. This phase protects the body against invading microorganisms. Accordingly, it can be divided into early stage and late stage, and is described by its five cardinal signals: pain, redness, increased heat, swelling, and loss of function [2].

In its early stage, it is characterized by the migration of neutrophils (reaching their maximum numbers in 24–48 h) plus activation of the complement; and in its late stage, monocytes migrate from the vessels and are termed macrophages once they reach the damaged site (after 48–96 h of the lesion). First, the neutrophils are marginalized by the attraction of chemotaxins/complement proteins (C3a and C5a) and rolled through the vase endothelium by selectins and integrins' connections. They are the first cells to arrive at the damaged site, doing phagocytosis and producing oxygen-derived reactive species that can help with bacterial destruction until the macrophages play their role [2,4].

At the late stage, the macrophages are fundamental to the success of the inflammatory phase. They proliferate and contribute through cytokine secretion, growth factors secretion, angiogenesis, phagocytosis of bacteria, fibroplasia, and extracellular matrix (ECM) synthesis [4,5]. As mentioned earlier, these cells were blood monocytes that transformed into tissue macrophages after the stimuli of cytokines such as PDGF and TGF-β, and leukotrienes. Macrophages are the most effective phagocytic cells, with a longer lifespan in comparison with neutrophils (as neutrophils undergo apoptosis after the phagocytosis and in the presence of the antigen). Moreover, they can effectively collaborate with the healing environment through tissue recovery stimulation by factors secretion, together with dendritic cells. At the end of this phase, IL-1 (Interleukin 1) is secreted from macrophages and other cells resulting in lymphocytes attraction to complete the immunological response of the inflammatory phase [2].

10.1.3 Granulation/proliferative phase

After the homeostasis has been established and the inflammatory/immunological response settled in place, the tissue recovering process goes further. The proliferative phase comprises four different stages: collagen deposition, angiogenesis, granulation tissue formation, and re-epithelialization. Physiologically, these stages overlap each other in an effort of the organism to resolve the exposition; however, we separate them didactically for a better understanding of the process. It begins from the fourth day after the exposition and extends approximately until the end of the second week [2,5].

First, there is an attempt of the epithelial cells to restructure the basal membrane and consequently the protective barrier. To achieve the healing site, angiogenesis is stimulated by tumor necrotic factor-alpha (TNF-α), PDGF, vascular endothelial growth factor, and TNF-β, and is characterized by the migration of endothelial cells (EC) and the formation of new capillaries.

Its final stage is the formation of the granulation tissue. The fibroblasts and EC are the main cells of this stage. There is a marked increase in epithelial cells mitotic rate to achieve reepithelialization after a few hours, and a migration flux from the wound edges to the center of the damage [2]. The fibroblasts are migrated from bordered tissues or from the blood (in 5–7 days); nonetheless, they need to be activated by PDGF to play their role. In the following steps, TGF-β stimulates the fibroblasts to produce collagen types I and III to proliferate and differentiate into myofibroblasts, promoting the contraction of the wound. On the natural wound healing, collagen type III initially predominates, but it is later changed for collagen type I [4,5]. Collagen is the most abundant protein in the human body and also the main component of the ECM of tissues. This extracellular matrix, also composed of glycosaminoglycans (GAGs), fibronectin, and proteoglycans, is essential for the healing process and supports cell migration through the site [2,6].

The collagen creates a network that is responsible for the scar tissue and is resulted from the processes of synthesis, fixation, and degradation of itself and its precursors. Procollagen helixes, each formed by three alpha strands linked by disulfide bonds, are polymerized by peptidases to form the collagen fibril. Varying on the amino acid composition of the alpha strands, there can be 19 different types of collagen with different characteristics [4].

10.1.4 Remodeling phase

This phase commences after the third week and may last for years. Its highlight is the reorganization of the collagen network, being responsible for the scar formation. The initial collagen (collagen type III) is reabsorbed and a thicker collagen (collagen type I) is organized through the tension lines. Collagenases secreted by leukocytes and fibroblasts degrade old collagen to promote the formation of a new structure in the wound site [4].

Within the remodeling phase, there is the differentiation of fibroblasts into specialized myofibroblasts that behave as contractile muscle cells. This behavior leads to the contraction ability of the wound, increasing the tensile strength with the collagen collection. As mentioned above, collagen is degraded and formed at an almost equilibrated rate, being always present in the wound. With time, the skin can recover to 50% or 80% of its original function, hence, it will never reach its full ability as it was before the damage. The final product of this process is a scar with a decreased number of cells (macrophages and fibroblasts undergo apoptosis) and vascularity, with a high tensile strength. Thereupon, complete wound healing and recovering of the local function is still a major challenge for doctors and researchers [2,4,7].

10.2 Types of wound healing

Overall, the wounds can be categorized according to several characteristics (Table 10.1). The time frame necessary for the most complete heal is crucial for its clinical success; therefore, it is the most clinically used criteria. At the same

TABLE 10.1 Wound healing process categorized according to different criteria, as stated in Velnar T., Bailey T, Smrkolj V, 2009 [2].

Wound healing classifications	Clinically	Acute
		Chronic
		Complicated
	Etiology	Contusions
		Abrasions
		Avulsions
		Lacerations
		Cuts
		Stab wounds
		Crush wounds
		Shot wounds
		Burns
	Contamination degree	Aseptic wounds
		Contaminated wounds
		Septic wounds
	Morphological characteristics	Closed
		Open

time, when considering multiple points such as cellular interactions, molecular mechanisms, and time necessary to heal, the four main categories of wound healing are: healing by primary intent, delayed primary healing, healing by secondary intent, and healing that transpires [8].

10.2.1 Primary intent

This type of healing is faster if compared to the others, and is a consequence of a surgical insult that leads to minimal cellular mortality. The edges of the wound are close to each other, facilitating the epithelialization process [4].

10.2.2 Delayed primary healing

It occurs when the edges of the wound are not immediately reapproximated. The main consequence is a longer process of healing, with the granulation phase having the possibility to extend itself and form granulomas. In some cases, this type of healing is desirable, especially when there is bacterial contamination involved. If the ultimate part of the process does not go under surgical assistance, it might result in chronic inflammation and lead to prominent scarring [4].

10.2.3 Secondary intent

This type of healing results in an even more intense inflammatory response, with a bigger amount of granulomatous tissue being formed in behalf of closing the wound. Henceforth, fibroblastic proliferation is enhanced and there is a pronounced contraction of wound [4].

10.2.4 Healing that transpires

This type of healing occurs in wounds that are more superficial, considered partially thick, involving only epidermis and superficial dermis. In this type of healing, the epithelialization, which is the migration and replication of epithelial cells to recover the barrier, is the major mechanism in which the healing occurs. Contraction and fibroblastic activity are uncommon [4].

10.2.5 Complications derived from the natural healing process

The utmost aim of the wound healing process is to achieve a complete repair of the damaged site. For this aim, the process follows a line of events combining molecular, biological, and immunological mechanisms that can end up successfully or not depending on extrinsic and intrinsic factors of the healing conditions. To be noted, intrinsic factors such as type of the damaged tissue, local contamination, low oxygenation of the site, tissue necrosis, and dimensions of the lesion can determine the outcome of a naturally healed wound. Equally important, the extrinsic factors such as the age of the patient, nutritional

and immunological stage, diabetes and other disorders, drugs use (specially steroids, smoking), alcoholism, and chemotherapy need to be considered when evaluating the healing ability of the patient on an intent to assess better strategies to outplay these disturbers [2,4].

Often, due to these intrinsic and extrinsic conditions, this process can go wrong and the fibroblasts set off into an exuberated proliferation stage resulting in a *hypertrophic scar* confined into the wound site. Higher exuberance can result in a *keloid scar*, which goes beyond the wound site, being commonly irregularly arranged and painful. On the contrary, if the healing is insufficient, the process can result in a hypotrophic or *atrophic scar*, which is linked to a lower degree of inflammation [9].

Debridement (the removal of necrotic tissue) is a clinical practice essential for cell renewal, and together with the correct use of a wound dressing it determines the success of the healing process. Recent efforts have been made on dressings in an attempt to modulate scar formation at a molecular level to completely avoid pathological scars [4]. These reparative strategies, mostly involving engineered dressings, either exogenous or endogenous, have been used lately—with highlights to polysaccharides.

10.3 Polysaccharides as dressings

Biopolymers are considered natural polymers given that living organisms synthesize them. Structurally these organic molecules are repeating sequences of nucleotides, monosaccharides, amino acids, or esters forming polyphenols, polysaccharides, peptides, or polyesters. Polysaccharides are a fascinating resource for wound healing dressings owing to their natural origin. The polysaccharides biopolymers properties are based on their diverse sources such as animals, fungi, plants, algae, and bacteria; therefore they offer benefits over synthetic polymers due to their biodegradability, biocompatibility, renewability, and lower antigenicity [10–14] (Fig. 10.1). Polysaccharides have been widely used in wound healing applications given their properties and natural abundance; moreover, they usually are nonimmunogenic and antimicrobial, two extremely important characteristics for wound healing materials [15–17]. Another appealing feature regarding polysaccharides is their chemical variance related to different molecular weights, chemical compositions, and charge, consequently making it possible to design specific wound dressings for different types of wounds just by changing the polysaccharide.

Wound dressings offer an appropriate environment for the wound to repair, protecting the area from the external environment while keeping the wound moist for suitable healing. Current dressings are being developed to not just protect the area but also to absorb the wound exudate, accelerating the healing process [18]. By the same token, novel dressings should actively play roles in the process; thus, the incorporation of bioactive therapeutics in the dressing platform could improve and accelerate the healing process. An interesting feature of

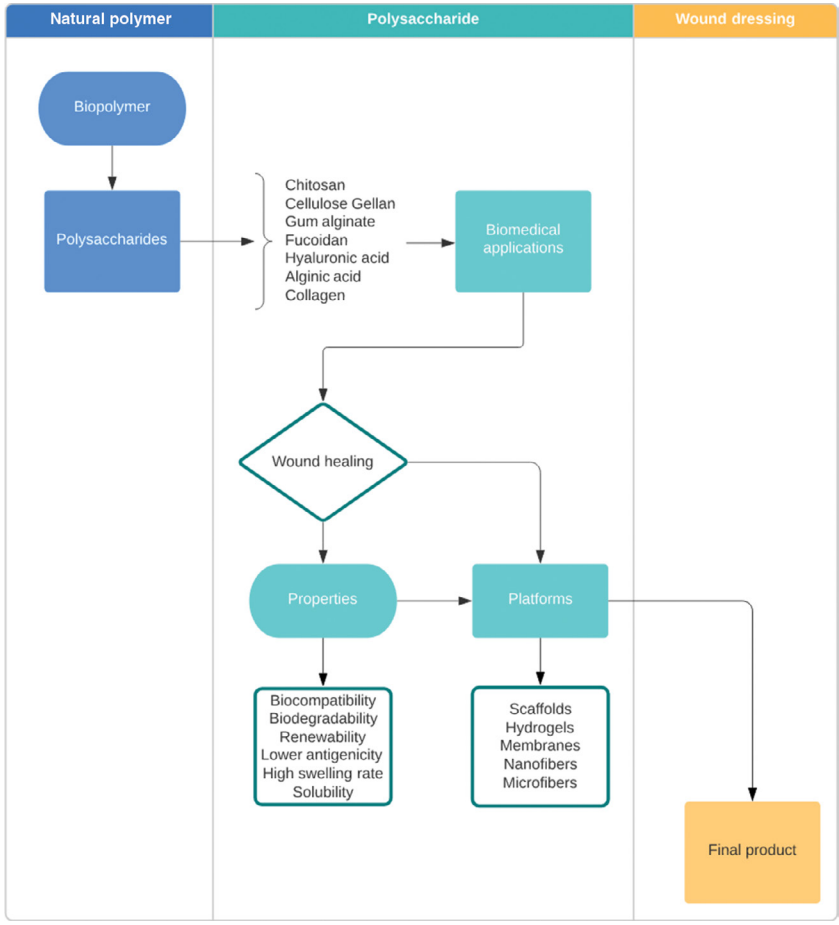

FIG. 10.1 Polysaccharide wound dressing scheme.

polysaccharides is their ability to activate macrophages to promote repair in the injured site [19]. Furthermore, they present GAGs in their composition, which are also part of the extracellular matrix, presenting an important role during wound healing by binding proteins involved in cell adhesion, signaling, differentiation, cell-matrix interactions and improving the healing process through augmenting the vascularization and promoting the reepithelialization [20,21].

10.3.1 Types of polysaccharides

Biopolymers such as polysaccharides are complex carbohydrates, composed of ten up to several thousand monosaccharides arranged in chains and are structurally diverse in terms of chemical composition, molecular weight, and charge. Polysaccharides provide a wide range of structural parameters

TABLE 10.2 Classification of polysaccharides based on their several sources.

Microbial	Algae	Plants	Fungi	Animal
Bacterial	Alginate	Cellulose	Chitin	Chitosan
Alginates	Galactan	Guar gum	Chitosan	Chitin
Dextran	Fucoidan	Gum Arabic	Glucan	Glycosaminoglycan
Gellan gum	Ulvan	Xylan	Cellulose	(hyaluronic acid)
Xanthan gum	Laminarin			

and properties for manufacturing wound dressings [22]. The main biological activities of the polysaccharides are proliferative, antiinflammatory, antimicrobial, and antioxidant properties; therefore, polysaccharide dressings can play a vital role during the healing process [23]. Chitosan, chitin, cellulose, alginate, and hyaluronic acid (HA) are the main polysaccharides applied for wound healing. They are naturally produced by sources such as fungi, animals, plants, microorganisms [24,25], and their classification is given in Table 10.2.

Additionally, biopolymers present biocompatibility, biodegradability, renewability, similarity to the ECM, and in some cases bioactivity, and are being widely used in the regenerative medicine field for the treatment of the wounds and burns as dressings [23,26].

One of the essential factors for wound dressing application is biocompatibility, because the wound might be potentially exposed to a cytotoxic environment that would intensify the healing process. Polysaccharides are normally biocompatible and are able to promote nonspecific activation of the immune system by activating macrophages [23,27,28].

A variety of biopolymers have been used for wound healing; their composition and biological role are shown in Table 10.3.

TABLE 10.3 Composition and biological role of polysaccharides in the healing process.

Polysaccharides	Composition	Biological role	References
Alginate	D-Mannuronic acid and L-guluronic acid residues linked by α-1, 4 glycosidic linkages.	Induces fibroblast proliferation and migration, hemostatic properties in exudation/bleeding	[29–33]

Polysaccharides	Composition	Biological role	References
Chitin	β-(1→4)-linkages (similar to the linkages between glucose units forming cellulose).	Induces proliferation and migration in fibroblast and keratinocytes	[25,34,35]
Chitosan	(1→4)-D-glucosamine and N-acetyl-D-glucosamine.	Induces proliferation and migration in fibroblast and keratinocytes	[36–39]
Cellulose	β-D-glucose linked by β-1,4-glycosidic linkage.	Matrix for chronic wound dressings, reducing pain, and shortening healing time	[40–42]
Curdlan	β-1,3-linked glucose residues.	Enhanced migration, proliferation, and wound closure	[43–45]
Dextran	α-1,6 glycosidic linkages between glucose monomers, with branches from α-1,3 linkages.	Stimulates fibroblast and keratinocytes and angiogenesis	[46–48]
Fucoidan	α-L-Fucose linked by α-1, 3 glycosidic linkages.	Antioxidant, antiinflammatory, and growth factor -dependent activities	[49–52]
Gum Arabic	D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid.	Absorption of exudates, retention of moisture, and stimulates fibroblasts	[53,54]
Hyaluronic acid	N-acetyl-D-glucosamine and glucuronic acid linked via alternating β-(1→4) and β-(1→3) glycosidic bonds.	Stimulates fibroblast and keratinocytes, regeneration and remodeling of human epidermis. Antiinflammatory	[55–57]
Pectin	D-galacturonic acid resides in an α-(1–4) chain.	Bacterial or viral barrier and controlling exudates	[58,59]
Schizophyllan	β-1,3 beta-glucan with β-1,6 branching.	Antitumor and immunomodulator properties	[60]
Ulvan	α- and β-(1,4)-linked monosaccharides (rhamnose, xylose, glucuronic acid, and iduronic acid).	Anticoagulant, antioxidant and stimulates the immune response	[61]

Biopolymers have material properties such as high adsorption efficiency and biodegradability that allow them to be molded into hydrogel, scaffold, and blended with other polymers, nanoparticles, and chemical compounds. These features provide several benefits as biomimetic properties, with increased mechanical strength and bioactivity, promoting the repair process.

The most common polysaccharides used for wound healing are as follows:

- Alginates
  Vital role: afford the moist environment and absorb exudates.
  Wound types: dermal, surgical incisions, infected, and postoperative.
  Advantages: hemostatic.
  Disadvantages: cannot be used for eschar and dry wounds and third-degree burns.
- Cellulose
  Vital role: afford the moisture-retaining properties.
  Wound types: burns, chronic, plastic/reconstructive surgeries.
  Advantages: antibacterial.
  Disadvantages: cannot be used for second- and third-degree burns
- Chitosan
  Vital role: stimulates hemostasis and accelerates tissue regeneration.
  Wound types: acute and pressure ulcers.
  Advantages: antimicrobial
  Disadvantages: poor mechanical performance and stability.
- HA
  Vital role: stimulates fibroblast proliferation, remodels ECM, and keratinocyte migration.
  Wound types: chronic, partial, and full-thickness.
  Advantages: flexible, highly biocompatible, and bacteriostatic.
  Disadvantages: none.

10.3.2 Platforms

Dressing strategies based on nanotechnological approaches have enabled the improvement of wound care products. Biopolymers-based materials present suitable mechanical features that allow them to be straightforwardly shaped into membranes, hydrogels, and scaffolds. In addition, blends of polysaccharides with other polymers offer numerous benefits for the design of skin-like materials including biomimetic characteristics and increased mechanical strength [62,63].

Different types of materials and combinations have been applied in the production of wound dressings, and also depending on the desired dressing appearance, the methodological approach used can change. The most common methods used in the development of dressings and their features are summarized in Fig. 10.2.

Some features are required to release drugs from membranes or films, such as porosity, viscoelasticity, pore size, and thickness [64]. These characteristics are greatly influenced by the selected methodology. One of the most relevant features to be considered while developing dressings is the mechanical

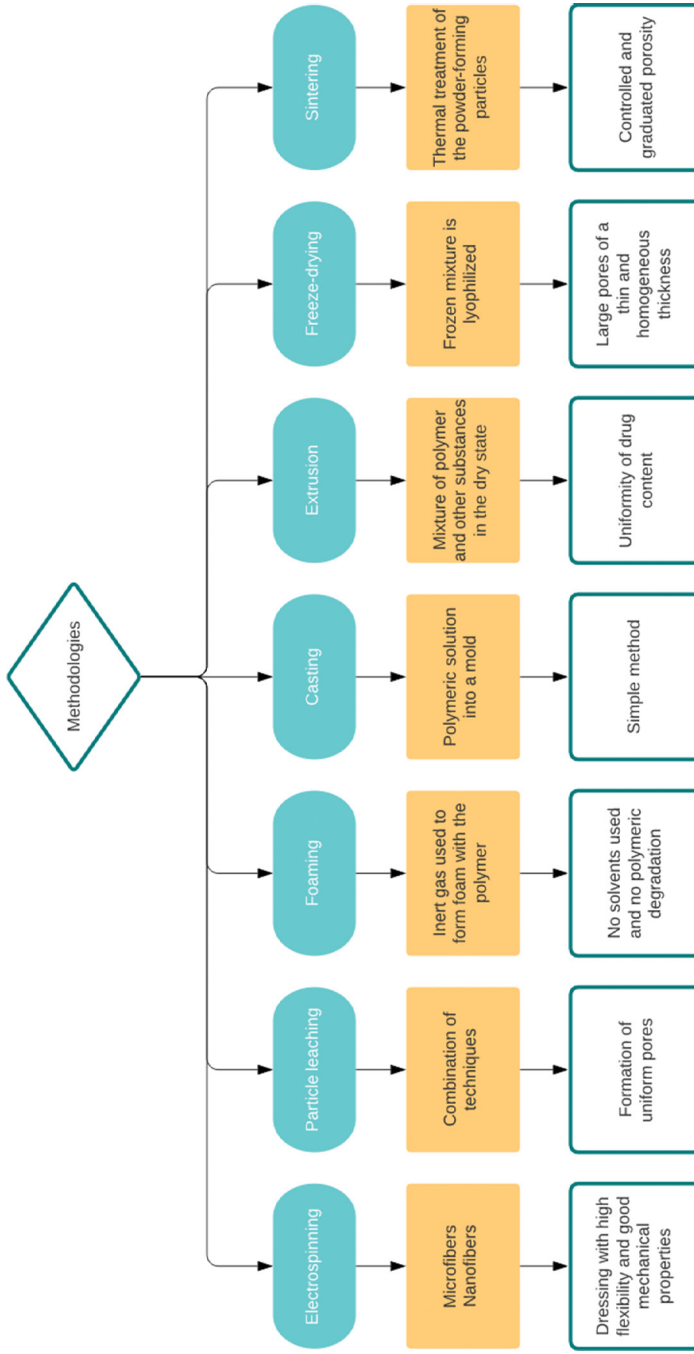

FIG. 10.2 Methodologies for obtaining dressings from polysaccharides.

properties as the material should be capable of resisting the patient movements. Therefore, several formulations are reinforced with synthetic polymers such as polyurethane and polyethylene forming a multilayer dressing [65].

Together with the mechanical properties, two features are significantly important and should be taken into account when choosing the dressing: the wound healing activity and the fluid-handling capacity. The first property is related to the healing activity that some polysaccharides have including proliferative and antimicrobial activities [66–70] and the second is related to the absorptivity and permeability abilities of the dressing. Given that distinctive kinds of wounds generate distinctive quantities of exudate and different phases of the healing process demand different environments (dry or moist), it is essential to determine this property.

10.3.2.1 Hydrogels

Hydrogel is a cross-linked polymeric dressing that presents decreased solubility in an aqueous environment. They are outstanding platforms for wound care owing to their significant ability to swell, promoting the wound exudate absorption, but keeping the wound moist.

In addition, they offer a nonadherent surface given their gel properties, avoid heat absorption, being comfortable for the patient, and can be very malleable [71–74] depending on the composition. Polysaccharides properties are important during the design of the dressing and they will dictate the degree of swelling of the product. Classically, high molecular weight polysaccharide hydrogels with abundant cross-link densities are hard and inflexible [75,76].

The formulations of polysaccharides in hydrogels are greatly versatile and straightforwardly manipulated enabling several biomedical applications [77]. These hydrogels have been largely used as drug delivery systems; nowadays researchers are greatly using polysaccharides as dressings for wounds owing to their structure that is similar to the ECM [78].

The main differences between the two cross-linking approaches, ionic and covalent, are listed in Table 10.4.

TABLE 10.4 Features of ionic and covalent cross-links [79].

Ionic cross-link	Covalent cross-link
Reversible process	Irreversible process
Greater swelling property	Small molecules, lighter, enzymes or monomers can be used
pH-dependent swelling	More stable networks
Ionic molecules creating a bridge within polymeric network	Toxic byproducts
Safe technique (no toxic catalysts)	Enables absorption without compromising mechanical integrity
Low stability	

One example of a positive polysaccharide that can be cross-linked is chitosan, which can be mixed with negatively charged cross-linkers such as phosphate-bearing groups [80] and metallic anions [81,82] aiming to create stable networks. Recently, Udhayakumar and colleagues developed a novel 3D hydrogel scaffold by combining chitosan and collagen [83]; thus, this mixture increased the biological and mechanical properties of the scaffold when compared to the individual materials owing to the ionic complex establishment between positively charged chitosan and negatively charged collagen [83,84]. The authors concluded that the combination of these molecules improved the porosity of the material providing an artificial ECM environment for fibroblasts adhesion and improving wound healing *in vivo* by promoting collagen synthesis and skin regeneration without scar formation [83]. Another important cross-link method is the freeze-thaw technique, which does not involve any additional cross-linking agents. Song et al. produced a self-healable chitosan/cordycepin hydrogel dressing by using freeze-thaw method that, due to its excellent mechanical strength and biocompatibility, greatly improved reepithelialization of skin wounds increasing collagen synthesis. Moreover, this hydrogel was able to adapt in irregular wounds without the need of remodel [85].

It is known that HA dressings present outstanding absorption properties therefore they can be used in wounds that generate excessive amounts of exudate. These dressings can be modulated into several different compositions and forms and they are capable of absorbing up to 20y times their weight [86]. Amid the several HA clinical uses, it is possible to observe wound dressings [87], joint lubricants [88], and skin substitutes [89]. One HA-based material, HYAFF-11, is a commercialized biocompatible HA matrix that has enhanced mechanical properties after an esterification cross-linking step [87], enabling a swelling up to 1000 times its weight for extremely exudative wounds while preserving mechanical integrity.

10.3.2.2 Electrospinning

Nanofibrous scaffolds can be synthesized by electrospinning method as it is cost-effective for generating continuous biomaterials. Electrospun nanofibers (NF) have a notably large surface area, allowing the incorporation of substantial drug amounts maintaining the therapeutics after encapsulation [90,91] (Fig. 10.3). Thus, it has provided efficient drug carriers for wound healing given its mechanical properties [92–116].

This method is an interesting approach to construct scaffolds due to the robustness of the electrospinning. It comprises a polymeric solution into a plastic support connected to a syringe, a pressure pump, an electric source of high voltage, a needle, and finally a conductive collector plate [117]. The production of nanomaterials starts when the solution is pumped by the system and a high-voltage electric field is applied. Owing to the voltage, a deformation occurs in a solution drop created at the needle, replacing its shape with a cone-like configuration entitled the Taylor Cone [118], then it is sprayed toward the collector plate.

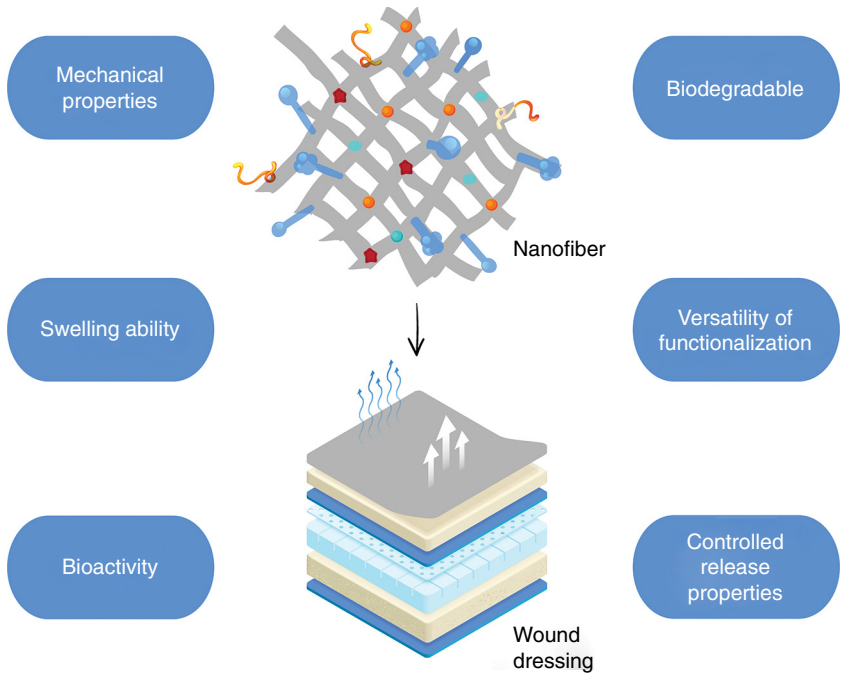

FIG. 10.3 Electrospun dressing features.

The consequential scaffold structure is commonly associated with the solution concentration, charge, and the solvent. To define the idyllic combination, it is indispensable to adjust the solution viscosity. If it is a less concentrated solution, there is a lack of chain entanglements and the spray is unable to stabilize; however, if it is a solution with adequate concentration, fibers can be formed as a result of a stable polymeric network [119–121].

Electrospun NF are an appealing approach as wound dressings considering their high surface and porosity that absorbs a great amount of exudate and keeps the wound moist [122]. Additionally, some NF features such as porosity allows a suitable environment for the cells in which they can exchange oxygen, and, at the same time, inhibits permeation of bacteria keeping the wound clean [122,123]. Another important characteristic is the viscoelasticity of NF, given that flexible materials provide comfort and compliance of patients [122].

As shown in Table 10.5, polysaccharide biopolymers have become extensively common as electrospun scaffolds for wound healing given their biodegradability, antimicrobial properties, natural abundance, and biocompatibility. Moreover, polysaccharides with GAGs can be electrospun into nanofibrous scaffolds that mimic skin tissue owing to their extracellular matrix.

Recently, Chen and colleagues [116] produced cross-linked pectin NF by oxidizing the polysaccharide and then covalently cross-linking pectin

TABLE 10.5 Electrospinning polysaccharide NFs.

Polysaccharide	Features	References
Hyaluronic Acid	Core-shell PCL-HA- EGF NF scaffolds enhanced regeneration of fully functional skin facilitated by epidermal regeneration by using full-thickness wound model	[92]
	HA-collagen NF evaluated in a chronic wound healing model revealed an accelerated wound closure rate, elevated collagen deposition, and enhanced maturation of vessels	[93]
Guar gum	PVA NF with encapsulated paramagnetic iron oxide nanoparticles presented adequate levels of cytotoxicity and cell adhesion/proliferation	[94]
Ulvan	For the first time, the preparation of a hybrid spider-web-like PCL or PEO Ulvan NF mat based on a marine polysaccharide was developed	[95]
Chitin	High surface area chitin NFs were produced by a simple method directly from biomass in a one pot system	[96]
	Chitin NF, alone or with extracellular matrix proteins such as collagen, improved cell attachment and spreading of normal human keratinocytes and fibroblasts	[97]
	Before electrospinning of the chitin solution, the polymer was depolymerized by gamma irradiation improving its solubility	[98]
Chitosan	PVA/chitosan/starch nanofibrous mats promoted cell migration	[99]
	Chitosan/PVA/zinc oxide nanofibrous mats presented antibacterial and antioxidant properties for diabetic wound healing	[100]
Schizophyllan	PVA-based nanofibrous scaffolds improved cell adhesion	[101]
Alginate	PEO-alginate NF improved ciprofloxacin hydrochloride model drug release	[102]
	PVA-alginate NF presented higher stability	[103,104]
	PVA-zinc oxide NF presented antibacterial activity	[105]

(Continued)

TABLE 10.5 Electrospinning polysaccharide NFs. *(Cont'd)*

Polysaccharide	Features	References
	Honey-loaded PVA membranes presented antibacterial activity and biocompatibility *in vitro*	[106]
Dextran	PU-dextran interacted favorably with the cells and the mat showed good bactericidal activity against both Gram-negative and Gram-positive bacteria	[107]
	PU-dextran dressing loaded with β-estradiol promoted neovascularization and skin regeneration in postmenopausal wound healing	[108]
Curdlan	PVA-curdlan promoted increased wound closure rate compared to pure PVA scaffold due to the immunomodulatory features of the polysaccharide	[109]
	PEO-curdlan scaffold provided a controlled release of tetracycline hydrochloride and an improved antibacterial activity	[110]
Collagen	Laminin-coated NF improved cell adhesion and migration of keratinocytes	[111]
	Sulfated xylorhamnoglycuronan scaffolds showed collagen alignment similar to normal skin	[112]
	PCL NF showed accelerated migration and proliferation of fibroblasts	[113]
ß-glucan	PLGA NF enhanced fibroblast proliferation and angiogenesis in nude mice	[114]
Cellulose	NF promoted collagen synthesis and neovascularization	[115]
Pectin	Pectin NF with high oxidation/cross-linking degree exhibit improved cell adhesion ability	[116]

EGF: epidermal growth factor; *HA*: Hyaluronic Acid; *NF*: nanofibers; *PCL*: polycaprolactone; *PEO*: polyethylene; *PLGA*: poly(lactic-co-glycolic acid); *PU*: polyurethane; *PVA*: polyvinyl alcohol.

macromolecules with adipic acid dihydrazide (Fig. 10.4). The cross-linked NF exhibit outstanding mechanical properties and improved degradability (up to 3 weeks), furthermore, the authors observed a significant improvement in cell adhesion when compared to ionically cross-linked calcium-pectin, suggesting that these scaffolds are a promising candidate for *in-vivo* applications.

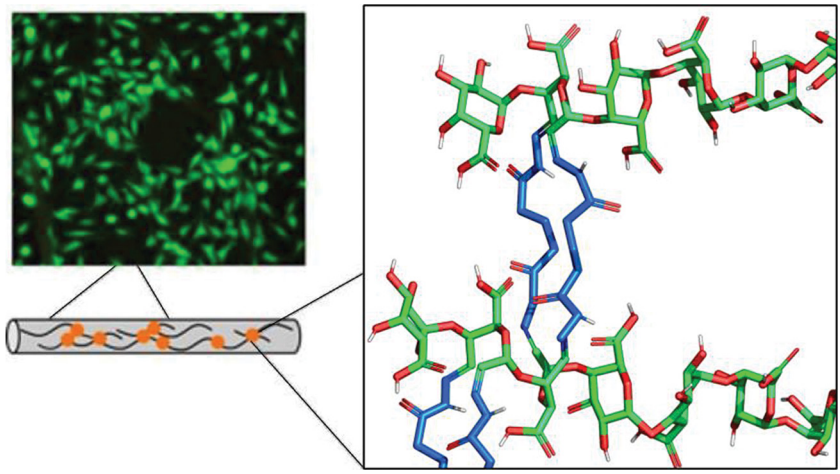

FIG. 10.4 Molecular modeling of cross-linked pectin nanofibers. Pectin polymers are represented in green and cross-linked in blue. CarbM web-based tool was used to build 3D structures of pectin and the Pymol program was used to model the complex. *Adapted from Sainan Chen; Sisi Cui; Hui Zhang; Xuejing Pei; Junli Hu; Yifa Zhou; Yichun Liu; 2018 [116].*

10.3.2.3 Commercially available dressings

Recently, pharmaceutical companies have pursued novel methods and materials to improve the dressings healing properties. Well-designed dressings require following:

1. Encapsulation of drug with wound healing or bactericidal activities.
2. Improvement of permeation features.
3. Easy application and removal.
4. Adequate porosity aiming to avoid contaminations.

Even with the continuous production of the traditional dressings, the dressing market is concerned about innovative and bioactive dressings owing to their enhanced healing speed [124]. The Surgical Dressing Manufacturers (SDMA) embraces multinational companies and family businesses that produce dressings and associated products. This association holds a website that records handy information on dressings and their content (www.dressing.org) for people who wish to explore and learn about this subject.

Amid the commercially available dressings, the polysaccharide-based dressings are shown in Table 10.6. Some of these dressings are not entirely composed of polysaccharides; however they may include other materials including synthetic polymers. It is known that most of these dressings are recommended for the treatment of trauma and surgical wounds, ulcers, burns, superficial and cavity wounds; nevertheless, it is also suggested to analyze the existing information on each dressing to select the most suitable one.

TABLE 10.6 Commercially available polysaccharides dressings for wound healing.

Polysaccharide	Features	Commercial dressing
Alginate	Good absorption of exudate, hemostat, aids autolytic debridement, promotes granulation in the wound.	ActivHeal Aquafiber - ActivHeal, UK.
	Forms a gel when it comes into contact with the exudate, keeps the wound moist.	Algisite M—Smith and Nephew Medical Ltd, UK.
	Forms a nonadherent gel in contact with the exudate, hemostat, nonimmunogenic, biodegradable.	Curatec Alginato de Cálcio e Sódio—Curatec, Brazil.
	Can be used throughout all phases of wound healing and in all types of wounds: acute, chronic, and stalled.	MediHoney Calcium Alginate—Derma Sciences, USA.
	Provides a broad-spectrum antimicrobial action, can be used in moderate to heavily exuding and in full-thickness chronic wounds.	SILVERCEL Antimicrobial Alginate Dressing—Johnson and Johnson, USA.
	Highly exudate absorption capacity, bacteria and debris are trapped in the gel, comfortable.	Suprasorb A—Lohmann and Hauscher Company, Germany.
	Forms an absorbent gel, can be used on heavily exuding wounds (absorbs up to 20 times its weight).	Tegaderm Alginate—3M, USA.
Alginate/carboxymethylcellulose	Metalloproteinases targeting and deactivation, easy to use, moisture manager.	Biostep—Smith and Nephew Medical Ltd, UK.
	Hemostatic properties, exudate management, moist wound healing.	UrgoSorb—Urgo Medical, France.
Carboxymethylcellulose	Secondary dressing on highly exuding wounds as a skin protection.	Aquacel—ConvaTec Ltd, UK.
	Indicated for low to moderately exuding ulcers and wounds, cost-effective, reduces healing time.	UrgoTul and UrgoStart—Urgo Medical, France.

Polysaccharide	Features	Commercial dressing
Cellulose and derivatives	Antiseptic paraffin impregnated gauze, used for minor wounds.	Bactigras—Smith and Nephew Medical Ltd, UK.
	Highly absorbent, nonwoven and antimicrobial gelling properties.	Durafiber—Smith and Nephew Medical Ltd, UK.
	Hypoallergenic, immediate pain relief, improved healing time, keeps the wound moist and free of adhesives.	Membracel—Vuelo Pharma, Brazil.
	Wound dressing for noninfected wounds, keeps wound moist, reduces pain, elastic structure.	Suprasorb X—Lohmann and Hauscher Company, Germany.
Chitin	Artificial skin applied to full-thickness burn wounds, more adhesive than cellulose films.	Beschitin W—Unitika, Ltd, Japan.
Chitosan	Hemostatic, mucoadhesion forming a mechanical barrier on the area.	Axiostat—Axio Biosolutions Private Limited, India.
	Hemostatic, nonwoven, prevents lethal bleeding rapidly.	ChitoSAM 100—SAM Medical, USA.
	Extremely adherent when in contact with blood, hemostatic, antimicrobial barrier.	HemCon—Tricol Biomedical, Inc, USA.
	Controls severe bleeding, easy to use, high tensile strength, biocompatibility, hemostatic.	Hemo-bandage—CoreLeader Biotech, Taiwan.
Cross-linked polysaccharide Starch	Forms an absorbent gel when in contact with exudate, release of iodine for antimicrobial activity.	Iodoflex—Smith and Nephew Medical Ltd, UK.
Esterified hyaluronic acid	Indicated for surgical wounds, trauma wounds, second-degree burns, flexible and nonwoven pad.	Hyalomatrix—Medline Industries, Inc., USA.

10.4 Biocompatibility

When performing biocompatibility assays, the study must take different steps whether evaluating in an academic level or performing regulatory experiments to introduce new products to the market. To achieve safety and efficacy in regulatory parameters, the analyst should take a deep look into the ISO-10993 recommendations and country-specific guidelines, such as those provided by the Food & Drug Administration (FDA) in the United States through the Code of Federal Regulations (CFR) and the Conformité Européenne (EC) in Europe. In economic unions such as the EU, there may be country-specific guidelines that add parameters besides what the EC suggests. Thus, it should be known precisely which country to target or prefer to fulfill the most rigid regulations and consequently satisfying a huge group of countries. Nonetheless, academic studies usually lack good laboratory practice and other important parameters for quality control, such as the guidance provided by the ISO 17025 [125,126].

10.4.1 *In-vitro* analysis

Standardized cell culture conditions should always be a priority, such as keeping cultures in a 37°C incubator environment with a 5% CO_2 atmosphere [127]. Choosing the right cell lines to carry out experiments needs to be critically evaluated, as they must be similar expected target tissue and whether high xenobiotic metabolism is necessary. Besides incubatory parameters, culture medium, and serum supplementation should be looked into, as the type of medium may vary along within serum type and concentration.

To retrieve high-quality data during *in-vitro* experimentation, the analyst should know that while performing assays that do not aim to fulfill regulatory frameworks, the same guidelines provided by the ISO, FDA, EC, and OECD may be useful, as the similar steps are to be carried out. First, it is extremely useful to understand the toxicity of the tested device through cytotoxicity experiments such as the neutral red uptake (NRU) or thiazolyl blue tetrazolium bromide (MTT) and define half maximal inhibitory concentration (IC 50) through the dose-response linear regression [128,129]. This step is rather simple and takes a few days to perform, usually being the first experiments carried out with new devices.

When evaluating cytotoxicity, the analyst must understand that the production of dressings may have left surface contaminants and residual chemicals on the material that may or may not be avoidable. For this, the ISO-10993 suggests sample elution with culture medium (serum supplemented) and exposing cells indirectly to the eluted solution [130,131]. Time and heat are important when performing the elution step and it is highly recommended to expose the tested sample for 72 h in a 37°C or 50°C environment. However, physical properties and clinical use of the dressing have to be considered, as degradation, integrity and structural properties may be lost at high temperatures, but

also will not represent what could happen clinically, making it unnecessary to test on higher suggested temperatures [130]. The same assay should be carried out with the device itself, by exposing it to the cell culture through culture medium. Experimentally, it is common to carry out both direct and indirect exposure together, gathering as much data as possible about whether the toxicity is from the device, drugs used in the polysaccharide dressing formulation, or residual substances. Both the NRU and MTT are extremely similar when it comes to the procedure itself.

First, 5×10^4 cells are plated into each well in a 96-wells microplate and kept incubating in ideal conditions until reaching around 80% confluence (Fig. 10.5A and B) [128]. Second, cells are washed with phosphate buffer saline solution and then exposed according to its group (Fig. 10.5C). Generally, MTT and NRU require six wells for each treatment to achieve a good statistical analysis at the end of the assay. After the exposure time, the washing procedure must be repeated once to eliminate every dead cell and further exposed to either the NRU or MTT dye and incubated for 20 min or 1–4 h in the dark, respectively (Fig. 10.5D). As the principle of both methods is the incorporation of the dye inside the lysosome (NRU) or the mitochondria (MTT), the intensity of either red or blue will be directly proportional to the amount of cells. Finally, cells are washed twice and exposed for 20 min to different desorption solutions according to each assay (Fig. 10.5E), which will solubilize the dye content inside the remaining cells. If any concentration results in toxicity, the dye intensity should be reduced compared to the control or safe doses groups. The plate now can be read in its proper absorbance wavelength through a spectrophotometer coupled to a computer (Fig. 10.5F). Considering the control group's mean absorbance as 100% of viability, the other cell viabilities can be reached through the following equation: $\beta \times \mathbf{100}/\alpha \times \mathbf{X}$ where β is the arithmetical mean of a treatment group, α is the arithmetical mean of the control group, and X is the cell viability result, displayed in percentage [132].

After retrieving cytotoxicity data, further steps can be achieved to determine pharmacological efficacy on wound healing through a simple method called "scratch" test. This assay commonly uses EC to simulate living skin tissue and is performed by simulating a wound exposed to the tested device [133]. To allow image analysis, the experiment requires a relatively wide area to cell colony formation and is usually performed on Petri dishes or polystyrene plates with large wells (1–3 wells plates). Thus, around 10^5 cells are plated and should be kept in cell culture conditions until around 90% of confluence is achieved (Fig. 10.6A and B). Then, a straight line in the middle of the dish must be scraped with a cell scraper, followed by the removal of the scratch edges with serum-supplemented culture medium and the time 0 (t_0) is defined (Fig. 10.6C). Subsequently, sample and vehicle should then be applied to the cell culture (Fig. 10.6D). The wound healing process usually takes 8–18 h *in vitro* (Fig. 10.6E and F). During this time, the culture should be held in the

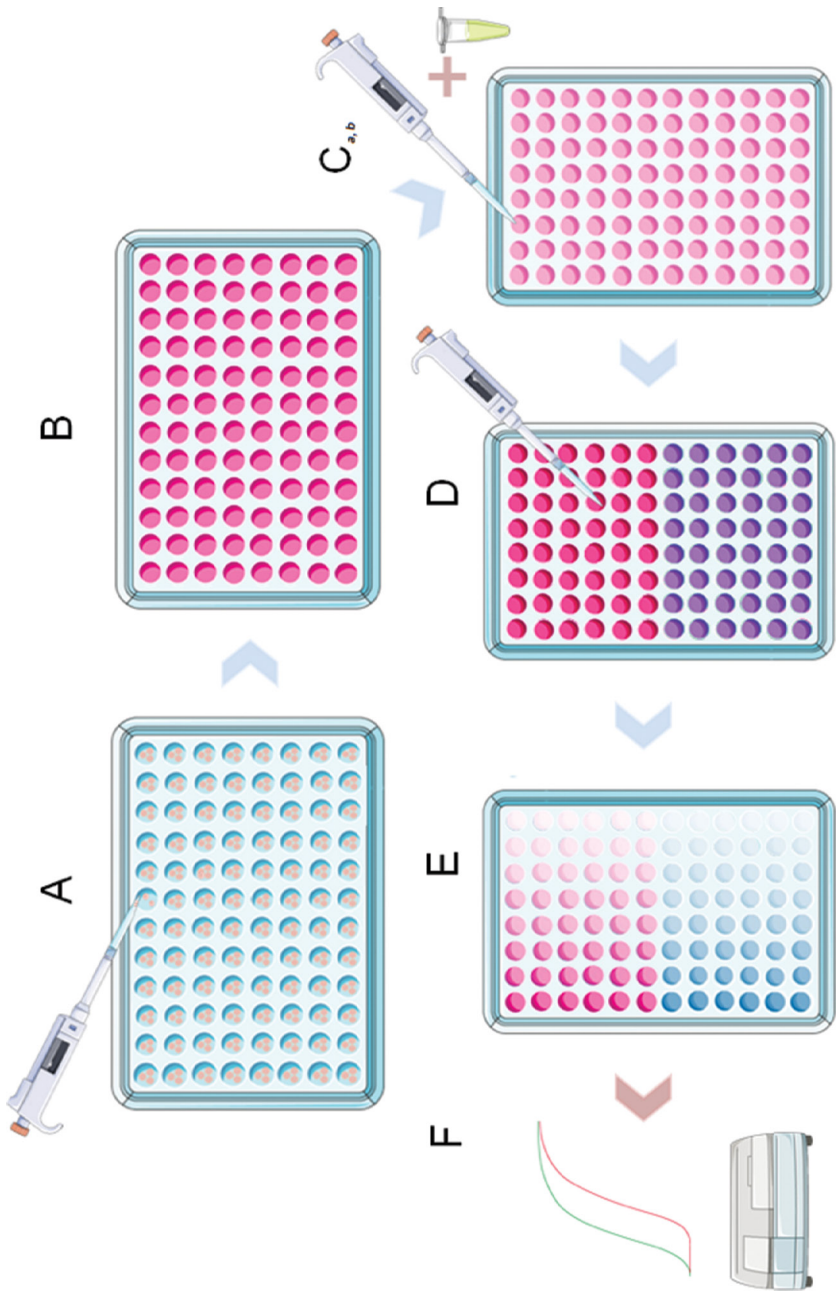

FIG. 10.5 *In vitro* wound healing flowchart. (A) Cell plating. (B) Cell growth. (C) Cell treatment (*a*: control; *b*: treatment). (D) Neutral red uptake (NRU) and (NRU) or thiazolyl blue tetrazolium bromide (MTT) incubation. (E) Desorption solution incubation. (F) Spectrophotometer reading.

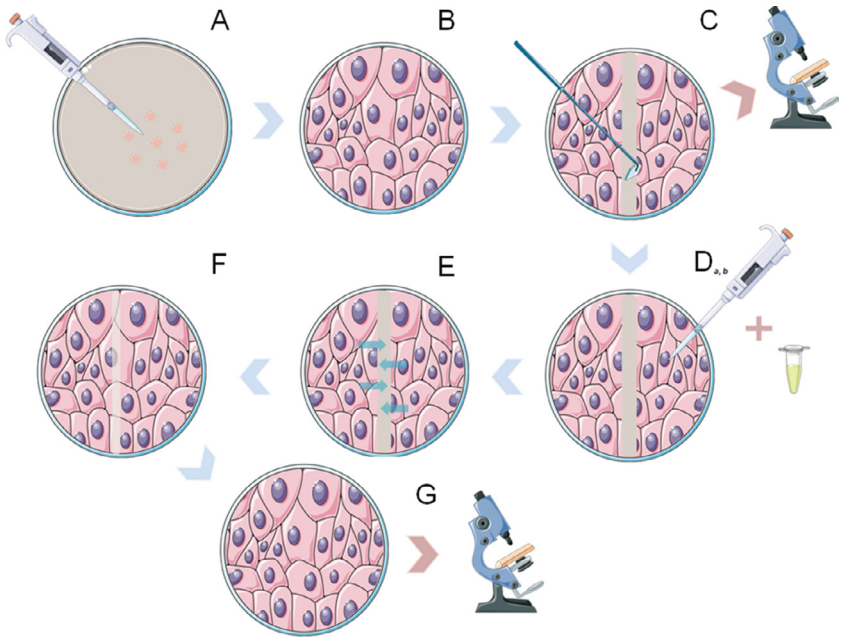

FIG. 10.6 Scratch flowchart. (A) Cell plating. (B) Cell growth. (C) Pipette scrap and t_0 image acquisition. (D) Treatment exposure (*a*: control; *b*: treatment). (E and F) Cell proliferation. (G) Wound closure.

same conditions as the proliferation step. Nonetheless, wound healing time may vary according to the chosen cell line and should be empirically decided by the analyst. After defining the analysis times, the dishes will be looked into through the same microscope as before to evaluate the healing process (Fig. 10.6G). Every image should be compared to each other and can be analyzed through different image software available to the laboratory. Aiming to avoid any bias, cells have to be treated with the vehicle, test sample, and a control group (medium and serum only), eliminating the possibility committing a mistake in the evaluation considering other cell proliferation inductors or inhibitors rather than the tested sample itself.

There are other parameters that can be retrieved from the scratch method, such as green fluorescent protein (GFP) marker and gene regulation [133]. Those assays will depend on the availability to the laboratory as they represent an increase in budget expenses because of the required reagents. GFP usually takes place during live cells imaging, making it possible to analyze signaling events and subcellular localization of proteins. On the other hand, transcriptional analysis will give detailed information on molecular behavior of different biochemical pathways, ranging from mitotic to coagulation cascades in mammals [134].

10.4.2 *In-vivo* analysis

Even though *in-vitro* assays are able to provide answers about the biocompatibility of a compound, the *in-vivo* analysis is essential mainly because polysaccharides can trigger immune reactions and allergy. When evaluating biocompatibility and wound healing processes *in vivo*, histology and surgical procedures are commonly chosen. The first decision to make is which animal species should be used. Usually, researchers prefer to work with rats rather than rabbits (lagomorph), due to the accessibility, time of experiment, and ease of test performance. Nonetheless, when skin sensibility and other physiological responses are being evaluated, a deeper look should be taken for species to be chosen. That information can be easily retrieved from the OECD Guidelines for the Test of Chemicals [135]. After choosing species, animals should be held for at least 5 days in the experimental room for laboratory acclimatization. There must be a maximum of five animals per cage, temperature should be around 22°C (± 3°C), humidity must fall between 30% to 70%, and the light cycle be split in 12 h of light and 12 h of dark [136]. Both water and food should be available ad libitum.

After the acclimatization period, animals are ready to be experimented on. They should be individually anesthetized into a deep state in which there are no pain reflexes (Fig. 10.7A). The anesthesia administration route may vary according to what is available to the laboratory. One to two 6-mm wounds are made in the dorsal skin of the rat and their extremity should be marked

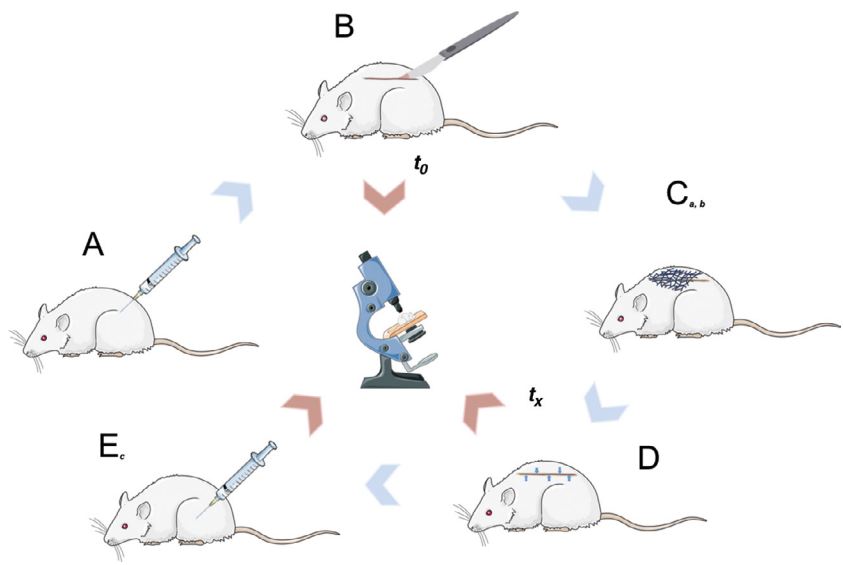

FIG. 10.7 *In vivo* wound healing flowchart. (A) Anesthesia. (B) Wounds infliction. (C) Dressing application (*a*: control and *b*: dressing). (D) End of exposure. (E) Euthanasia (*c*: gross and detailed necropsy, according to the experimental procedure).

with suture, nontoxic dye, or any available device that will not interfere in the healing or skin toxicity process (Fig. 10.7B, t_0). In the following step, animals should be divided into vehicle control and sample test groups and have the sample applied on each wound (Fig. 10.7C). According to the scratch method described before, pictures of each wound should be taken in the t_0. Further pictures should be taken according to the experimental planning, defined by the analyst itself and depending on the total time of exposure (Fig. 10.7D, t_x). At this point, it should be considered whether the animals will be exposed only once or repeatedly, according to what is aimed by the study and the dressing physical properties, such as degradation time and drug release rate (if applicable). It is relevant to state that any bias caused while taking the pictures should be avoided, such as camera height, zoom, and focus even when wound surroundings are already marked, avoiding any possible kind of data misinterpretation.

After taking the last picture of the experiment, animals should be euthanized through a humanized method (Fig. 10.7E). To evaluate histological features of the wound, such as immune system cells recruitment, inflammatory profile, granulomas, and any other parameter planned to analyze, the wounded tissue must be collected and properly stored to avoid loss of integrity [137]. If there is more than one assay to perform in the wounded tissue, the analyst must know if the storage process differs in each method, such as cell morphology, histochemical, and any other analysis in mind. Simple chemical pathology methods can help to understand the healing process, such as general hematology and urinalysis, being usually considered as blood and urine are easy to collect and to store.

10.5 Final considerations

Dressings for wounds have an important role in the management and progress of healing. It is known that, given the continuous improvement of dressings quality, natural polysaccharides have been proven to be promising candidates for promoting wound healing process, predominantly in severe wounds, owing to their impressive biological and mechanical properties (Fig. 10.8). Nevertheless, despite their merit as a treatment for atopy people, polysaccharides can induce allergies and promote an overreaction of the immune system; therefore, the adequate selection of the material may overcome this drawback,

Nowadays, 3D NFs have been extensively studied and developed for personalized tissue engineering and regenerating; therefore it is possible to design a scaffold material with a required function. Finally, nanotechnology together with polysaccharides seems to be an outstanding approach for wound management and several other medical applications.

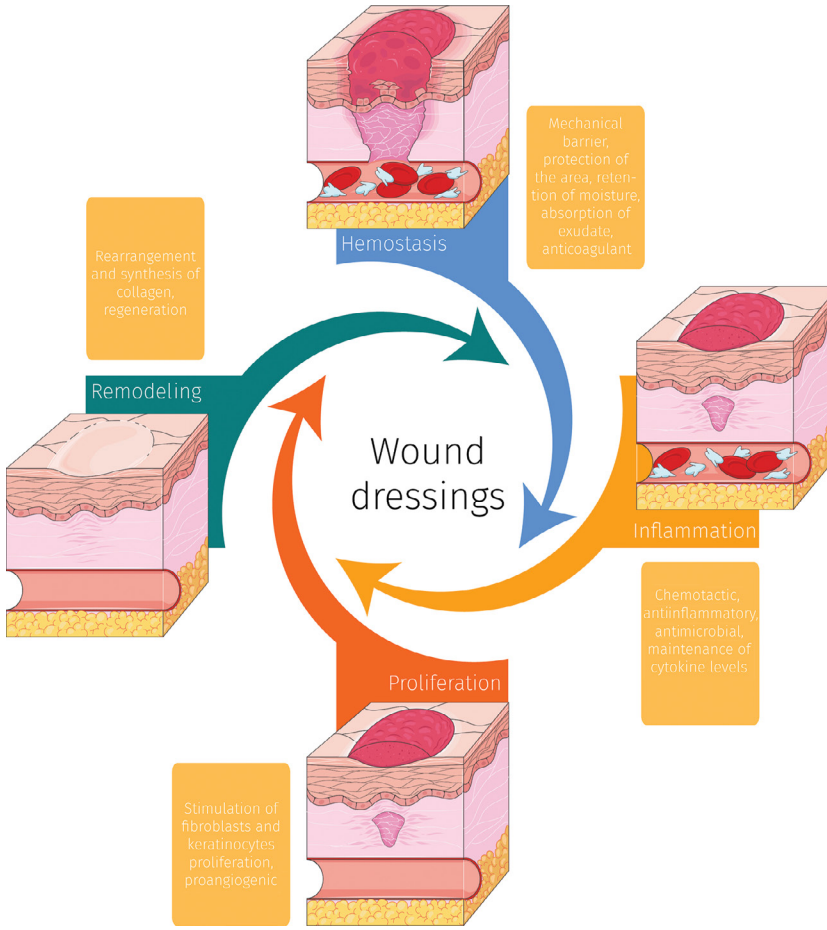

FIG. 10.8 Polysaccharide dressings role in wound healing.

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3.2. Capítulo 2: *Plantago australis* Hydroethanolic Extract-Loaded Formulations: Promising Dressings for Wound Healing

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ORIGINAL ARTICLE

Plantago australis Hydroethanolic Extract-Loaded Formulations: Promising Dressings for Wound Healing

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Abstract
The productions of wound dressings involve three key goals: wound protection, suitable environment, and acceleration of the healing process. This study was based on our previous findings of a *Plantago australis* Lam., Plantaginaceae, hydroethanolic extract and its pronounced wound healing activity, leading to the development of wound dressings containing the extract. The hydroethanolic extract was produced by an ultrasound method, using leaves followed by HPLC characterization. A nanofiber containing the extract was produced by using polyvinyl alcohol and electrospinning and had its mechanical and physical properties characterized. The hydroethanolic extract was found stable after electrospun and the nanofiber provided a continuous drug release. An ointment containing the extract was produced in lanolin:vaseline base by spatulation. *In vitro* and *in vivo* wound healing activities were assessed by 3D cell culture and by inflicting wounds in Wistar rats and placing the dressings up to 14 days, respectively. *In vitro* proliferation assay showed that exposure to hydroethanolic extract nanofiber increases the size of the spheroid (sum of the spheroid area and cell proliferation area) compared to the extract and blank nanofiber. *In vivo* testing indicated enhanced wound healing efficacy of animals treated with the hydroethanolic extract nanofiber and ointment when compared to the hydrogel containing the extract and blank formulations. Thus, these dressings could be used to maximize the healing process, improving the treatment outcome.

Keywords Electrospinning · Nanofiber · Polyvinyl alcohol · Ointment · Wound dressing

Introduction

The World Health Organization considers medicinal plants as those popularly used to prevent, alleviate, cure, or modify physiological or pathological conditions, or as a source and raw material for drugs and medicines (WHO 2013). The pharmaceutical industry has been rescuing the phytotherapeutic segment, with considerable investments in this research field. *Plantago australis* Lam., Plantaginaceae, is a perennial plant distributed in Latin America, widely found in southern Brazil and popularly known as "tansagem." Its leaves and seeds are used in the management of several diseases and symptoms, and its chemical composition includes numerous metabolites and, among them, verbascoside (**1**) has been widely studied (Sperotto et al. 2018; Henn et al. 2019). The ethnopharmacological knowledge of *P. australis* is widespread, and its use is related to the treatment of renal and bladder diseases, with antiviral, antimicrobial, and anti-inflammatory activities for the throat and ovaries, as well as healing, antiulcer, and antidiarrheal properties (Flores et al. 2016; Sperotto et al. 2018). Likewise, *P. australis* has some proven pharmacological activities. Its aqueous extract was able to inhibit vesicular stomatitis virus replication in *in vitro* assays without cytotoxic effects, whereas the hydroalcoholic extract of leaves, seeds, and fruits demonstrated analgesic and anti-inflammatory properties in rats. The gastroprotective activity of the ethanolic extract of the leaves of the species was reported in different

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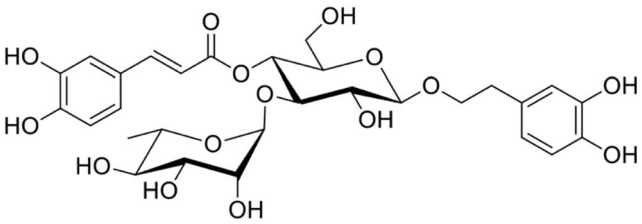
models in rats and was able to reduce the ulcer index and even increase the secretion of the stomach mucoprotective layer (Palmeiro et al. 2002).

Recently, our research group developed a hydroethanolic extract of the leaves of *P. australis* (PAHE) standardized in verbascoside (**1**) using two different methods: ultrasound-assisted extraction (UAE) and percolation. The UAE method showed the highest concentration of **1** (6.2604%) and a similar yield % when compared to the second method (9.92% and 10.43%, respectively). Toxicological evaluation of the extract obtained by UAE method revealed that it is safe to use, not presenting genotoxicity and mutagenicity in rats after acute and subchronic treatments (Henn et al. 2019). Additionally, both extract and **1** presented healing activity *in vitro* and *in vivo*, accelerating the process of cell migration, suggesting that **1** can inhibit inflammatory mediators, and both compounds can reverse the oxidative process (Sperotto et al. 2018). Thus, we presume that with an effective drug delivery system, PAHE biological effects could be improved.



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The development of wound healing drug delivery systems is an essential research field for biomedical sciences, fundamentally targeting applicable wound healing treatment capable of reducing undesired side effects and accelerating healing (Mekonnen et al. 2013). Several studies have demonstrated the efficacy of nanoproducts used as curatives (Kim and Lee 2017; Liu et al. 2017). Therefore, the development of dressings with natural bio-active compounds such as *P. australis* and verbascoside (**1**), which possess wound healing properties and are relatively easy to produce, seems to be a promising approach for wound healing management. Originally, dressings were made from natural compounds, such as plant fibers. Nowadays, the products have evolved and therefore, artificial materials can be produced by different technologies creating multifunctional dressings (Liu et al. 2017). Nanofibers (NF) are nanoscale materials that are widely used as dressings given their ability to release drugs locally and to store and maintain therapeutic agents in their structure efficiently, allowing the prolonged release of these drugs (Liu et al. 2017).

Polymeric NF are one of the most promising approaches owing to their biocompatibility and simple drug encapsulation during production (Pourgholi et al. 2016). In this context, polyvinyl alcohol (PVA) is an interesting polymer choice, since it is non-toxic, biocompatible, and has a remarkable record of biomedical purposes, specifically in the form of hydrogels and nanoparticle materials used for clinical applications (Chen et al. 2015; Reinhardt et al. 2020; Steffens et al. 2020). Among the different forms of nanotechnologies to produce NF, electrospinning creates nanoproducts with complex nanostructures on an industrial scale simply and directly (Liu et al. 2018), providing great flexibility in the production of materials with customizable size, porosity, drug concentration, and release rate (Zhang et al. 2016). Besides, this method has attracted much interest due to its versatility to manufacture nanofibrous membranes for dressings that promote healing, given their capacity to create moist environments around wound areas (Liu et al. 2017), and their mechanical properties resemblance with the human skin (Lee et al. 2014). Given their excellent matrices for drug delivery, electrospun NF have been used combined with different compounds for wound healing purposes such as antibacterial agents, anesthetics, antioxidants, enzymes, anti-inflammatory drug, and growth factors (Charernsriwilaiwat et al. 2012; Heo et al. 2013; Augustine et al. 2014a, 2014b; Lai et al. 2014; Monteiro et al. 2015); however, this work is the first study to produce NF with a natural plant extract with healing and anti-inflammatory activities.

Furthermore, since the production of polymeric nanoproducts as NF can result in a final high-priced product, this work also evaluated other cost-effective products: an ointment and a hydrogel. Ointments promote a healing environment in the wound surface because they form semi-occlusive protection, maintain the area in a wet condition, and promote drug release in the damaged area (Gupta et al. 2017). Moreover, ointments can be manufactured in compounding pharmacies due to their low-cost of production (straightforward technique and affordable reagents), facilitating their distribution. On the other hand, hydrogels are extensively used for wound dressing given their water retention and non-adhesiveness providing a humid environment for healing whereas being comfortable to the patient (Francesko et al. 2018). Due to the socio-economic relevance and the wide use of medicinal plants for improving the quality of life of a large part of the population, the present study proposes the production and characterization of a hydrogel and NF for the encapsulation and directed release of PAHE (Sperotto et al. 2018; Henn et al. 2019) and an ointment containing PAHE, which can be used as dressings to accelerate the healing process.

Materials and Methods

Extraction

The leaves of *Plantago australis* Lam., Plantaginaceae, were collected in Santa Cruz do Sul, Brazil, in October 2014, and identified by Dr. Gustavo Hassemer (botanic specialist from

the Natural History Museum of Denmark, University of Copenhagen, Denmark). The specimens are deposited in the ICN herbarium at the Federal University of Rio Grande do Sul, Brazil (voucher number: ICN 179648). The plant material was extracted according to Henn et al. (2019). Briefly, grounded *P. australis* leaves were mixed in a proportion of 1:10 w/v leaves-hydroethanolic solvent (PAHE, 70% ethanol and 30% water). The mixture was submitted to ultrasound bath (Unique Group USC 1800, São Paulo, Brazil), consisted of a rectangular container with 37-kHz transducers annealed to the bottom, at 25 °C for 40 min. The extracts were paper-filtered and concentrated in a rotary evaporator at low temperature (< 40 °C) under vacuum, until the solvent evaporated. The mass yields (y%) were calculated, and the extracts were stored at − 20 °C until further use. Aiming to optimize the concentration of verbascoside (**1**), additional optimization was accomplished by complete factorial design (2^2, focusing on extraction time and temperature) and response surface methodology (RSM) to optimize the effect of these variables by a central composite design. HPLC coupled with Diode Array Detector (SPD-M20A, Shimadzu, Japan) analysis aiming compound **1** identification was also performed as previously described (Henn et al. 2019).

Hydrogel and Nanofiber Production

Polyvinyl alcohol (PVA) solutions were produced by dissolving high Mw PVA (10%, w/v) at 90 °C with constant stirring in distilled H_2O. Following the complete solubilization of PVA, the semi-solid PAHE was weighed (4%, w/v) and added at 50 °C with constant stirring. After cooling down to room temperature, ethanol (10% v/v) was added to adjust the surface tension of the solution for the electrospinning (Felice et al. 2015; Reinhardt et al. 2020). Then, to shape the hydrogel, the solutions were let dry at room temperature and rehydrated before use. To produce the NF, the solutions were also electrospun using a blunt-end 20-gauge needle. The flow rate was set at 0.5 ml/h using 15 kV and the needle tip to collector distance was 5 cm. Pure PVA solutions were also prepared and electrospun for comparison reasons.

Nanofiber Morphology and Size

The morphology of PAHE NF was evaluated by using a scanning electron microscope (SEM, Tescan Mira XMU, TESCAN, Brno, CZ). The NF were sputtered with gold and the backscattered-electron mode was used with × 20,000 magnification. The ImageJ software was used (ImageJ Version 1.48v, National Institute of Health, Bethesda, MD, USA) to evaluate the mean NF diameter of approximately 500 NF.

Solid-State NMR

The PAHE stability after electrospinning was evaluated by NMR (nuclear magnetic resonance spectroscopy). The assessment was accomplished by using a Bruker 400 MHz Avance III HD (Billerica, MA, USA) set with a 3.2-mm H/X CPMAS probe.

Hydrogel and NF Mechanical Properties

The hydrogel and NF mechanical properties were studied using a Discovery HR-2 rheometer (TA instruments, DE, USA). By using the parallel plate mode, the analyses were executed with a 60-mm steel plate as the top geometry. After 15 min of soaking in PBS buffer (pH 7.4), the NF and hydrogels were placed on the Peltier plate. After an equilibration step, apparent viscosity flow curves of individual samples were produced.

Drug Release Analysis *In Vitro*

The drug dissolution profiles of NF and hydrogel were studied in a Distek Model 2500 Dissolution System (Distek, Inc., NJ, USA). The samples (10 mg) were added into sample baskets and incubated at 37 °C in 500 ml of PBS (pH 7.4) per vessel and the stir rate was set to 50 rpm. Samples were taken at fixed times and evaluated using a Shimadzu UV 1280 (Kyoto, Japan) spectrometer at 270 and 320 nm. PAHE concentration was calculated by using a standard curve of PAHE prepared in PBS. Sink conditions were used to allow the complete dissolution of the samples while the same amount of PBS media was used to ensure un-impaired dissolution. Blank NF and hydrogel were used as the blank controls for the spectrophotometer analysis.

Infrared Spectroscopic Study

ATR-FTIR (attenuated total reflectance Fourier transform infrared spectroscopy) was used to investigate the possible chemical interactions between PVA and PAHE. By using a Perkin Elmer Spectrum One (Waltham, MA, USA) attached to a universal ATR sampling accessory, the samples were assessed in the spectral range of 4000–650 cm^{-1} using 4 scans per sample cycle. Post-analysis was performed using the Spekwin 32 software.

PAHE Ointment Production and Characterization

Plantago australis hydroethanolic extract (PAHE) ointment 4% (w/w) was produced in lanolin:vaseline (2:3). The PAHE was dissolved in distilled H_2O and carefully added in solid vaseline. Finally, in a Petri plate surface, the lanolin was combined into a homogeneous mixture by spatulation, with

continuously heaping them together. A blank ointment was also produced without PAHE. The pH was determined using 2-g dispersion of the formulation in ultrapure water with continuous agitation by a magnetic stirrer. This solution was heated to a temperature of 40 °C, cooled, and then filtered with a 0.45-μm filter. To determine the *in vitro* drug release of the ointment, 10 mg of the samples was incubated at 37 °C in 50 ml of PBS in a magnetic stirrer set at a low speed (50 rpm) and samples were taken at set periods and evaluated by a spectrophotometer (SpectraMax M2e, Molecular Devices, San Jose, USA) at 270 and 320 nm. Sink conditions were also used to allow the complete dissolution of the ointment while the same amount of PBS media was used to ensure un-impaired dissolution. The result was compared with PAHE and its major compound, verbascoside (**1**). The maximal absorption peak was used to verify the release over 5 days. To ensure that the ointment was homogeneous, and no phase separation would happen, 1 g of PAHE and blank ointments were submitted to 30 min of centrifugation at 870×*g*. Blank ointment was used as the blank control for the spectrophotometer analysis.

3D Cell Culture Proliferation Evaluation

U87 cells were thawed into a 25 cm^2 flask and let grown until 90% of confluence under standard conditions in DMEM 10% FBS; then, to magnetize the cells, 200 μl of magnetic nanoparticles (NanoShuttle™-PL, Nano3D Biosciences, Houston, TX, USA) was added to the flask. After 24 h at 37 °C, cells were placed into a cell-repellent 96-well plate (CELLSTAR, Greiner Bio-One, Frickenhausen, Germany) at 100,000 cells/well and incubated for 24 h with a magnetic drive of 96 magnets positioned under each well. By positioning the magnet under the multi-well plate, the magnetized cells were attracted to the middle of each well and spheroids were formed by cell aggregation. After the incubation period, cells were exposed to full media (negative control), PAHE (100 μg/ml), PAHE NF, and blank NF for 5 days. Spheroid size was analyzed by using an EVOS FL Auto 2, Imaging System microscope (Thermo Fisher Scientific, Waltham, MA, USA), and calculated by using the ImageJ software (National Institute of Health, Bethesda, MD, USA).

Animals

Male Wistar rats weighing 280 ± 50 g and aging 60 days were obtained from the Animal House of UFCSPA. The rats were maintained at 23 ± 1 °C under a 12-h light/12-h dark cycle receiving water *ad libitum* and standard food. All experimental procedures were performed according to Sperotto et al. (2018).

Wound Healing Analysis

The animals were anesthetized by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg) prior to mechanical hair removal (Philips Series 1000 Beard trimmer, Netherlands). The wound infliction (20 mm^2 in dorsal region skin) was excised by surgery. The animals were randomly divided into six groups of eight animals: control NF, PAHE NF, control hydrogel, PAHE hydrogel, control ointment, and PAHE ointment. They were treated for 7 and 14 days. To estimate wound closure, the wounds were photographed on days 0, 7, and 14 by using an iPhone X and analyzed by using the ImageJ software (National Institute of Health, Bethesda, MD, USA). Wound closure percentage was described as a diminution of wound area when compared to the initial area (100%). After the conclusion of the experiments, on the 7 or 14 days after lesion induction, the animals were anesthetized by intraperitoneal injection of ketamine and xylazine and, by using a 10% buffered formalin saline, the wound lesions with adjacent normal skin were fixed for histological evaluation. Sections of 3 μm were separated from the paraffin blocks and stained with hematoxylin and eosin or Masson's trichrome in order to evaluate the wound histopathological features and collagen production from the tissues.

Statistical analysis

Data were shown as mean ± standard deviation (SD). Each *in vitro* experiment was repeated at least three times. Statistical analysis was achieved using one-way analysis of variance (ANOVA) followed by Tukey post-test or two-way ANOVA followed by Sidak or Bonferroni post-test using the GraphPad Prism 5 software (La Jolla, CA, USA). Results that were statistically significant presented a p value ≤ 0.05.

Results

Extraction and Standardization

The PAHE chromatographic fingerprint registered by HPLC-DAD (Fig. 1) established verbascoside (**1**) as the principal component matching 6.8 ± 0.25% of the extract. Similarly, the presence of baicalein, aucubin, oleanolic, and ursolic acids was explored (Fig. S1) in PAHE; nevertheless, only **1** was detected (Henn et al. 2019).

Formulation development and characterization

In our previous studies, we have assessed the toxicological profile of PAHE (Henn et al. 2019), showing that the extract does not present chronic and acute toxicity when given orally, and it does not induce genotoxicity and mutagenicity *in vivo*.

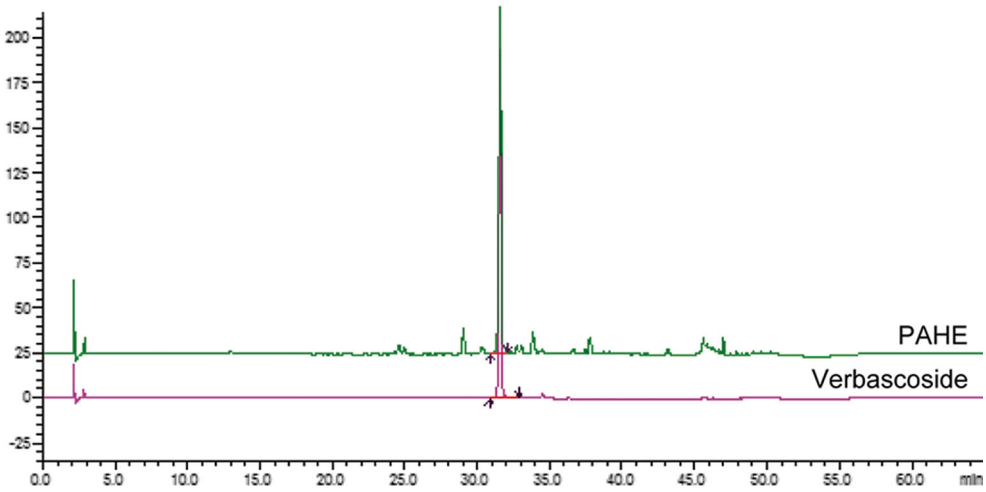

Fig. 1 HPLC-DAD chromatograms for the characterization of *Plantago australis* hydroethanolic extract (PAHE) solution presenting verbascoside (**1**) with a retention time of 31.562 min and chromatogram of standard **1** solution in ultrapure water showing its retention time of 31.563 min. The characterization was performed three times by using different solutions

Thus, the concentration of PAHE in the dressings was based on an oral dose that promoted wound healing *in vivo* (Sperotto et al. 2018).

A solution screening was performed to select the ideal concentration of PVA, in which the spinning was stable, and the samples were uniform, for the production of the NF (data not shown). PAHE NF presented a smooth structure and a mean size of 2822 ± 638 nm analyzed by SEM (Fig. S2B-C), whereas the hydrogel presented a homogenous structure with low porosity (Fig. S2A).

The stability of PAHE after electrospun with PVA was examined by using FTIR and NMR. FTIR fingerprints of PVA associated with hydroxyl and acetate groups were detected in all samples (Fig. S3A) and no significant shift alterations were observed, demonstrating no chemical interactions between PAHE and PVA (Reinhardt et al. 2020; El-Feky et al. 2015). PAHE presented bands related to O–H stretching (around 3200 cm^{-1}), C–H stretching (around 2900 cm^{-1} and 2800 cm^{-1}), C=O stretching (around 1740 cm^{-1}), C=O stretching (around 1630 cm^{-1}), and C–O stretching (around 1020 cm^{-1}, predominantly carbohydrates) (Fig. S3A). A significant aspect related to adequate drug delivery systems is the crystalline degree of the used polymer (El-Feky et al. 2015). It was possible to observe that the intensity of the PVA peak in 1141 cm^{-1} position is present in the hydrogel sample but it is missing in the NF (Fig. S3A; black arrow), this band is attributed to the crystalline structure of PVA because it is associated with C–O (stretching vibration) and symmetric C–C (stretching mode) in the polymeric chain where hydrogen bonds are formed between the OH groups of the same side of the chain and the reduction of this band indicates a limited substitution of hydrogen bonding among PVA chains by hydrogen bonding amid water molecules, increasing the material solubility and decreasing its crystallinity (Mohammad et al. 2016; Yang et al. 2017). Figure S3B shows the results of ^{13}C NMR analysis where PVA fingerprint peaks (CH_2 45 ppm and CH–OH – I, II, and III around 60 and 80 ppm) were observed and PAHE main peak (around 37 ppm) appeared in NF sample indicating suitable stability; this peak could be related to C-7 of verbascoside (Xie et al. 2012); however, given PAHE's complex composition, it is challenging to predict with accuracy its fingerprints peaks.

Rheometer analysis (Fig. S3C) showed higher viscoelasticity of hydrogel samples when compared to NF. PAHE addition slightly increases the products' viscoelasticity, but without significant changes. The *in vitro* drug release results (Fig. S3D) revealed a continuous PAHE release profile over 8 days provided by the NF samples, while PAHE hydrogel presented a burst initial release; however, only around 50% of the extract was release after 192 h, probably due to the low degradability and diminished solubility of the hydrogel when compared to the NF. Our previous studies suggested that it is possible to achieve a prolonged release by using high Mw PVA when producing NF (Steffens et al. 2020).

Development and Characterization of Ointment

PAHE ointment presented a satisfactory homogenization, good spreadability, and no apparent phase separation. In addition, in the centrifugation test, no phase separation was observed, and the ointment exhibited a characteristic lanolin smell (Table S1). The absorbance spectrum presented a peak around 270 and other between 320 and 340 nm (Fig. S4), which correspond to verbascoside (**1**), the major compound of PAHE (Henn et al. 2019). No peaks were observed above this wavelength (data not shown). The release assay demonstrated that PAHE ointment under agitation releases the extract rapidly, whereas under no agitation, the extract is gradually released, indicating satisfactory compatibility in the lanovaselin matrix (Fig. S5A-B) since the dissolution in the aqueous phase was minimal. The agitated ointment samples showed a rising release in the first few hours, while the non-agitated samples released PAHE slowly (Fig. S5C). The pH of

the samples did not demonstrate changes during the 5 days of testing; the lowest pH of PAHE ointment found was 6.6 (Fig. S5D).

Wound Healing Activity Evaluation

In Vitro

The cell proliferation activity of PAHE NF was investigated *in vitro* by using 3D cells. We have used the U87 cell line because these cells can quickly form regular spheres and our research group established a routine protocol to produce these spheroids. U87 cells treated with PAHE for 5 days presented increased spheroid size (Fig. 2a), expansion spheroid area (halo of live cells) (Fig. 2b), and total spheroid size (spheroid area plus halo area) (Fig. 2c). The hydrogel and ointment were not tested *in vitro* given their ability of water absorption and swelling. These characteristics would be toxic to cell testing due to the physical and environmental disruption of cell growth producing a diffuse outer layer of cells.

In Vivo

Following the healing activity experiments, we tested if the PAHE products present efficacy *in vivo*. No animal showed signs of pain after the procedure and throughout the evaluation. Our previous studies have shown that PAHE extract is safe for use and it does not induce any side effects *in vivo* (Sperotto et al. 2018; Henn et al. 2019). After 7 days of treatment, animals treated with NF containing PAHE exhibited an accelerated healing process compared to animals treated with blank NF; moreover, PAHE NF and PAHE hydrogel demonstrated diminished wound size when compared to PAHE hydrogel (Fig. 3a–c). After 14 days of treatment, the animals presented practically total wound closure (Fig. 3b, c), indicating that the nanoproducts were able to accelerate the process.

The histological analysis (Fig. 4) revealed that the healing process is more developed in the PAHE NF (Fig. 4A–D) and ointment groups (Fig. 4I–L) when compared to the controls. It was observed a dense granulation tissue with fibroblasts proliferation and new delicate thin-walled capillaries in a loose extracellular matrix, usually with a mixture of occasional inflammatory cells, corresponding to more advanced stages of healing. Especially in the ointment group, there was greater progress in epidermal junction with almost complete ulcer closure and reepithelization within 14 days (Fig. 4L, black arrow; Fig. S6, box). In the hydrogel groups, the inflammatory process, the vascular proliferation, and the formation of scab are similar between the PAHE and control groups, corresponding to earlier stages of the healing process (Fig. 4E–H).

Discussion

PAHE hydrogel exhibited a low porous and homogenous structure (Fig. S2A) when compared to previously described hydrogels (Sornkamnerd et al. 2017). The NF

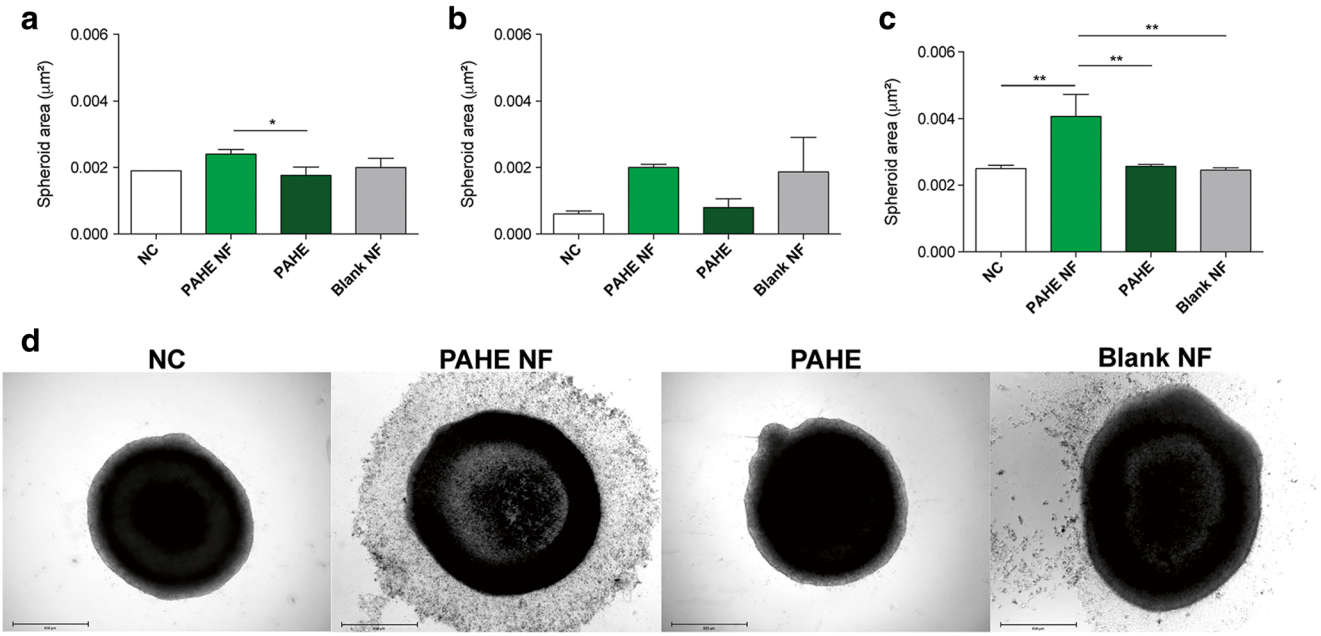

Fig. 2 3D cell proliferation analysis. **a** Spheroid size, **b** expansion spheroid area (halo of live cells), and **c** total spheroid size (spheroid area plus halo area). **d** Images of spheroids after 5 days of treatment. Results are showed as mean spheroid area ± SD. Statistical analysis was accomplished using two-way ANOVA followed by the Bonferroni post-test. Significant results were considered at $*p < 0.05$ and $**p < 0.01$. NC, negative control

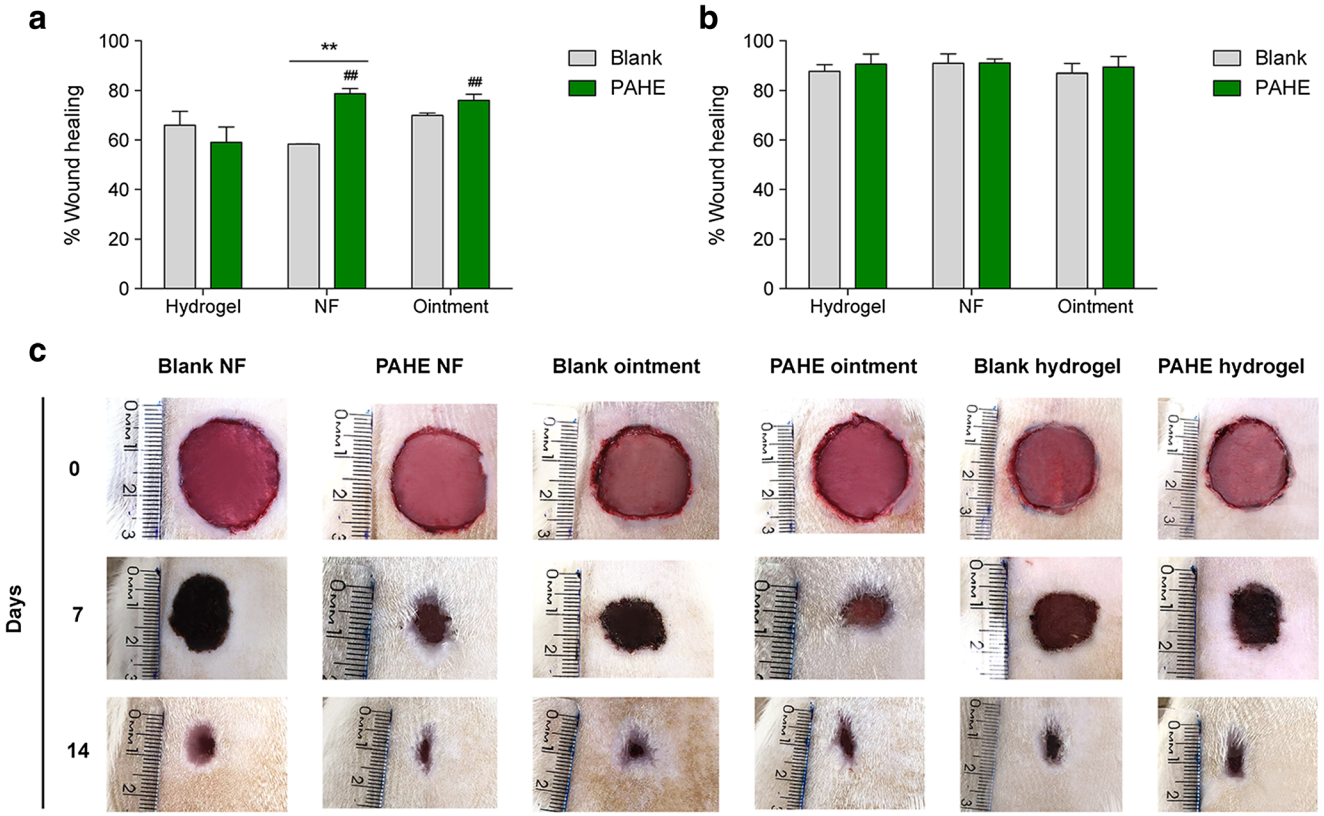

Fig. 3 Wound closure after 7 and 14 days of treatment. **a** Percentage of wound closure after 7 days of treatment with hydrogel, NF, and ointments. **B**. Percentage of wound closure after 14 days of treatment with hydrogel, NF, and ointments. **c** Wounds images immediately after the procedure and after 7 and 14 days. Results are expressed as the mean percentage of wound closure ± SD. Statistical analysis was achieved using two-way ANOVA and Sidak's multiple comparisons test. Data were considered significant at $^{**}p < 0.01$ between the same group and $^{\#\#}p < 0.01$ when compared to PAHE hydrogel

(Fig. S2B-C) displayed a smooth configuration showing large fibers (Gomes et al. 2015; Nemati et al. 2019; Bae et al. 2020) and a dispersed diameter range size probably given the distance between the middle and the edge of the collector plate since short electrospinning jets usually produce larger electrospun nanoproducts (Felice et al. 2015). Additionally, the NF presented average porosity and spacing between fibers and these are critical features for dressings that act as a microbial barrier (Augustine et al. 2015).

FTIR studies (Fig. S3A) revealed that NF presented improved solubility and decreased crystallinity suggesting that this system could also have enhanced drug release properties and exudate absorption (Mohammad et al. 2016; Yang et al. 2017). Furthermore, NMR analysis (Fig. S3B) indicated good stability of the plant extract and the polymer after electrospinning, without chemical interactions. The rheometer analysis (Fig. S3C) suggested that NF are more flexible and less viscous than hydrogel, which is in accordance with the *in vitro* drug release profile of the sample, given that the NF provided a continuous PAHE release over 8 days (Fig. S3D) while hydrogel presented a slower release profile and only approximately half of the total amount of PAHE was release after 192 h, showing that NF improved PAHE solubility providing its sustainable release. As aforementioned, the development of polymeric NF can result in a high-priced product; thus, in this study, we showed the production and characterization of a cost-effective option, an ointment. PAHE ointment exhibited satisfactory properties including its constant pH, which is compatible with biological systems (Fig. S5D).

The *in vitro* evaluation of the NF wound healing activity was carried out by exposing 3D cells to a 5 days' treatment and then measured spheroid properties. The results (Fig. 2) indicated that the NF can stimulate cell proliferation probably given its slow release of PAHE. Generally, the spheroid area and morphology are determined by proliferation rate and tightness of cell contact; cells that agglomerate into tight spheroids usually present high E-cadherin expression and low proliferation rate and Ki67 expression (Schmidt et al. 2016). PAHE NF–treated spheroids agglomerated into loose spheroids possibly due to their increased proliferation stimulation; hence, PAHE NF could be used as a scaffold for cell proliferation and stabilization of the injured epithelial tissue.

Following the efficacy evaluation of PAHE NF *in vitro*, the efficiency of the PAHE-containing systems was assessed *in vivo*. Wounds inflicted in Wistar rats were treated with the

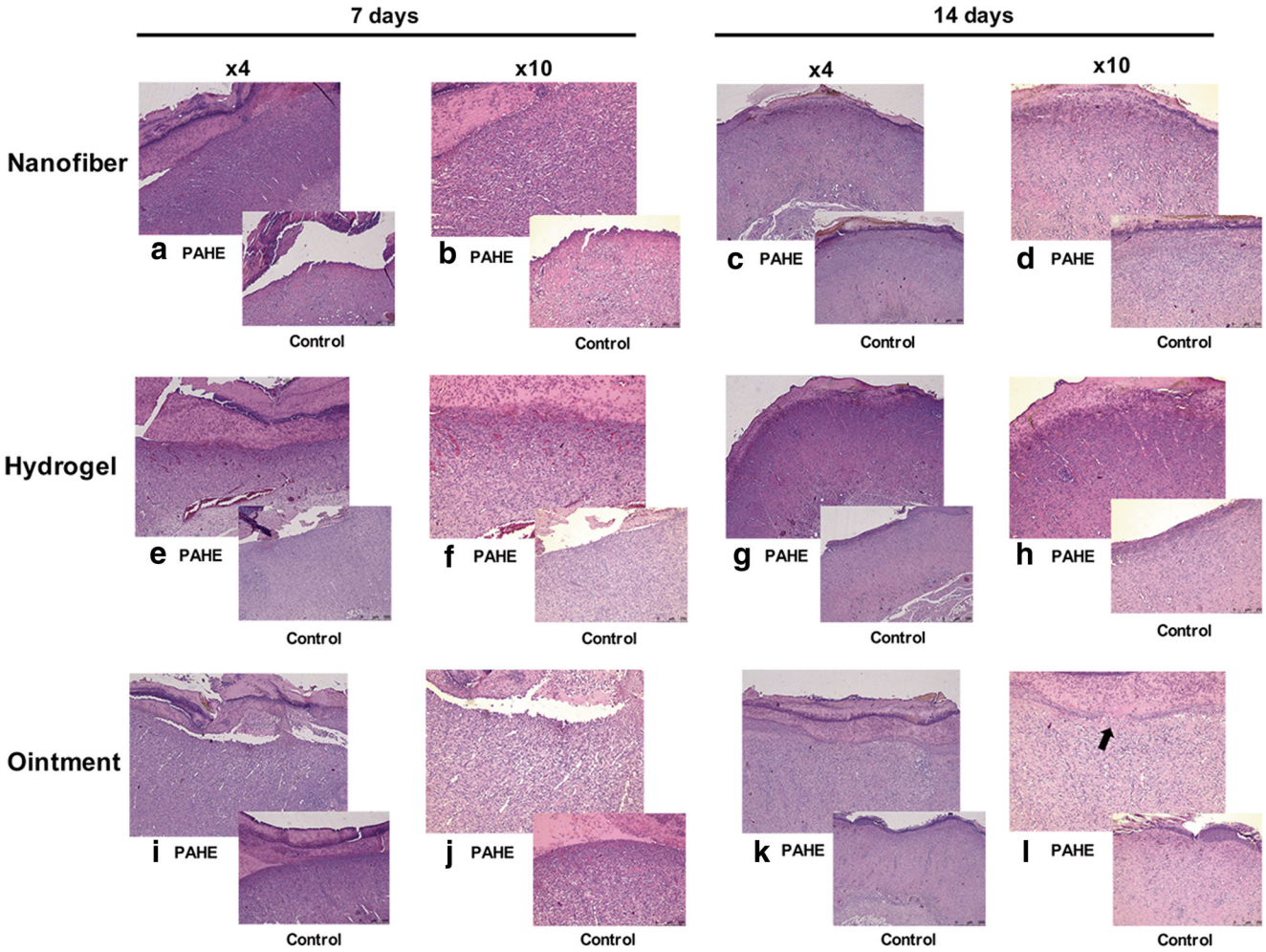

Fig. 4 Wound histological evaluation after 7 and 14 days of treatment. Upper panel: A–D *Plantago australis* hydroethanolic extract (PAHE) NF-treated rats compared to blank NF (control). Middle panel: E–H PAHE hydrogel-treated rats compared to blank hydrogel (control). Lower panel: I–L PAHE ointment-treated rats compared to blank ointment (control) (black arrow shows the ulcer closure and reepithelization)

dressings (Fig. 3); PAHE NF and ointment demonstrated an improved and accelerated healing. Regarding the hydrogel, the wound closure percentage was not significant when compared to the control samples probably due to two factors: (i) the release of the extract is lower than the other nanoproducts (the hydrogel retains the extract) and (ii) the hydrogel is stiffer than the fibers and ointment; consequently, its application is challenging. In many animals, when the hydrogel dried, the dressing detached from the wound, reducing the time of contact with the wound. In our previous studies, we observed approximately 90% wound closure with an initial area of 10 mm^2 after PAHE oral treatment (Sperotto et al. 2018). Here, it was possible to detect about 80% wound closure by analyzing wounds with 20 mm^2 of the initial area after 7 days of treatment, showing a great improvement of PAHE local application (Fig. 3).

It was observed the same efficacy of wound closure of PAHE NF and ointment. It is known that vaseline keeps lesions moist, clean, and covered, protecting the skin from germs and hydrating the wound (Czarnowicki et al. 2016); therefore, a mixture of PAHE and vaseline in an ointment seems to be an advantageous approach for stimulating the healing process given their complementary activities. Moreover, the production of ointments has a low-cost when compared to more complex products making them an appealing product for mass production. However, wound dressings as PAHE NF are more adequate for superficial wounds given their precise control over PAHE release rate preserving its bioactivity for a prolonged time and easy application (Weng and Xie 2015). Furthermore, it maintains an appropriate wound environment for healing and protects the lesion, while ointments do not provide a homogenous application and it can feel greasy. Besides, in an industrial scale of production, preservatives would be added to the ointment and that could cause allergic reactions to sensitive skin.

The histological evaluation of the skin of the rats (Fig. 4) demonstrated a more advanced wound healing stage in rats exposed to NF and ointment when compared to hydrogel and controls. The improved vascular support in the wound promoted by the NF and ointment is related to the angiogenesis amplification during the first steps of healing (Fig. S6, black arrows) that plays an important role due to the formation of a new microvascular network improving the blood flow and promoting repair (Honnegowda et al. 2015).

It is common knowledge that commercial wound dressings including films (OpSite®), foams (PolyMem®), hydrogels (IntraSite gel®), and gauzes have been broadly used in the clinic. Nevertheless, numerous drawbacks are related to these products, such as poor absorption of drugs and the necessity of supplementary dressings (Hassiba et al. 2016). Electrospun NF can bypass these disadvantages given their mechanical and release properties (Mei et al. 2016); however, dressing NF are still exclusively being used in research laboratories instead of being commercialized. In this study, it was demonstrated that NF have advantages when compared to hydrogels to encapsulate drugs and to be used as wound dressings. Finally, the two essential requirements for modern dressings include rapid hemostasis and bactericidal activity (Liu et al. 2017). *P. australis* extracts have been studied for several antimicrobial related uses such as kidney and bladder diseases, anti-inflammatory, antibiotic, general infections, cystitis, throat and ovarian inflammation, wounds, and vaginal discharge (Flores et al. 2016; Sperotto et al. 2018). Our research group has revealed that PAHE mechanism of action is related to verbascoside properties and is associated with the activation of antioxidant enzymes such as superoxide dismutase and catalase and, in lipopolysaccharide-induced cells, PAHE was able to decrease pro-inflammatory mediators including tumor necrosis factor-alpha (Sperotto et al. 2018). Therefore, together with our results, it seems that PAHE NF and PAHE ointment are ideal dressings for wound healing, showing that this plant extract is effective under a simple and functional ointment approach as well as under a novel and robust NF and that these systems could be used in primary health care for injured skin repair.

Conclusions

Commercial wound dressings exhibit several application drawbacks and usually, they are not biofunctionalized. In this study, we developed and analyzed three approaches for the delivery of a plant extract: NF, hydrogel, and ointment and their effectiveness comparison. We demonstrated that NF has advantages to be used as dressings including mechanical properties, continuous-release profile, and easy application when compared to a hydrogel with the same composition. Furthermore, PAHE formulations, NF and ointment, demonstrated suitable characteristics for *in situ* application and they can accelerate the wound healing process, promoting cell proliferation and practically closing the wounds after 7 days. Thus, PAHE NF is a distinguished pharmacological approach for wound healing, obtained by using a plant that has already been used in folk medicine and therefore could be clinically applied given that these dressings can keep wounds protected and cleaned, accelerating the healing process. In addition, PAHE ointment is an interesting and low-cost option.

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Authors' contributions LSR and AMM developed and characterized the products; LSR, JGH, AMM, NDM, and MBF conducted the experiments; AVR and AUSP conducted the histopathological evaluations; ZC performed the scanning electron microscope analysis; LSR wrote the original draft; LSR, MN, and DJM reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there are no conflicts of interest.

Ethics Approval The *in vivo* experiments were approved by the Animal Care Committee of the Federal University of Health Sciences of Porto Alegre (UFCSPA) (protocol number 602/18).

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3.3. Capítulo 3: Nanopolymeric Systems to Improve Brain Cancer Treatment outcomes

Capítulo de livro publicado no livro Advances and Challenges in Pharmaceutics Technology.

CHAPTER 12

Nanopolymeric systems to improve brain cancer treatment outcomes

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1 Introduction

Cancer is a complex disease and one of the main reasons for human morbidity. The projection of new GLOBOCAN 2012 cases worldwide was 14.1 million per year, with an estimated increase to 19.3 million by 2025 [1]. Surgery, radiotherapy (RT), chemotherapy (CT) and immunotherapy are the main approaches used for cancer therapy [2]. To eradicate localized cancers, surgery and RT works relatively well, however, when metastasis is associated, CT and immunotherapy are the main functional treatments [3]. However, high toxicity, an insufficient amount of therapeutic agents released, water insolubility of some drugs, erratic circulation, non-specific biodistribution and drug release for both cancerous and non-tumor cells are the main limitations of CT [2, 4]. Consequently, intelligent and efficient strategies are needed to decrease these mortality statistics [1, 5] through a more specific delivery to the cancer targets.

2 Glioblastoma overview

Brain tumors develop from several different types of cells. Gliomas, for example, are brain tumors primarily similar to typical glial cells, such as astrocytes, oligodendrocytes, and ependymal cells [6]. In general, these tumors are incurable and highly resistant to CT and RT. Gliomas can infiltrate through brain regions and are essentially categorized according to their morphological similarity with corresponding glial cell natures, cytostructure and

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immunohistology [6]. The glioma classification system, according to the World Health Organization (WHO), distinguishes astrocytomas in four degrees (I, II, III and IV) and oligodendrogliomas, in two degrees (II and III). Grade IV astrocytoma, known as glioblastoma (GB) is the most prevalent and poorly forebode glioma [6].

The National Cancer Institute (INCA) estimates that for each year of the 2018/2019 biennium, there were 11,320 new cases of brain tumors/central nervous system (CNS), 5810 in men and 5510 in women in Brazil [7]. These numbers correspond to an estimated risk of 5.62 new cases per 100,000 men and 5.17 per 100,000 women. GB is one of the most frequent and aggressive types of CNS cancer in adults [8], comprising 16% of primary brain tumors. Because it is a predominantly recurrent primary malignant brain cancer, it accounts for 77% of all malignant brain cancers [9, 10].

Clinical features and malignancy are used to categorize gliomas, with GB having histopathology comprising cell pleomorphism, high mitotic rate, and cellularity with unusual nuclei. Furthermore, GB can be distinguished by the heterogeneity of cells, the resistance of cancer cells to treatment with chemotherapeutics, development of necrosis and angiogenesis [11]. Although GBs essentially develop only in the brain, they can occur in some cases in the spinal cord, cerebellum, and brain STEM (American Association of Neuroscience Nurses [12]). Initially, it was believed that GB was exclusively derived from glial cells, but more recent studies suggest that GBs can develop from several cells with neural trunk characteristics [13].

GBs develop primarily in patients with a mean age of 64 years [10]. The mean survival time in patients with GB is less than 50 weeks, even with exhaustive management which includes surgical resection, CT and RT [14]. The current clinical protocol for GB is a combinatory procedure of RT doses and systematic delivery of anticancer drugs [15]. The BC Cancer Treatment Management Guidelines (protocol: CNAJ12TZRT) for GB treatment includes concomitant treatment of temozolomide (TMZ) and RT for 5 days, with 2 days of RT, totaling 7 days of exposure. This cycle on the clinical protocol of patients must be repeated for 6 weeks [16], although combinatorial treatment is most effective, it leads to frustrating side effects. Given the complex features of GB cells and the presence of physiological barriers, particularly the blood-brain barrier (BBB), the cure of this cancer remains a challenge [17, 18].

2.1 Molecular aspects of glioblastoma

GBs have a complex genetic profile. The Atlas Cancer Genome project analyzed the GB genomic profile of 200 tumor specimens and approximately

600 genes were overexpressed [19]. Based on this analysis, three major protein signaling pathways were established: (a) tumor protein 53 (p53), (b) receptor tyrosine kinase/Ras/phosphoinositide 3-kinase and (c) pathways related to retinoblastoma [20]. Due to modifications in these protein pathways, primary and secondary tumors predominantly have uncontrolled cell proliferation. This allows malignant cells to avoid cell cycle checkpoints and cell death processes, markedly senescence and apoptosis [19].

Primary and secondary GBs exhibit complex patterns of gene expression: epidermal growth factor receptor (EGFR) overexpression, phosphatase and tensin homolog (PTEN) mutations, and loss of the long arm of chromosome 10 are examples of genetic and molecular characteristics of primary GB. On the other hand, mutations in isocitrate dehydrogenase 1 (IDH1) and p53, as well as the loss of the long arm of chromosome 19 are commonly observed in the secondary GB [21–23].

Nek1 is a protein that is associated with tumor resistance in gliomas [24]. It has recently been reported that Nek1 is overexpressed in different gliomas and, interestingly, the level of expression is directly related to the degree of tumor severity, proliferation rate, and TMZ resistance. In light of analyzing patient samples, the association of Nek1 expression with decreased survival prognosis ($p = 0.03$) was observed [24]. Inhibitors of the kinase activity of this protein have already been tested [25] to inhibit the DNA damage response signaled by Nek1. Although the results indicate that Nek1 is somehow related to the tumor process, much information is still missing to identify its true role in tumor resistance.

Given all the complex changes in the signaling pathways of these tumor cells, GBs have become a major obstacle in oncology with several progression and survival outcomes. These molecular changes are extremely important to understand GBs and could potentially be used for targeted therapy [26–28].

2.2 The challenge imposed by the blood brain barrier (BBB)

The blood brain barrier (BBB) is a special tissue that is crucial in the biodistribution of drugs from the general organism and the CNS (central nervous system), comprising a challenge on GB treatment. Due to its lipophilic character, cells peculiarity and permeability limitations, the BBB constitutes a challenge on the delivery of medicines through it. The four main reasons can be listed as:

(a) The cerebral vasculature of the BBB cell composition is formed by narrow endothelial lines. These endothelial cells can develop a strong

connection between neighboring cells. Therefore, the paracellular transport of the brain is restricted by this physical limit [29];

(b) The presence of specific enzymes that degrade some drugs, preventing them from reaching specific sites in the brain;

(c) The active efflux transporters, which sends the drugs back to the blood, resulting in their metabolism and consequent inability to reach their destination [30];

(d) The astrocyte extensions form a lamella network being close to and adhered to the outer surface of the BBB endothelium [31].

The narrowed junctions between the cells in the BBB are essentially composed of occludins and claudins [32]. The latter group is critical for the restriction of small molecules. For example, claudin 5 restricts molecules smaller than 800 Da. Also, impairment in the functioning of some claudins, for example, claudin 3 is associated with increased penetration of BBB in the vasculature of cancer [33]. However, in infiltrating and micrometastatic gliomas, the barrier remains intact. This important aspect indicates that the modulation of permeability at these sites is critical and can be used to increase the effectiveness of the treatment.

The two main forms of transport through the BBB are carrier-mediated transport and receiver-mediated transport. Plus, molecular transport through BBB is specifically limited to particles larger than 12 nm [30]. It is estimated that almost all large molecules cannot cross the barrier, and that is why several promising drugs do not reach the tumor site in sufficient concentrations [34].

Because of this, BBB prevents the route of numerous antitumor drugs to brain sites, making CT in GB one of the biggest challenges among current cancer treatments. Intending to improve drug transport or avoid the BBB, many research groups have been developing new technologies to overcome these obstacles. An option to transport drugs to the brain bypassing the cited limitations is the use of nanocarriers [35]. Biochemical changes in drugs and the use of nanocarriers have been proposed by researchers, allowing the local release of drugs at sufficient concentrations, avoiding exposure to the system and overcoming BBB limitations [3].

2.3 Current strategies in GB treatment

The treatment of recently diagnosed GB comprises multidisciplinary tactics. The possible tactics include surgery, RT, CT, Immunotherapy, and hyperthermia. Currently, the standard therapy for GBs uses surgical resection followed by simultaneous RT and CT with TMZ, known as Temodal®

(National Comprehensive Cancer Network [36]). Remaining and infiltrating malignant cells persist consistently within the brain, leading to advanced tumor progression and even the relapse of the disease [22]. There are several complications related to the management of GB, including insufficient drug delivery, damage to healthy tissues and resistance to CT. Among the existing approaches, CT, immunotherapy, and RT can, to a certain extent, assist patients with GB. However, beyond their benefits, these strategies have unfavorable characteristics [37].

2.3.1 Surgery

GBs are usually invasive tumors and are located regularly in important places in the brain; in particular, at the areas responsible for sensory control, motor function and speech, hence surgical resection is risky though still used. To say nothing of, these tumors are highly invasive; making complete resection of the tumor mass unfeasible.

2.3.2 Immunotherapy

In the last decade, interest in the development of immunotherapeutic ways to deal with tumor malignancy has increased. Among the various immunotherapeutic methods, stimulated lymphocytes, viral drugs, peptide-based vaccines, cytokines, and dendritic cell treatment appear to be successful approaches for use in GB therapy [38]. A large number of studies focus on the administration of bevacizumabumab, a humanized monoclonal anti-vascular endothelial growth factor antibody (anti-VEGF), which acts on angiogenesis [39]. Even so, when combined with TMZ in newly diagnosed patientsit was not able to increase overall patient survival [39].

Another treatment strategy is the use of an anti-EGFRvIII vaccine. This bioproduct, called rindopepimute, was demonstrated satisfactory in the phase II study combined with TMZ with a survival of 21.8 months [40], but the phase III study was discontinued in 2016 after the second partial analysis demonstrate failure to increase survival compared to standard (20.1 *vs* 20 months) [41]. Another immunological treatment is the use of an antibody that inhibits the programmed T cell death receptor 1 (PD-1) or its linker (PD-L1) [42]. The PD-1 inhibitor (pembrolizumab) has already led to satisfactory responses in patients with advanced melanoma, non-small cell lung cancer and renal carcinoma [43]. In gliomas, a clinical study was started in 2014, with an estimated end date for 2018, but still has no partial results (NCT02017717).

Although immunotherapies are interesting options for GBs treatment, there are some complications, frequently patient selection to develop clinical trials, monitoring of body response, evaluation of side effects and clinical consequences [38]. Equally important, some issues need to be assessed: first, effective preclinical outcomes should be carefully considered, as cross-cutting variations are crucial; second, determination of the maximum acceptable dosage is not achievable in most cases, and finally, immune responses during *in vivo* monitoring are very complex.

2.3.3 Radiotherapy

Radiotherapy is the most common initial treatment option for GB patients is RT. However, there are numerous drawbacks regarding the use of this method. An important aspect is that the reaction of the tumor to RT is influenced by its size. Given the multifaceted biology of the tumor, large ones almost do not respond to RT. In contrast, with smaller samples, the earlier the treatment begins, the better the result, given that RT is most effective in the first 5 years after resection [14]. The side effects of RT include both acute and chronic effects. Acute side effects include sterility, gastrointestinal abscesses, and damage to the epithelial surfaces of the mouth and throat. Late effects include heart disease, hair loss, fibrosis and lymphedema [37].

2.3.4 Chemotherapy

TMZ is the main drug of choice for the treatment of GB [44], consisting of a second-generation Imitatrazazine, and its toxic effect is due to DNA methylation [45]. TMZ is a prodrug, which means that it is spontaneously hydrolyzed at physiological pH and converted into the active drug, 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) [45].

TMZ is administered orally, being regularly used in the early stages of GB treatment. Even though TMZ has benefits in GB therapy, there are numerous complications. To be cited, the existence of BBB which limits the delivery of drugs to specific sites, as well as O6-methylguanine DNA methyltransferase (MGMT) which can lead to DNA repairing. The latter consequence can modify the cellular phenotype and, consequently, increase the cellular resistance to TMZ [46]. These phenotypes can alter the expression of key proteins, to enumerate p53, PTEN, EGFR, Mouse double minute 2 homolog (Mdm-2) and galectin-1. To overcome these complications, new drugs that can target these proteins are needed. For example, to inhibit MGMT it is possible to use O6-benzylguanine as well, some tyrosine

kinase inhibitors may suppress EGFR and finally, nutlin-3 can act as an inhibitor of Mdm2 [47, 48].

Dacarbazine (DTIC) is another antitumor agent that despite having erratic absorption and low solubility [49], it has been studied in recurrent GBs, proving to be effective [50]. However, DTIC causes severe toxicity in healthy tissues [49]. Thus, new strategies and formulations are required to decrease systemic toxicity.

CT has numerous harmful effects, notably nausea and vomiting, rash, diarrhea, hair loss, insomnia, infertility, and nerve damage. Some reports infer that CT in association with different strategies, especially RT, offers the most efficient GB treatment strategy [51, 52].

Immunotherapy, RT and CT have both beneficial and disadvantageous results. It is believed that the combination of these treatments may positively improve the effects. van Linde et al. [53] applied a neoadjuvant management approach to the newly identified GB, which comprises a chimeric monoclonal antibody, bevacizumab, followed by RT together with TMZ. Their results revealed that this combinatorial treatment was safe; however, patients presented a low response rate [54].

2.3.5 Hyperthermia

Hyperthermia is an alternative technique for GB management [55] based on the elimination of malignant cells dependent on heat production at the site of the tumor. This treatment, therefore, induces functional changes in the cells and this can lead to cell death [56]. Hyperthermia occurs at a temperature of approximately 45 °C and stimulates various intra- and extracellular mechanisms including cell degradation, cellular organelle aggregation, and protein unfolding, which may lead to apoptosis [57].

Treatment parameters, chiefly the temperature at the site of the tumor and the duration of exposure, can significantly alter the efficiency of this treatment [58]. Among the conventional methods of hyperthermia induction, the most common are infrared irradiation, microwave, and ultrasound [59]. However, all of these methods have limitations, notably low heat diffusion for tumor metastases. Particularly in tumors that are well vascularized, heat can be dissipated by the blood and this can be a problem because healthy tissues can be affected. To solve these problems, in 1957, magnetic particles began to be used for hyperthermia [60], making it known as magnetic hyperthermia therapy (THM). The limitations that come with using this method are the preparation of the materials and their cost [60]. Yet, over the years, much research has been done to prove that hyperthermia is the

only strategy lacking for the success of GB therapy. However, very few combinatorial studies have been reported. Some of the clinical studies conducted so far have described that hyperthermia could be safely applied with minimal side effects [61].

To develop targeted treatment complexes with low toxicity to healthy tissues, several different techniques are being investigated, such as radioimmunotherapy, stereotactic radiosurgery, hyperfractionation, and iodine-125 brachytherapy. However, these methods have not significantly increased patient survival [62]. Due to some deficiencies, expressively low tissue accumulation of drugs, low specificity, and toxicity, the conventional management of GB has restricted its use in patients. Consequently, the use of new nanotechnologies is an encouraging approach, since they present promising results in the treatment of GB.

3 Drug delivery systems (DDS)

Numerous obstacles in brain cancer therapy are being addressed in this work, such as low absorption, bioavailability, and low drug biodistribution since proper clinical results are not found using conventional DDS [63]. To overcome these problems, the development of new DDS capable of improving the drugs pharmacological properties is an urgent need. At this point, DDS-associated nanotechnology could be the best technology in which the created systems should prevent side effects in healthy cells [64] since upgraded DDS are capable of improving compliance with patient treatment. Furthermore, they can increase the useful life of the drug and, consequently, reduce the costs associated with medical care [65]. Subsequently, the development of DDS which exhibit controlled properties is demonstrating great potential and various nano-DDS formulations are being offered for cancer treatment [66–72].

To develop an efficient DDS, some features are essential, to enumerate:

(a) a specific target;
(b) a useful drug carrier;
(c) a suitable route of administration.

First of all, the physical-chemical characteristics of the DDS system and the drug should be clearly understood as the goal is to target the cancer cells and avoid harming normal cells. In this topic, another important aspect is drug concentration, nano-DDS may contain minor amounts of drugs, decreasing toxicity, and consequently side effects [3].

As outlined by Wen and Reardon [73], despite all efforts to treat GBs, no new approach has modified the prognosis of the disease, justifying the need for studies on promising systems using nanocarriers.

3.1 Electrospinning technique for DDS production

There are several methodologies associated with the production of DDS, notably the electrospinning technique. With this method, there is the removal of the usual emulsion step that can trigger loss of bioactivity of sensitive drugs, a point that differs and highlights it from other methods used to produce nanocarriers [74].

This technique allows the production of polymeric NP and NF with diameters varying from 3 nm to 5 μm [75]. These nanoproducts have several applications in nanocatalysis, tissue engineering, protective clothing and filter membranes [75, 76].

Electrospinning is an interesting technique given the robustness of the equipment. It consists of a support with the polymer solution, a syringe connected to a needle, a pressure pump whose purpose is to control the flow of the solution, an electric source of high voltage and a collector carrier produced from a conductive material [77]. The development of the Nano product occurs when the solution is pumped by the system and a high voltage electric field is applied to induce repulsion between the charges in the polymer solution. Due to the applied voltage, deformation occurs in the drop formed at the end of the needle, changing its shape to a cone-like structure called the Taylor Cone [78]. When the electrostatic force exceeds the polymer surface tension at the needle end, a jet (spray or spin) is formed toward the collector plate while the solvent evaporates allowing the material to solidify [79].

The final characteristics of the fibers and particles produced, such as their diameter, morphology, degree of alignment and mechanical strength may be interfered by various adjustable parameters in the art. To clarify these effects, several studies have been carried out elucidating the correlation between the parameters used and the resulting fiber. Pham et al. [79] in their review indicated variations in fiber morphology and diameter as a function of changes in electrophilic parameters [79]. Lee [80] studied the influence of molecular weight on the structure and properties of polyvinyl alcohol (PVA) nanofibers and demonstrated optimal conditions for obtaining uniform fibers [80]. Among the parameters that can affect the process are: the polymer used and the conformation of its chain, solvent, elasticity, conductivity, polarity and

solvent surface tension, applied an electric field, the distance between capillary, viscosity and solution concentration [76].

The resulting structure of the nanoproduct, is usually related to the polymer concentration. Spherical particles are fabricated at lower concentrations of polymers. To find the ideal mixture, it is essential to modify the viscosity of the solution. If its concentration is extremely low, there is no suitable chain entanglement within a drop to stabilize the NP development during electrospray, nevertheless, if the concentration is too elevated there is an increased amount of chain webs, creating polymer networks and fiber production [81].

4 Nanotechnological approaches

The category of technology that engages in employment, production, and analysis of nanometer-sized materials is known as nanotechnology. This category uses nanomaterials for practically all aspects of use [63, 65, 82–88]. Drug delivery systems (DDS) based on nanotechnology have improved pharmacokinetic characteristics. Among them, it is possible to mention high clearance rate, distribution of a large volume of drugs and high bioavailability of drugs in the tumor, due to the greater effect of enhanced permeability and retention (EPR). Coupled with it, nanomedicine-based methods can transport drugs through biological barriers [89].

The most searched nanocarrier formulations are emulsions, dendrimers, micelles, liposomes, and polymers. Polymeric nanoproducts can be divided into different systems, including nanospheres, nanocapsules, nanoparticles (NP) and nanofibers (NF) [90]. The first group is composed of solid colloidal particles in which drugs can be dispersed or adsorbed onto a matrix made of one or a mix of polymers. The size of nanospheres varies from 100 to 200 nm [91]. Nanocapsules, on the other hand, are vesicular systems. In this type of systems, the drug is encapsulated by a polymer capsule. The nanocapsules are a common system to be used in the entrapment of hydrophobic substances and, consequently, some homopolymers that may prevent the opsonization of these systems can be used in the formulation, including polylactic acid (PLA) [18]. Finally, NP and NF are solid nanoproducts (size between 1 and 1000 nm) in which it is possible to encapsulate or dissolve drugs [92].

NP and NF have interesting qualities since it is possible to use these carriers for various purposes, for instance, diagnosis and imaging, gene delivery and drug release. Due to the incorporation of the drug into the material, the

common side effects of the drugs can be decreased and also the rapid enzymatic degradation of the drug.

Additionally, these biofunctionalized products can point the target tissues in higher concentrations, being a good choice in the delivery of biological substances proteins, peptides, nucleic acids and antibiotics [93]. Further, depending on the composition developed, such products, especially NP, may be administered by various routes, including intravascular, oral, nasal, transdermal and intraocular [94, 95]. Therefore, nanotechnology-based DDSs appear as useful approaches in therapies related to CNS diseases.

Polymer properties, chiefly crystallinity, solubility, composition, molecular weight, polydispersity, and hydrophobicity are important features for developing a DDS [2]. Therefore, studies of physicochemical characterization and bio interactivity considering the different fluids, barriers and environments of the human body are crucial for building advanced knowledge at this matter. Altogether, NPs is the most commonly approached system when talking about GBs treatment, with a rising up area on NF for controlled and local delivery.

4.1 PNPS in the treatment of glioblastoma

Considering the numerous benefits of polymeric NP (PNP), markedly targeted drug administration, improved bioavailability, decreased drug side effects, drug protection against degradation, improved drug solubility and sustained release of the drug, its use in the diagnosis and treatment of CNS diseases is worthwhile [96]. The targeted DDS can be described as a material capable of delivering a medicament in a precise place with an appropriate dose. This method can be separated into passive and active [97]. In the first system, the release depends on differences between healthy and unhealthy tissues. It is known that damaged tissues present several changes in a physiological condition through the EPR effect [98, 99]. However, tumor sites usually have high concentrations of drugs when compared to normal sites [100]. The second system, using nanotechnology techniques, can be performed by adding specific ligands on the surface of the PNPs [101]. Conjugation of specific nucleic acids, peptides, and antibodies coating PNP leads to specific targeting (Fig. 1). Not to mention, this enables the modification of the tumor cells signaling pathways [15]. The ability to readily modify the surface is a supplementary outstanding property of the PNPs [87, 102]. However, to accomplish the desired delivery of the drug, it is important to understand the nature of the PNP and the properties of the drug.

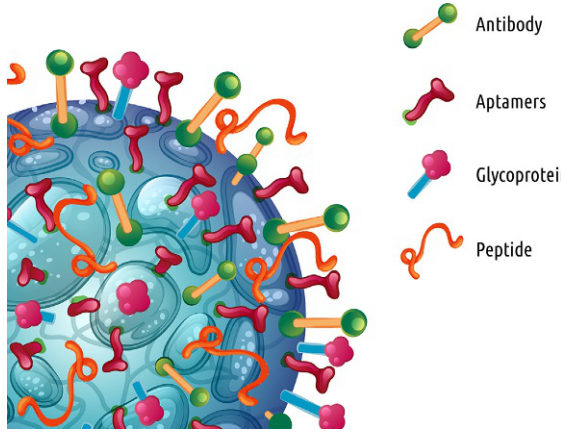

Fig. 1 Schematic representation of polymeric nanoparticles and biofunctionalization options.

Polymers such as poly (lactic-*co*-glycolic acid) (PLGA), poly (methyl methacrylate) (PMMA), poly (ethyleneimines) (PEI), polyesters, poly (methylene malonates) (PMM) and poly (alkyl cyanoacrylates) (PACA) were used as drug delivery carriers [103]. The correct selection of the polymer will depend on the purpose of the treatment. For example, PEG is a biocompatible, inert and hydrophilic polymer used to design PNP, increasing its stability [104]. However, oxidative damage avoids its long-term uses [105]. PLA and PLGA are the most effective polymers used to date since their metabolites are eliminated by the Krebs cycle and are therefore non-toxic [106]. PVA, a non-toxic hydrophilic polymer approved by the Food and Drug Administration (FDA), is an interesting option for designing nanoproducts since this polymer has low cytotoxicity and is biodegradable. The PVA also has an excellent history in biomedical applications, mainly hydrogels [107–110].

The NP could be efficient in GB treatment due to its various properties, including biocompatibility, small size and simple, fast and low-cost production [18]. Furthermore, NPs can enhance various aspects of the drug, including stability and release [111]. There are numerous assortments of NP construction and different design strategies. The need to develop effective DDS, both safe and biodegradable systems, has made PNPs one of the most encouraging technology, achieving wide recognition in the field of DDS, particularly in cancer therapies [88, 94, 95].

PNPs are submicron-sized colloidal particles that can encapsulate or adsorb drugs, imaging substances and biological agents within or on their surface. Considering that the structure of the PNP matrix allows the addition of drugs, the surface facilitates its distribution in the body. Besides, PNPs offer resolutions to CT limitations, including resistance to usual medications and undesirable effects [18].

PNPs can be synthesized from synthetic polymers. To point out, PVA, (poly (ethylene glycol)-PEG, poly (acrylamide)-AMP, ε-poly (caprolactone)-PCL and poly (acrylate)-PA) or natural compounds like chitosan, gelatin, polysaccharides and albumin [112]. Synthetic PNPs such as PLA, PLGA and PGA are biocompatible and degrade hydrolytically [113]. Among the biocompatible PNPs, the FDA approved PVA, PLGA, and PCL. In order to deliver agents to brain tumors, mainly cationic polymers have been used, especially for transporting nucleic acids, since they have positive charges that allow them to interact with DNA and RNA [94, 95, 114].

PNPs can be allocated in biodegradable or non-biodegradable PNPs. Due to the low immunogenicity, high bioavailability and stability, low cytotoxicity and administrable release of the drug, biodegradable PNPs are generally used for DDS and are the appropriate option for the encapsulation of hydrophobic drugs [18]. Moreover, these PNPs show better stability in biological fluids [115], and their biodegradable versions are often employed for the purpose of improving the solubility and bioavailability of bioactive substances [100].

Although PNPs are extensively studied for GB drug delivery, BBB remains a challenge [116]. Significant results were obtained when an intravenous delivery of poly (*n*-butyl-2-cyanoacrylate) NP (PBCA) was tested [117]. Moreover, different polymers, notably PGA and PLA, were similarly investigated as carriers increasing drug delivery efficiency [118]. Using an unconventional route, intranasal administration that is able to bypass the BBB, Sekerdag et al. [119] analyzed the lipid-fused pseudo-thiosalicylic acid-FEG-PEG-PLGA hybrid NPs in rats and these PNPs achieved a substantial reduction of the tumor area.

Recently, Baghirov et al. [120] produced a new platform with focused ultrasound (FuS) and poly (2-ethyl-butyl cyanoacrylate) microbubbles that could space the BBB and accumulate in the cerebral parenchyma. In this area, Mead et al. [121] studied in rats a combined method with the delivery of genetic material with a continuous release. Remarkably, this system achieved important results without inducing toxicity or activation of astrocytes.

The first PNP used for drug delivery to the CNS was PBCA PNP [122]. Subsequently, the NP design has been based on the ability of PNPs to target BBB and to deliver drugs to the tumor site using receptor-mediated endocytosis (Table 1). This mechanism causes endogenous materials to pass through the BBB. Besides, changes in the surface of PNPs, for instance, surfactant coverage or ligand binding, significantly increase cell uptake, especially when the linker is associated with receptor-related proteins, which enhances receptor binding to CNS cells [3].

Surface changing with surfactants is a very common modification. In 2010, Gelperina et al. demonstrated that NP of PLGA with a poloxamer-188-modified surface showed more substantial results than PNP without modification. In another study using glioblastomas from 101/18 rats, Wohlfart et al. [135] showed that PLGA NP with poloxamer-188 and doxorubicin (DOX) were able to reach the brain and cross the BBB. By the same token, polysorbate-80 (P-80) modified poly (isohexyl cyanoacrylate) (PIHC) was tested *in vitro* against GB cells, showing a promising use as a non-invasive treatment of GB. An alternative to NP coating is glutathione-coated PNP. Geldenhuys et al. [136] presented PLGA NPs with paclitaxel (PTX) and coated with glutathione, which resulted in an increased capacity to reach the passage through the BBB.

Additional modifications, namely protein binding, can lead to better results. Transferrin (TF), a brain-directed glycoprotein ligand which has a receptor on endothelial cells, was used by Ren et al. [127, 134] to functionalize PEG-PLA NP with results demonstrating that the formulation reaches C6 cells and crosses the BBB. Lactoferrin glycoprotein (LF) used in PEG-PLGA NP was also effective in reaching the brains of mice [128]. Son et al. [147] studied NP dissociates of disulfide modified with rabies virus glycoprotein (RVG) (SS-PEI) that were used to deliver microRNA to GB.

A red blood cell membrane-coated NP with the neurotoxin candoxin (CDX), a peptide that shows high affinity for receptors expressed on CNS endothelial cells, including nicotinic acetylcholine receptors, have also been shown to be efficient [148]. Because these NPs are very flexible, this methodology system could be used for several types of membranes, serving different medical purposes.

Fang et al. [149] used chlorotoxin (CTX), a neurotoxin that binds to cell membranes, on the surface of a PNP that contained a chitosan nucleus functionalized with biotin and PEG and was covalently conjugated to TMZ. TMZ-CTX-PNP showed higher uptake by GB cells and were more efficient in treatment than PNP without CTX. They were also able to cross

Table 1 Polymeric nanoparticles in the treatment of glioblastomas.

NP	Surface coating	Ligand	Drug	Results	References
MPEG	PLA	Folate	PTX	Folic acid induced increased cell absorption	Wang et al. [123]
PLGA	PEG	AS1411, a DNA aptamer	PTX	Extended drug circulation and accumulation of PTX in tumor tissue. Increased inhibition of glioma growth in rats and increased animal survival	Guo et al. [98, 99]
PLGA	PEG	F3 and tLyp-1 peptides	PTX	Drug accumulation in tumor tissue, increased tissue penetration and increased animal survival	Hu et al. [124]
PEG-*co*-PCL	MMP-2/9		PTX	Increased PTX efficacy. Increased animal survival	Gu et al. [125]
PLGA	Poloxamer 188/ polysorbate 80		DOX/ loperamide	Increased treatment effectiveness due to formulation parameters such as surfactants, drug and stabilizer	Gelperina et al. [126]
PLA	PEG	Transferrin		Functionalized NP were able to penetrate the tumor tissue	Ren et al. [127]
PLGA	PEG	Lactoferrin		Viability assays demonstrated low systemic toxicity	Hu et al. [128]
GNRs	PEG	Arg-Gly-Asp		Increased toxicity due to avβ3 binding	Verma et al. [44]

Continued

Table 1 Polymeric nanoparticles in the treatment of glioblastomas—cont'd

NP	Surface coating	Ligand	Drug	Results	References
PEG-PCL			PTX	Increased toxicity due to microtubule stabilization	Xin et al. [129]
PLGA			Celecoxib	COX inhibition	Vera et al. [130]
PEG-SLN			c-Met siRNA	C-Met inhibition and decreased cell proliferation	Jin et al. [131]
Liposomal PEG			DOX	Increase of DOX infusion through BBB	Birngruber et al. [132]
PLGA	Poloxamer 188		DOX	DOX accumulation in glioma	Agarwal et al. [133]
PAMAM dendrimer			mir-21 5-FU	Increased efficacy of 5-FU and apoptosis in glioblastoma	Ren et al. [134]
PLGA	Poloxamer 188		DOX	Effective DOX delivery through BBB	Wohlfart et al. [135]
PLGA	Glutathione		PTX	Increased transport ability through BBB	Geldenhuys et al. [136]
SS-PEI		RVG	micro-RNA	Increased transport ability through BBB	Jin et al. [131]
PIHC	Polysorbate 80		DOX	Powerful vector for use in noninvasive therapies	Wohlfart et al. [135]
MNP	PEG	CTX		Increase of drug efficacy	Sun et al. [137]
SPION	PEG	CTX		Increased cell absorption and decreased tumor invasion	Veiseh et al. [138]
MNP	PEG and chitosan	CTX	siRNA	Ideal system for delivery of genetic material in the presence of a magnetic field	Veiseh et al. [139]
PLGA			Celecoxib	Increase of antitumor activity	Suzuki et al. [140]
PEG-*co*-PTMC	2-Deoxy-D-glucose			Increase of cellular absorption	Jiang et al. [141]

MNP	PEG, chitosan and PEI	CTX	GFP	Increased cell absorption and GFP expression	Kievit et al. [142]
MNP	PEG	Arg-Gly-Asp		Target-specific in glioblastoma cells	Cabada et al. [143]
MNP	PEG		DOX	Drug release promoted by infrared light	Agarwal et al. [133]
AuNP	PEG			Increased DNA damage to glioblastoma cells after radiotherapy	Joh et al. [144]
AuNP	PEG		DOX	Increased cell absorption and drug half-life	Ruan et al. [145]
PEG-PCL			PTX Angiopep	Increased transport ability through BBB	Dilnawaz et al. [146]

NP: nanoparticles; MPEG: methoxypolyethylene glycol; PLA: polylactic acid; PTX: paclitaxel; PLGA: poly(lactic-*co*-glycolic acid); PEG: polyethylene glycol; PCL: polycaprolactone; COX: cyclooxygenase; MMP-2/9: matrix metalloproteinase-2/9; DOX: doxorubicin; GNRs: gold nanorods; SLN: solid lipid nanoparticle; c-Met: tyrosine-protein kinase Met; BBB: blood-brain barrier; 5-FU: fluorouracil; PAMAM: poly(amidoamine); SS-PEI: disulfide–polyethylenimine; RVG: rabies virus glycoprotein; PIHC: polymer-infiltrated hybrid ceramic; MNP: magnetic nanoparticles; SPION: super-paramagnetic iron oxide nanoparticles; CTX: chlorotoxin; PTMC: trimethylene carbonate; GFP: green fluorescent protein; Au: gold.

the BBB and transport the drug to the tumor site, demonstrating a promising option to transport TMZ in significant concentrations and avoid toxicity in healthy cells [149].

Comparatively, Liu et al. [150,151] constructed a liposome containing TF (TF-CPP-SSL) in the nucleus *via* PEG. These TF-CPP-SSL PNPs showed efficient BBB penetration, increased drug circulation *in vivo* and were able to target the tumor. Coupled with it, they showed high cellular uptake and were able to avoid lysosomal degradation.

An important aspect to analyze the prognosis in GB patients is the necessary resection. However, it is difficult to establish a safe level of resection due to GB malignancy. In a recent study, the authors developed fluorescence-guided surgery, allowing a safer resection of GB. Tang et al. conjugated an aptamer 32 to a pegylated NP producing an aptamer probe capable of specifically binding to cancer cells [152].

The discovery of promising molecular targets to treat GB is a research challenge; however, it has stimulated the development of genetic treatments (Table 1). The low success rate of the available therapies is related to the inability of the vectors to reach the brain [3].

PTX is used to prevent mitosis because it promotes congregation and stabilization of microtubules. However, PTX is a highly hydrophobic substance and is not able to cross the BBB. To solve this problem, PTX can be absorbed into a PNP [153]. Angiopep (ANG1005) is a peptide with 19 amino acids that is conjugated with the taxane, a mitotic inhibitor. PEG-PCL-NP conjugated to angiopep and PTX were tested in glioma therapy, reducing GB cell proliferation and increasing cellular apoptosis [129]. Furthermore, using PLA NP, Wang et al. [123] produced a monomethoxy-PEG-PLA-PTX-NP modified folate target which increased cytotoxicity against HeLa and C6 cell lines.

In a study using AS1411 (Ap), a DNA aptamer, the authors produced an NP for siRNA delivery that significantly decreased cell proliferation due to prolonged drug release and showed better biodistribution of the drug at the tumor site compared with Taxol® [98, 99]. In a different study with PEG-PLA-NP, the F3 peptide, which similarly to Ap, binds to nucleolin, was used to target tumor cells by promoting an increase in cell uptake. These PNPs exhibited increased accumulation, tumor site infiltration and survival of the animal model [124].

Gu et al. [125] conjugated the low molecular weight activated protamine MMP-2/9 with PEG-*co*-PCL-NP, these NP improved uptake into cells, while *in vivo* results showed accumulation of NP at the tumor site. Also, mice

with C6 implants treated with the formulation had a longer survival time when compared to the control groups.

A cyclooxygenase inhibitor (COX-2), celecoxib, presents its action promoting cell death and decreasing cell proliferation [140]. In a dose-dependent manner, PLGA-NP with celecoxib showed activity against U87MG and C6 cell lines. These results reveal these NP as an efficient choice for drug delivery in GB [154].

Another promising strategy is polyamidoamine dendrimers (PAMAMs) used as transporters of 5-fluorouracil (5-FU) and antisense oligonucleotide miR-21 (as-miR-21) to specific sites in GB [155]. When tested on U251 cells, co-delivery of 5-FU and as-miR-21 significantly increased cytotoxicity and cell death. Therefore, co-administration of drugs and molecular vectors, especially if the tumor has a known gene overexpression, is an interesting and effective approach in the treatment of GB [127, 134].

Equally important is the down-regulation of the protein tyrosine kinase Met (c-Met) which is a tyrosine kinase receptor that can interact with hepatocyte growth factor and this interaction leads to an increase in cell proliferation, corroborating with invasiveness and resistance to CT. Therefore, with the use of siRNA it is possible to suppress c-Met and this allows therapy improvement. The PEG/c-Met-siRNA formulation significantly inhibited tumor drug resistance and cell proliferation [131].

Liu et al. [150,151] presented a PNP that successfully transports and protects siRNAs. The formulated PNPs involved PEGylated AuNPs coated with PEI and chitosan. The siRNA for Ape1 was added to the formulation and the NPs were tested on brain cancer cells showing that the reduction of Ape1 expression is related to an enhancement of DNA damage after exposure to radiation.

Recently, Wadajkar et al. [156] formulated particulate nanocarriers using PLGA and PEG binding the Fn14 receptor, generally overexpressed in GB invasive, on the surface. The increase in observed efficacy suggests that these PNPs optimized the supply of drugs to treat invasive GBs. In another study, the researchers developed a PNP using polystyrene (PS) with a surface functionalized with an antibody that binds to Fn14. The results showed that this PNP was able to penetrate tumor tissue by selectively targeting GB cells [157]. Although these studies with PNP appear promising, the usual methods of preparation are sometimes inconsistent and complex, which impairs serial production. On the other hand, electro-spinning is an interesting method to produce NP without the use of surfactants and on a large scale.

4.2 PNFS in the treatment of glioblastoma

Recurrent GBs are less responsive to CT than the original tumor and are extremely invasive [158]. Despite the application of all treatment strategies, GB remains an incurable disease and the overall survival of the patient between 12 and 15 months after the initial diagnosis [159]. Autopsy studies suggest that recurrent GB are mainly local and appear within 2 cm of the initial site of the tumor [160]. Therefore, localized CT by using polymeric NF that can be administered during surgery provides an alternative DDS for the treatment of GB, significantly reducing systemic side effects and preventing tumor recurrence.

Although *in loco* treatment option is extremely promising, few studies have been performed using NF in the treatment of GB (Table 2). As previously mentioned, PTX presents several difficulties in its application [161], so a local application of the drug is an interesting choice to improve its effectiveness. In one study, PLGA and PTX were used to make submicron and microfibers. Both formulations showed a prolonged release of PTX *in vitro* over 80 days, in which submicrofibers presented augmented release rates matched to microfibers due to the higher polymeric degradation rate and surface area. The superiority of NF over the systemic administration of Taxol® in terms of apoptosis has also been reported. Not to mention, inhibition of the tumor growth in mice disclosed that the animals treated with the fiber presented smaller tumors after 24 days and 32 days post tumor inoculation compared to animals treated with Taxol®, indicating significant advantages of prolonged release of PTX [162]. In a parallel report, PTGA NF loaded with PTX with a high drug release showed a drug penetration into the rat brain at 5 mm from the implant site, even after 42 days post-implant. Furthermore, NF demonstrated tumor inhibition and a low rate of tumor proliferation after 41 days of treatment in animal models compared to placebo [163].

Recently, Ramachandran et al. [167] developed PLZ-PLA-PCL NF containing TMZ implanted in an orthotopic model of GB, which demonstrated a continuous drug release of 116 mg per day with a small extravasation to the peripheral blood (less than 100 ng), demonstrating 1000-fold higher drug concentration at the tumor site compared to peripheral blood.

BCNU [1,3-bis (2-chloroethyl)-1-nitrosourea] (carmustine) is one of the most used antitumor drugs for GB treatment that is capable of penetrating BBB [180]. The mechanism of action of BCNU is based on the formation of DNA and RNA crosslinks leading to inhibition of DNA synthesis,

Table 2 Polymeric nanofibers in the treatment of glioblastomas.

NF	Drug	Results	References
PLGA	PTX	Extended PTX release up to 80 days in which sub-microfibers demonstrated faster release than microfibers	Ranganath and Wang [162]
PLGA	PTX	Increased drug penetration (5 mm) observed after 42 days of implantation	Ranganath et al. [163]
PPC-Ca-alginate MPs	PTX, TMZ	Synergistic effect of drugs	Ni et al. [164]
PCL-Diol-*b*-PU	TMZ	Extended TMZ release up to 30 days	Irani et al. [165]
PCL	TMZ	NF upregulated p53 and Bax more than only TMZ	Tavakoli et al. [166]
PLGA, PLA, PCL	TMZ	Animals treated with extended release NF showed longer survival (> 4 months) than those treated with short release NF (74 days)	Ramachandran et al. [167]
PCL	TMZ	Inhibition of tumor growth and favoring neuron differentiation for tissue reconstruction	Huang et al. [168]
PCL-Diol-b-PU-CS NP	TMZ and Au NP	Increased antitumor activity	Irani et al. [169,170]
PCL-Diol-*b*-PU	TMZ and Au NP	Increased antitumor activity	Irani et al. [169,170]
PEG-PLLA	BCNU	BCNU showed toxicity only after 72 h due to its controlled release	Xu et al. [171]
PLGA	BCNU	NF showed BCNU release for more than 6 weeks in rat brains	Tseng et al. [172]

Continued

Table 2 Polymeric nanofibers in the treatment of glioblastomas—cont'd

NF	Drug	Results	References
PEO, PLA	Rapamycin	Decreased cell viability after treatment	Wang et al. [173]
PC, PVP	Mycophenolic acid	Fibers prepared with coaxial electrospinning demonstrated gradual drug release	Han et al. [174]
PCL	Daunorubicin	Controlled and extended drug release	Lian and Meng [175]
PLGA	BCNU, irinotecan, cisplatin, combretastatin	Decreased tumor malignancy and tumor growth, and increased survival of the animal model	Tseng et al. [176]
PCL-GT	SN-38	Increased cytotoxicity after 72 h	Zhu et al. [177]
PLGA,PEI	MMP-2 mRNA and PTX	Significant regression of tumor growth in drug and gene fiber treatment	Lei et al. [178]
PLA	TRAIL	NF released TRAIL protein, leading to reduced tumor volume in mice	Bagó et al. [179]
PCEC	Curcumin	Blockage of cell proliferation signaling pathways	Guo et al. [98, 99]

NF: nanofibers; PLGA: poly(lactic-*co*-glycolic acid); PTX: paclitaxel; PPC: polypropylene copolymer; Ca: calcium; MPs: magnetic nanoparticles; TMZ: temozolomide; PCL: polycaprolactone; PU: polyurethane; p53: tumor protein 53; Bax: Bcl-2-associated X protein; PLA: polylactic acid; CS: chitosan; NPs: nanoparticles; PEG: polyethylene glycol; PLLA: poly-L-lactide; Au: gold; BCNU: bis-chloroethylnitrosourea; PEO: polyethylene oxide; PC: polycarbonate; PVP: polyvinylpyrrolidone; GT: glutathione; PEI: polyethylenimine; PCEC: polycaprolactone/poly (ethylene glycol)/polycaprolactone.

RNA production, and translation of RNA [171]. Gliadel® is a biodegradable poly (carboxyphenoxy-propane/sebacic acid) wafer that releases BCNU; it can be implanted into the tumor cavity after surgical resection [181]. This wafer has been approved by the FDA for clinical use and has shown some improvements in the survival of patients up to 2 months longer than those who did not receive the treatment [181]. Nevertheless, fast release of the drug and wafer stiffness are major disadvantages. In view of this, Tseng et al. [172] produced PLGA NFs releasing high amounts of BCNU for a prolonged time (>6 weeks) in the rat brain, coupled with no observation of inflammatory reaction in the brain tissue.

In one study, MMP-2 was chosen as a therapeutic target. An RNA plasmid suppressing the expression of MMP-2 in cancer cells was designed and complexed with PEI. This plasmid together with PTX were encapsulated in PLGA NF aiming to achieve prolonged release of both. It demonstrated substantial tumor growth suppression evaluated in intracranial tumor models and compared to NF with PTX and commercial PTX, demonstrating synergistic therapeutic effects of the gene and PTX [182].

In another research, different CT drugs such as bis-chloroethyl nitrosourea, irinotecan, cisplatin and an antiangiogenic drug, combretastatin, were loaded into PLF NF. CT drugs were rapidly released from NF, while combretastatin exhibited a prolonged release up to 2 weeks. This formulation was considered efficient in decreasing tumor progression and extending survival in GB rats compared to NF without combretastin [176].

Although there has been an increase in the interest of researchers in developing NF that are able to provide gradual release of drugs over a long period to improve the effects thereof, the concentrations used in the formulations are often toxic. In addition, the experimental procedure tested in the animals does not reflect what happens in the clinic, considering that surgical resections are not performed.

5 Adverse effects of nanocarriers

Typically, most studies that develop nanoproducts use biocompatible and biodegradable materials. Therefore, the harmful effects of nanocarriers should be minimal. However, some described adverse effects might be related to the surface area of the product, because this surface can result in the formation of reactive oxygen species (ROS), markedly superoxide anion and hydrogen peroxide [183]. Due to the existence of electron receptor sites and donors on the transport surface that tend to react with oxygen

molecules, this consequently leads to the oxidation of nearby chemical compounds or even organelles [183]. Consequently, an essential factor to analyze when evaluating the side effects of nanoproducts is their surface area.

In like manner, a common side effect is the hypersensitivity reactions that some patients describe following intravenous injection. This adverse effect is common not only for NP formulations but also for several others drugs and can be avoided by administering different medication to patients or by decreasing the infusion rate [184]. Generally, hypersensitivity reactions are greater in the administration of free drugs when compared to DDS formulations. An interesting example is the administration of DOX. It is known that this drug induces a cardiotoxicity effect which can be decreased when a DDS formulation is applied. Equally important, the evaluation of the possible toxicity related to the remaining carrier substance needs to be highlighted so the production of biodegradable polymers with a known life expectancy is aimed to produce safe formulations [3,185].

6 Clinical trials

Although several nanopolymeric systems are available in the market, only a few clinical trials focusing on brain cancers are being developed, all in phase I or II (Table 3). One of them was a phase I completed the trial (NCT00734682) of intravenous liposomal irinotecan in 34 patients with recurrent high-grade gliomas (HGG). Dosing was given IV every 3 weeks, the maximum tolerated doses were established between 120 and 150 mg/m^2 and the toxicity profile was felt acceptable, guaranteeing the continuation of this study to the phase II [186]. Also, a phase II study (NCT01663012) using PEGylated liposomes containing etirinotecan pegol (NKTR-102; the active compound of irinotecan) to treat bevacizumab-resistant HGG was carried out. Among the 20 patients treated for 3 cycles, three of them had partial MRI responses and 10 patients had stable disease and, once this study did not explore the efficacy of NKTR-102 over other therapies, it might warrant further investigation of this molecule against HGG [187].

Other two studies, NCT01386580 and NCT01818713, were based on a glutathione PEGylated liposomal doxorubicin (2B3-101/targeted NC), which demonstrated a 5-fold higher brain delivery in rats as compared to Caelyx® and Doxil®, two commercial pegylated liposomal DOX formulations. Phase I studies were carried out in 37 patients with HGG ($n = 13$) or solid tumors in advanced stages and brain metastases ($n = 24$), and the results

Table 3 Clinical trials related to nanoparticles in the treatment of GBM.

Clinical trials identifier number	Treatment name/platform	Intervention	Phase
NCT01386580	2B3-101/targeted NC (PEGylated liposomes)	DOX	I/IIa
NCT01818713	2B3-101/targeted NC (PEGylated liposomes)	DOX	II
NCT01663012	NKTR-102 In Bevacizumab-Resistant High Grade Glioma (PEGylated liposomes)	Etirinotecan pegol	II
NCT00734682	Nanoliposomal CPT-11 (NL CPT-11) (PEGylated liposomes)	Irinotecan	I
NCT00944801	RNOP-09: Pegylated Liposomal Doxorubicin	DOX	II
NCT01806675	18F FPPRGD2 PET/CT or PET/MRI Imaging of αvβ3 Integrins Expression as a Biomarker of Angiogenesis	2-Fluoropropionyl-labeled pegylated dimeric RGD peptide	II

indicated a good tolerability up to a dose of 15 mg/m^2/week with or without a combination with trastuzumab and 16 patients showed stable tumor progression after 2 cycles of treatment. In phase IIa, the safety, tolerability and intracranial anti-tumor activity of the same liposomal formulation were evaluated in patients with HGG ($n = 18$) and breast cancer brain metastases ($n = 10$). Results up to March 15th 2014 revealed that in both groups the side effects were similar to the treatment with Caelyx® and Doxil®, and 31% of patients with HGG presented stable disease [188]. In the same way, the phase II trial NCT00944801 investigated the outcomes of the administration of RNOP-09 (PEGylated liposomal doxorubicin) and prolonged TMZ coupled with radiotherapy in 63 patients at the newly diagnosed GBs. Altogether, results indicated that the toxicity of the three methods combination was well tolerated, and the progression-free survival and median global survival did not result in an expressive improvement at the outcome of the patient in comparison with databases [189].

Finally, the phase II study NCT01806675 consists in a prospective trial using a new tracer (18F FPPRGD2 PET/CT or PET/MRI) for prognosis

and early assessment of response to anti-angiogenic therapy in patients with GBs and other types of cancer-based on $\alpha_v\beta_3$-positive cells. This trial involves 25 patients and is still in progress. Although new data have not been released, some studies concerning other types of cancer revealed that this approach could provide comparable diagnostic information as compared to conventional methods. Thus, a similar outcome might be observed in the diagnosis of GBs cases [190].

7 How to study drug and polymer interactions with multiscale computational methods

Polymers present particular aspects that differ from the angstrom level of a bond between atoms to nanometers of the chain, micrometers, millimeters, and polymeric nanofibers. The different time scales for each material properties may vary from femtoseconds to seconds or hours (phase separation in blends). On the literature, there are many examples of multiscale nature of polymer systems [191–198]. Because of this, several computational methods were developed in order to address these issues [199–207]. These new methods introduce new possibilities to construct, optimize and predict the properties and structures of polymers. Not only that, these methods allowed the study of these materials with other molecules [208–212]. Therefore, it is possible to predict polymer-drug interactions in nanosystems by using *in silico* methods.

Until the present moment, no computational method can cover different size scales of polymers [213]. Thus, the multiscale simulation approach is one of the best choices to deal with this issue. The multiscale strategy combines various methods and is considered one of the most important subjects in computational chemistry. To perform a multiscale method, different theories and models are combined. Following the scales that are divided the different methods (Fig. 2):

- The quantum scale: In this method, the nuclei and electrons are part of the calculation and quantum mechanics (QM) calculations are used to model the system. This allows the study of several events associated with chemical reaction, in particular, the disruption and formation of chemical bonds between atoms, the transitions in electrons in the nucleus, and others important phenomena on polymers material that need to be modeled at the quantum scale.
- The atomistic scale: In the atomistic calculations, all atoms are present, defined and treated by single spheres. The force field, a typical system

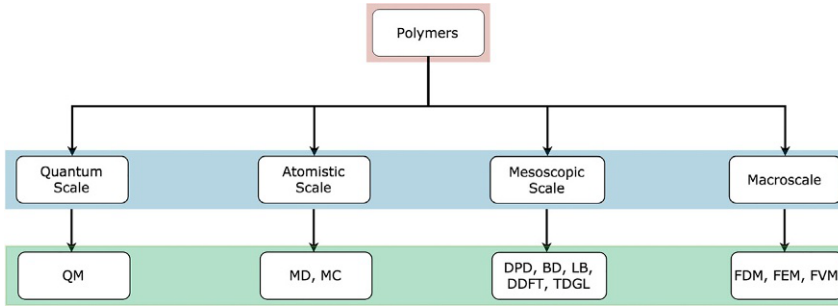

Fig. 2 Different theories and models to study polymeric materials with multiscale approach.

interaction, is responsible for the potential energy and these interactions include the bonded interactions such as the bond angle, the bond length, and the dihedral angle potentials between atoms. Moreover, force fields also contain non-bonded interactions. These interactions act between atoms in other molecules and in the same molecule. Within force fields, non-bonded interactions can be divided into two types: Van der Waals interactions and electrostatic interactions. Monte Carlo (MC) and Molecular dynamics (MD) simulations are examples used at this level to study molecular processes covering a larger system (high number of atoms), for example, proteins, membranes, and nucleic acids.

- The mesoscopic scale: In this approach, the molecules are defined as particles, known as beads. Some details of the system are implemented implicitly**,** allowing the possibility to simulate some systems on higher time scales when compared to atomistic simulations. An example is a coarse-grained model, where the particles are accumulated in beads. During the calculation, for definition of the system, interactions between beads are used. Several techniques were made to investigate the structure of polymers at mesoscopic scale, among them lattice Boltzmann (LB), Brownian dynamics (BD) dissipative particle dynamics (DPD), time-dependent Ginzburg-Landau (TDGL), and dynamic density functional theory (DDFT).
- The macroscale: In this methodology, the system is considered as continuous and the details of atoms and molecules are ignored. The constitutive laws are responsible for the behavior of the system. The conservation laws are associated with constitutive laws to simulate the systems. The main functions, namely stress and velocity are continuous. On the other hand, a finite number of locations which separate

continuity regions is considered discontinuous on these calculations. An excellent analogy to describe this scale would be to replace a heterogeneous material (details of atoms and molecules) with an equivalent homogeneous model (macroscale). To perform molecular simulations at this scale, the following methods can be used: finite difference method (FDM) and finite volume method (FVM), and finite element method (FEM).

8 Final considerations

GB is an extremely aggressive disease, however, current therapy to treat GBs does not suggest efficient results and cure is a distant reality. The use of nanotechnology can be seen as a promising strategy to GB therapy; at the same time, several unknown responses need to be further investigated. Researchers are not yet aware of all possible effects when using DDS, in general, toxicity and efficacy parameters. Nowadays, cancers such as GB, which are particularly malignant and very resistant to CT, can be treated with nanocarriers that improve the efficacy of existing and new medicines. Currently, several vectors are being investigated, in contrast, only some formulations using this innovative approach are commercially available. Even so, nanocarriers are a promising strategy for the effective delivery of stable release of drugs at tumor sites for a sustained period.

Because GBs can invade functional areas of the brain, the second surgical resection is jeopardy. Autopsy studies suggest that recurrent GBs are mainly local and appear within two centimeters of the initial site of the tumor. Therefore, direct and controlled CT *in loco* can be provided with the alternative DDS for the treatment of GB. To emphasize, it can significantly reduce systemic side effects and can prevent tumor recurrence.

While many studies like this have established good results, further research is essential to the developing of successful DDS. In this regard, nanocarriers with biofunctionalized surfaces and molecular targets are necessary to improve delivery *in vivo*. In addition, developing functionalized nanocarriers can provide multiple targets and overcome BBB problems. Other important aspects that need to be better understood during the fabrication of DDS are:

(a) The microenvironment of GB, because it is important to elucidate the molecular implications that can affect the carrier;
(b) Responses of the immune system.

Clinical trials must be conducted to analyze the pharmacological characteristics and body response of formulations. Only with these types of assessments, it is possible to obtain optimized therapies. In general, it is also essential to develop intelligent methods that can evaluate and follow up on the results of the DDS formulation. It is extremely necessary to understand cell interactions after transporter uptake and changes in the tumor microenvironment.

The development of polymers structure and the study of their interactions with other biomolecules, as anticancer drugs, require a comprehensive knowledge of the phenomena at different time and length scales. Because of this need, the theoretical and computational methods present great progress, allowing the study of these systems. The development of multiscale methods enables the design of polymeric materials simultaneously on many scales instead of trial-and-error experimentation.

Multiscale simulations approaches may be useful strategies for assessing the behavior of molecules and their molecular alterations after carrier treatment since such technologies can predict dynamic changes on important molecules for cancer therapy. Finally, approaches based on nanotechnology can be used to improve and direct the delivery of new and known drugs for the management of various human diseases; the days are prosperous for strategies based on nanotechnology.

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3.4. Capítulo 4: Bionanocomposites for In Situ Drug Delivery in Cancer Therapy

Capítulo de livro publicado no livro Biomedical Composites.

Bionanocomposites for In Situ Drug Delivery in Cancer Therapy: Early and Late Evaluations

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Abbreviations

α-CD	A-cyclodextrin
BBB	Blood brain barrier
CT	Chemotherapy
CTC	Circulation tumor cells
DCA	Sodium dichloroacetate
DOX	Doxorubicin
DPPE	Dipalmitoyl phosphatidylethanoiamine
MWCNT	Multi-walled carbon nanotubes
NF	Nanofiber
NP	Nanoparticle
PCL	Polycaprolactone
PDLLA	Polylactide
PDT	Photodynamic therapy
PEG	Poly (ethylene glycol)
PEI	Poly(ethylene imine)
PEO	Polyethylene glycol
PLA	Polylactic acid
PLGA	Poly (lactic-co-glycolic acid)
PLLA	Poly-l-lactic acid
pNIPAM	Poly(N-isopropylacrylamide)
PS	Photosensitizer
PTX	Paclitaxel
PVA	Polyvinyl alcohol

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ROS	Reactive oxygen species
RT	Radiotherapy
SPIONs	Superparamagnetic iron oxide NPs
T1	Two-photon absorption compound
TMZ	Temozolomide
VEGF	Vascular endothelial growth factor

1 Cancer Outline

Cancer is a general word for a complex category of diseases described by unregulated cellular processes such as growth and death [1]. Theoretically, all cancers develop as an outcome of mutations in the DNA sequence, which may be hereditary, provoked by environmental dynamics or a consequence of replication errors of DNA [2]. These explanations are elucidated by the carcinogenesis multistep model (Fig. 1) comprising initiation, promotion, and progression stages [3, 4]. Initiation begins when healthy cells are damaged by contact with physical, biological, or chemical carcinogens resulting in mutations in the DNA [5]. Promotion is described by the clonal growth of initiated cells, inhibiting apoptosis [6]. Lastly, tumor progression is described by a malignant phenotype expression, including characteristics such as uncontrolled growth, genomic instability, metastases development, and modifications in the morphological and biochemical features of cells [6, 7].

Conventional cancer management embraces surgical procedure, systemic chemotherapy (CT), and radiotherapy (RT). The CT therapeutics frequently damage normal cells developing severe side effects [8, 9]. An alternative to the treatment is a nanotechnological intervention since it has transformed the cancer therapy by overcoming existing restrictions of conventional CT, and offering better targets with sustained and controlled drug release also improving both distribution and pharmacokinetics [9–11].

2 Drug Delivery for Cancer: General Reflections

Targeted drug delivery for cancer therapy refers, quantitatively and selectively, to drug accumulation within tumor tissue [12, 13]. Subsequently, targeted medicine or targeted therapy means precise drug efficiency combine with minor side effects and interaction, at the molecular level, between a biomolecule and a drug. As a matter of fact, the concentration of drugs and biomolecules must be high at the tumor site, while its concentration in healthy non-target tissue should be almost undetectable preventing systematic negative reactions [12, 13].

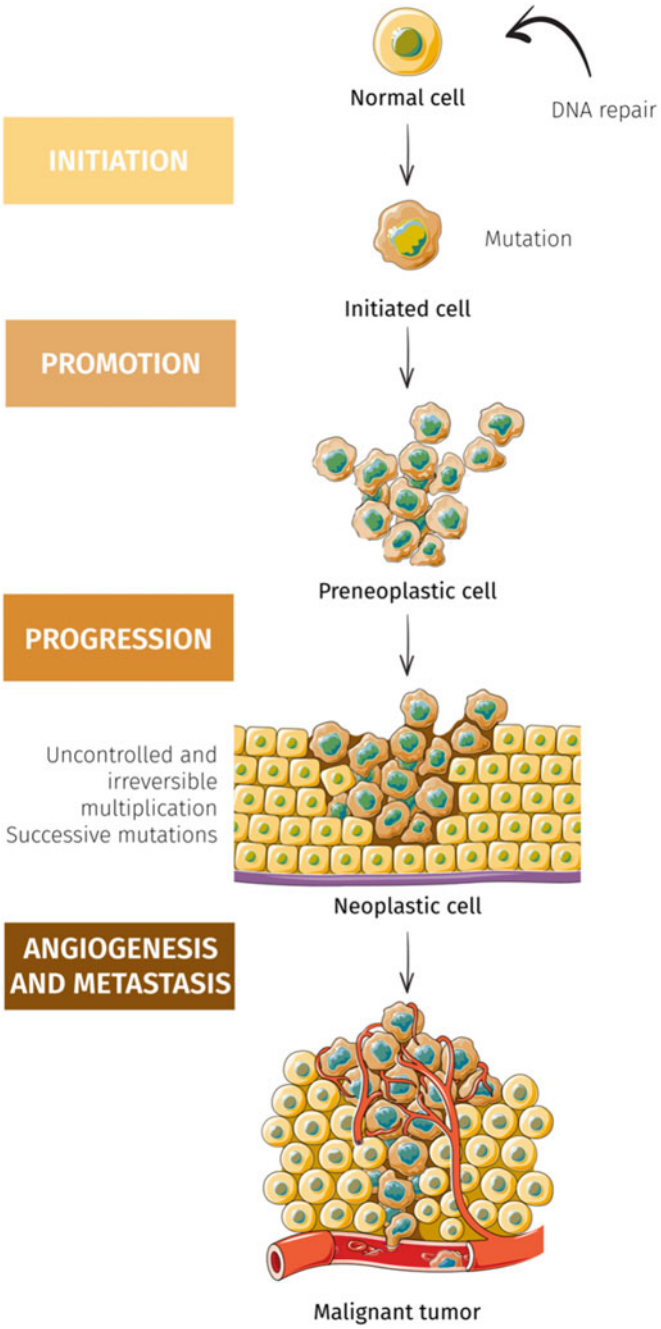

Fig. 1 Carcinogenesis multistep model comprising initiation, promotion, and progression stages

Preferably, for formulations to be efficient in cancer therapy, they should be biofunctionalized including simple features as size and surface charge and multifunctional features as biomolecules combination. Furthermore, they need to fulfill the requirement to:

a. Enhance drug pharmacodynamics and pharmacokinetics profiles.
b. Selectively eradicate tumor cells without affecting healthy cells.
c. Prolong and control the release of the active form of drugs.
d. Improve the cellular uptake of delivered drugs.
e. Diminish dose-limiting toxicities [14, 15].

In all assurance, to reach a successful CT without harming healthy tissues has been the key effort of targeting approaches. It is estimated the enhancement of drug delivery to cancer cells with an improved well-developed nanoproduct [12, 13], however, even with intense research developing in the bionanocomposites field, no significant results with meaningful changes in the life of patients have been accomplished. It is a challenge to bypass drug delivery issues such as early drug degradation, bloodstream circulation time, tissue membranes, and systemic toxicity, therefore, for some cancers, the main promising approach to achieve suitable drug delivery is an in situ platform. In fact, several nanoproducts present an initial burst release that can cause acute toxicity or release the total amount of a therapeutic compound before reaching the tumor cells, fortunately, both of these issues can be addressed by using local drug delivery.

3 Bionanocomposites

It is known that biopolymers possess some limitations in contrast to synthetic polymers in terms of stability, transparency, and mechanical properties nevertheless, these drawbacks can be overcome by combining nanosized materials while producing bionanocomposites. Among all advantages of bionanocomposites, this approach can avoid nanoparticle (NP) agglomeration during nanocomposite preparation by using a polymeric matrix where the NP can be dispersed [16], moreover, nanocomposite biodegradability increases after producing a composite with nanosized materials [17].

By rule, bionanocomposite is a two-phase system, where, at least one part had a nanosized dimension (lower than 100 nm) and the word “bio” indicates the use of biodegradable material [18]. The main feature of bionanocomposites is a large surface area owing to nanosized material resulting in enhanced interaction between its components with the composite matrix (Fig. 2) [19].

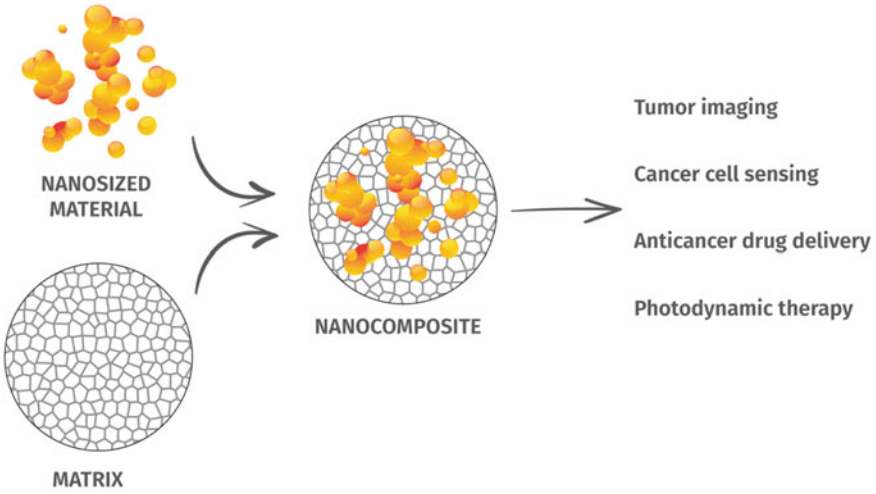

Fig. 2 Features of bionanocomposites

3.1 How to Choose the Right Polymer

In the last decade, the use of synthetic biodegradable polymers as drug delivery systems increased significantly [20], mainly because synthetic polymers made of petrochemicals are toxic and take a long time to degrade. Biodegradable polymers (Table 1) can be synthetic such as polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone (PCL) and poly-l-lactic acid (PLLA), or natural such as collagen, chitosan, and silk [35, 36].

Features of biocomposite materials such as optical and mechanical properties and conductivity usually are improved from bulk material although matrix composition is the same [37]. Polymers as PCL, poly (ethylene glycol) (PEG), PLA, and poly (lactic-co-glycolic acid) (PLGA) that are synthetic and biodegradable are commonly used as substrates for the production of scaffolds. Moreover, during the past years, other polymers with stimuli-responsive behavior such as poly(*N*-isopropylacrylamide) (pNIPAM) and poly(4-vinylpyridine) have been investigated as thermo and pH-responsive [38].

It is also essential, during the development of biocomposites as drug delivery devices, the solvent selection, which needs to offer adequate solubility for both drug and polymer. The compatibility of polymeric mixture components and their physicochemical properties such as surface tension, conductivity, and viscosity have key effects on the resulting morphology of the carrier [39, 40]. The state of both components, their crystallinity, and their distribution within the composite have significant influences on the drug release kinetics. Furthermore, the establishment of both phases can be influenced by the addition of amphiphilic molecules, which can facilitate the release of hydrophobic drugs to the aqueous environment [41, 42].

Table 1 Features of biodegradable polymers

	Polymer	Biodegradation	Mechanical properties	Physicochemical properties	Key-points	References
Synthetic polymers	PLGA	Hydrolysis	Strong	Amphiphilic	Good solubility, weak toxicity, application in several biomedical devices and fast biofilm formation	[21, 22]
	PLA	Hydrolysis	Strong	Hydrophilic	Weak toxicity and application in several biomedical devices	[23, 24]
	PCL	Hydrolysis	Strong	Hydrophobic	Application in long-term implantable devices	[25]
	PVP	Hydrolysis	Strong	Hydrophilic	Good solubility and application as antimicrobial nanofibers	[26, 27]
	PVA	Enzymatic	Strong	Hydrophilic	Good solubility, nontoxic, and non-carcinogenic	[28, 29]
	PEG	Enzymatic	Weak	Hydrophilic	Good solubility, high range of viscosity, contains toxic impurities, and is nephrotoxic if applied to damaged skin	[30]
Natural polymers	Collagen	Enzymatic	Weak	Hydrophilic	Weak toxicity	[31]
	Chitosan	Enzymatic	Weak	Hydrophilic	Molecular weight dependent solubility, weak toxicity, and several applications in pharmaceutical industry	[32, 33]
	Silk	Enzymatic	Strong	Hydrophilic	Moderate toxicity	[34]

PLGA poly (lactic-co-glycolic acid), *PLA* poly (lactic acid), *PCL* poly (Ɛ-caprolactone), *PVP* polyvinylpyrrolidone, *PVA* polyvinyl alcohol, *PEG* polyethylene glycol

3.2 How to Investigate Polymer-Drug Interactions

Polymers present particular characteristics which range from the angstrom level of an individual bond between atoms to nanometers of the polymer chain, micrometers, millimeters, larger in solutions, and polymeric nanofibers. Due to its particular characteristics, the best way to investigate the polymer-drug interactions is through in silico studies [43–47]. The different time scales for each material properties may range from femtoseconds to seconds or even hours. On the literature, there are many examples of the multiscale nature of polymer systems [48–55]. Because of this, several computational methods were developed in order to address these issues [56–64]. These novel methods introduce new possibilities to design, optimize, and predict the structures and properties of polymers. Not only that, these methods allowed the study of these materials with other molecules [43–47]. In the case of this chapter, is the possibility of studying the polymer-drug interactions. One of the main methods to study polymer-drug interactions, nowadays, is the atomistic calculations where all atoms are explicitly represented and treated by a single sphere. The force field, typical interactions in the system, is responsible for the potential energy of the system. These interactions include the bonded interactions that are the bond length, the bond angle, and the dihedral angle potentials between atoms. In addition to the bonded interactions, force fields also contain non-bonded interactions. Non-bonded interactions act between atoms in the same molecule and those in other molecules. Force fields usually divide non-bonded interactions into two: electrostatic interactions and Van der Waals interactions.

Molecular dynamics (MD) simulations are one example used to model molecular processes involving a larger group of atoms, such as drugs, biological compounds [65], carbohydrates [66–69], proteins, membranes [68, 69], nucleic acids, and polymers [43–45]. On the MD simulations studies, usually, to analyze the polymer-drug interactions the root means square deviation (RMSD) [43], solvent accessible surface area (SASA) [43], interaction energy [44, 45], number of contacts [46], and number of hydrogen bonds [46, 47] are the main choices. With these analyses, it is possible to study the polymer behavior in the presence and absence of the drug, verify the drug exposition and interaction to the solvent, verify the drug affinity for the polymer, and calculate the interaction number of contacts between the polymer and drug. The study of polymer-drug interactions requires a comprehensive knowledge of the phenomena. The computational methods, mainly MD simulations allow the study of these systems at the atomistic scale. With this method it is possible to verify some important features on the polymer-drug interaction, solving problems that experimental studies are not able to.

3.3 *Current Achievements in Bionanocomposites for In Situ Drug Delivery*

Recently, several researchers are developing bionanocomposites for in situ drug delivery aiming to address issues caused by systemic CT (Table 2). A significant drawback in cancer therapy is that 90% of patients with cancer die from cancer metastasis [75, 76]. Metastasis is a complex multistep process comprising tumor cells release and their proliferation at a secondary site [77, 78]. Nowadays, nanotechnology has been addressing this issue with controllable and functionalized structures able to recognize circulating tumor cells (CTCs) [79, 80], nevertheless few products are able to induce in situ CT in blood circulation. Chunmiao Liu and colleagues (2019) developed a multifunctional nanocomposite by conjugating FePt NPs onto the surface of Fe_3O_4 @SiO_2 NPs, which, in tumor pH, was able to release Fe^{2+} catalyzing reactive oxygen species (ROS) formation and inducing ferroptosis in tLyP-1 receptor-positive CTCs such as MCF-7, HeLa, HepG2, and 4T1. Thus, this system could effectively separate and capture CTCs suggesting it as a promising approach for in situ cancer treatment [73].

Angiogenesis displays a critical role in tumor proliferation and development in which, vascular endothelial growth factors regulate the development of new blood vessels and act binding tyrosine kinase receptors including vascular endothelial growth factor (VEGF) receptor 1 and 2 [81, 82]. As expected, these receptors are overexpressed in several tumors and have been studied as a target for treatments [83, 84]. Recently, Carenza et al. developed PLGA nanocapsules containing superparamagnetic iron oxide NPs (SPIONS) and VEGF165 in a polymeric shell able to

Table 2 Bionanocomposites for in situ drug delivery

System	Drug	Outcome	References
Silica-calcium phosphate nanocomposite (SCPC75) discs	Cisplatin	Lower tumor growth in the rats treated with SCPC75-cisplatin discs when compared to systemic cisplatin	[70]
CuS nanoparticles-graphene oxide with polyethylene glycol (PEO-GO/CuS)	DOX	75% cancerous cells death after irradiation at NIR laser	[71]
Albumin, PLGA and magnetic nanoparticles with diphenylhexatriane	5-Fluorouracil	Reduction of tumor size	[72]
FePt nanoparticles	–	Ferroptosis induction in tLyP-1 receptor-positive CTCs	[73]
PLGA nanocapsules with SPIONs	VEGF165	Improved accumulation in the tumor cells	[74]

CuS copper sulfide; *DOX* dooxorubicin, *PLGA* poly (lactic-co-glycolic acid), *FePt* Iron–platinum, *CTCs* circulation tumor cells, *SPIONs* superparamagnetic iron oxide nanoparticles

accumulate in the tumor cells [74], showing that magnetic bionanocomposites could be used in targeted and controlled drug delivery.

Multifunctional nanocomposites can be produced as biofunctionalized drug delivery systems that combine the drug transport with biomolecules such as proteins, enzymes, antibodies, nucleic acids, and membrane receptors [85]. Biomolecules are not able to penetrate some body barriers such as the brain-blood barrier (BBB), which creates the necessity of novel drug delivery routes for solid tumors treatment.

3.3.1 Injectable Hydrogels

Photodynamic therapy (PDT) stands for a therapeutic that sensitizes cells to a particular wavelength of light. The radiation and the photosensitizer (PS) are inoffensive when used separately however when combined, they can form several ROS and trigger local inflammation, cell apoptosis, and localized ischemia. Therefore, PDT is very effective as cancer in situ therapy [86]. Recently, Luo and coworkers (2019) established a micellar thermosensitive nanocomposite gel for intra-tumoral two-photon PDT by developing methoxy PEG-polylactide copolymer (mPEG-PDLLA) micelles loaded with PS and two-photon absorption compound (T1). The liquid characteristic of the micelles enabled an in situ gelification after injection maintaining the composite into the breast cancer tumor model and it was shown that the radiation promoted deep tissue penetration and enhanced tumor inhibition in around 50% [87].

Another study aiming PDT developed an injectable nanocomposite hydrogel with chitosan micelles loaded with paclitaxel (PTX) and PEGylated gold nanorods to treat local hepatocellular carcinoma by using xenograft tumor model [88]. After laser irradiation, the photothermal-induced damage was restrained to the tumor without damaging the adjacent healthy tissue and the sustained release of PTX enhanced the antitumor effect of treatment prolonging mice survival compared to PDT alone [88]. These studies indicate that photothermal-CT associated with nanocomposites present improved antitumor effects by suppressing tumor recurrence and prolonging survival.

Glioma is a common central nervous system tumor that presents no significant improvement in therapy in the past years, therefore, the average of patients' survival sits still around 14 months [89]. The challenges in drug delivery for this type of cancer include the existence of the BBB that provides an impediment to standard CT routes of delivery [90]. It was confirmed by Bodell et al. [91] that in situ drug delivery enhances by 113 times the drug concentration when compared to intravenous injection. By exploiting this approach, Ding and colleagues (2017) developed a nanocomposite thermoresponsive hydrogel using mPEG-dipalmitoyl phosphatidylethanoiamine (mPEG-DPPE) calcium phosphate NP and the double emulsion method for PTX and temozolomide (TMZ) delivery. Their results suggest that the autophagy induced by the combination of the drugs regulated tumor cell apoptosis and that the local delivery of the gel on C6 tumor-bearing rats improved significantly the treatment antitumor efficacy [92].

An injectable hydrogel system based on the polypseudorotaxane development between cationic mPEG-b-PCL-b-poly(ethylene imine) (PEI) and α-cyclodextrin (α-CD) was developed for the delivery of plasmid DNA, more specific the Nur77 gene. Given the addition of cyclic α-CD, this biocomposite could form solid hydrogels in situ, owing to the poly pseudorotaxane establishment between α-CDs and PEG. The authors showed that the controlled drug release was able to improve the tumor resistance in vivo [93].

Even that hydrogel studies seem promising for local therapy of cancer, there are some drawbacks to their application. If solid, the samples are usually very stiff and this makes the administration challenging in soft tissues and if liquid, the variable release and distribution could trigger side effects in healthy cells. Thus, different approaches are required for improving biocomposite hydrogels or introducing malleable materials with enhanced release properties such as nanofibrous drug delivery systems.

3.3.2 Electrospinning

The electrospinning method can be used for fibrous bionanocomposites as nanofibers (NF) combined with nanocarriers. The equipment consists of a conductive collector plate, a pump, a high voltage power apparatus, a tip needle, and a syringe containing the polymeric solution [37]. When the solution is loaded in the equipment, the high voltage is applied and the drop in the edge of the needle is transformed into a Taylor cone producing nanofibrous material jetted in the collector plate. By using this method, some features of the nanoproduct can be modulated such as diameter and drug release rate by choosing the adequate properties of the polymeric solution composition as polymeric concentration, viscosity, elasticity, conductivity, and the distance between the collector and the needle [38].

Generally, electrospinning is used for producing NF blends of drugs and polymers, however, it can be used to fabricate fibrous nanocomposites. These systems are constructed by the incorporation of other nanocarriers including nanoparticles (NP), vesicles, and micelles into the NF (Fig. 3). Table 3 shows several recent studies with promising NF for cancer applications.

It is known that the simple incorporation of drugs into nanocarriers commonly leads to burst drug release and to overcome this disadvantage the introduction of these nanocarriers into NF can expand the applicability of nanocarriers as cancer drug delivery, improving their release rate, stability, and acute toxicity. Lately, NF systems aiming at local drug delivery for both tumor therapy and prevention have been significantly studied by researchers worldwide. Table 4 presents an overview of studies focused on the design and evaluation of fibrous bionanocomposite in vivo. Within these studies, the treatment was focused on solid tumors therapy. These NF can increase the specificity of drugs associated with the tumor microenvironment and given their release properties tumor recurrence can be diminished.

One of the most frequent forms of gynecological tumors is cervical cancer, presenting an incidence rate of 25 years in young adults [103]. This type of tumor is

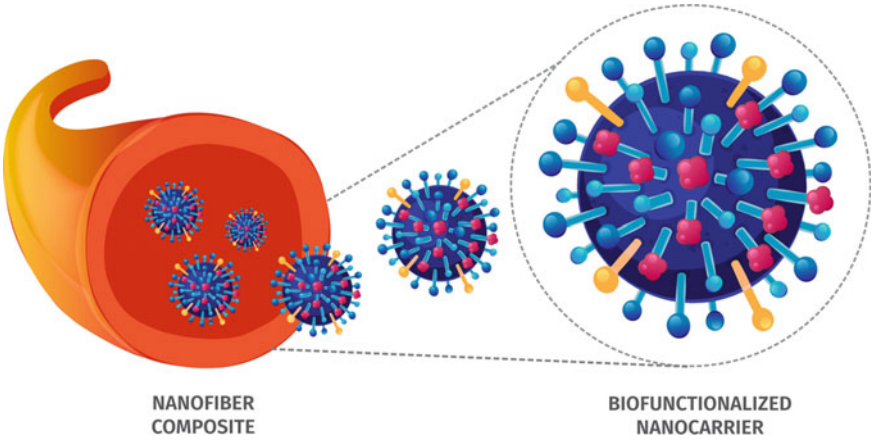

Fig. 3 Fibrous nanocomposites scheme constructed by the incorporation of nanocarriers into nanofibers

developed as a consequence of oncogenic types of human papillomavirus infection [104] and regardless of the mortality and incidence rates are considerably decreasing over the last few years in developed countries, patients with stage IV tumor have a survival rate of only 5 years occasioning in more than 270,000 deaths each year around the world [105]. The cervical cancer therapy depends on the cancer stage varying from surgical resection and RT at stages I to III and CT as a palliative treatment of stage IV (advanced stage or recurrent tumor). Amid the drugs used for the therapy the most common drugs are cisplatin (Pt), ifosfamide, doxorubicin (DOX), and sodium dichloroacetate (DCA) [106]. Albeit platinum compounds present high efficacy against cervical carcinoma, their toxicity on healthy tissues and their systemic toxicity is a drastic therapy problem. To overcome this issue, Zhang and colleagues [107] produced a system by using polyvinyl alcohol (PVA) NF loaded with DCA and Pt(IV)-backboned micelles [107]. Firstly, DCA release induced cell death by apoptosis through the inhibition of pyruvate dehydrogenase kinase and the micelles could highly accumulate in tumor cells via enhanced permeation and retention effect. In tumor pH, Pt(IV) is reduced to Pt(II) that is its active form which can cause DNA crosslinks and consequently inhibition of DNA replication. The nanoproduct presented improved in vitro cytotoxicity against HeLa cells when the two drugs were combined showing their synergistic effect. The in vivo evaluation was performed by using Kumming mice injected with the cervical cancer cell line U14 in the armpit, and the results showed an enhanced drug accumulation on the tumor of mice treated locally with NF when compared to intravenously treated mice. The tumor volume analyses after 16 days of treatment revealed that micelles-DCA-NF reduced tumor volume when compared to single-drug NF and intravenous injection. This study showed the importance of local treatment with synergic drug effects implying that this implantable bionanocomposite could be useful for successful therapy reducing system risks [107].

Table 3 Fibrous bionanocomposites for in situ drug delivery

System	Drug	Cancer	Outcome	References
Core-shell NF Core: iron oxide nanoparticles Shell: PS and collagen NF	–	SKOV-3 ovarian cancer cell line	After the application of an AMF during 10 min to the mats, the cancer cells deposited on the Fe_3O_4-in-fibers were eliminated	[94]
Core-shell NF Core: cobalt ferrite and titanium oxide nanoparticles Shell: DOX-loaded chitosan NF	DOX	B16F10 melanoma cell line	Higher cell death was observed when hyperthermia and chemotherapy were combined, achieving a synergistic effect, which enhances the cytotoxic effect and allows a reduction of side effects	[95]
Core-shell NF Core: vesicles of CTAB and SDBS with 5-Fluorouracil and paenolum Shell: PEO	5-Fluorouracil and paenolum	–	The release investigations concluded that the hydrophilic drug was released in an increased manner when the molar ratio of CTAB/SDBS was higher	[96]
Core-shell NF Core: three different vesicles composed of didodecyldimethylammonium bromide, CTAB/SDBS (7/3) and CTAB/SDBS (3/7) introduced into chitosan and sodium alginate nanocapsules Shell: PEO	5-Fluorouracil	–	The different drug delivery systems showed different release rates and pH-responsive behaviors	[96]
Core-shell NF Core: pluronic (F127) vesicles Shell: PCL NF	Rhodamine-B	L929 murine fibroblast cell line	Reduction in the release rate and increased cell viability	[97]

(continued)

Table 3 (continued)

System	Drug	Cancer	Outcome	References
Electrospun of a mesh using 10% of a hydrophobic PGC-C18 and 90% of PCL loaded with the SN-38	SN-38	HT-29 colorectal cancer cell line	Decreased cell viability	[98]
Core-shell NF Core: colloidal structures formed by a block copolymer composed by mPEG-PLA with 5-Fluorouracil and cefradine Shell: chitosan and PEO	5-Fluorouracil and cefradine	HepG-2human liver cancer cell line	Decreased cell viability	[99]
Core-shell NF Core: silica and hydroxyapatite nanoparticles containing DOX and hydroxycamptothecin Shell: PLGA	DOX and hydroxycamptothecin (topoisomerase inhibitor)	HeLa cell line	Increased thermal stability and inhibited tumor cells growth	[100]
Core-shell NF Core: silica nanoparticles with DOX Shell: PLLA	DOX	HeLa cell line	Sustained and prolonged release and higher antitumor efficacy	[101]
TMZ and polycaprolactone diol combined with gold nanoparticles	TMZ	U-87 MG glioblastoma cell line	The nanoformulation achieves a greater cell death overtime in contrast with free TMZ (25% more)	[102]

NF nanofiber, *PS* polystyrene, *AMF* alternating magnetic field, *DOX* dooxorubicin, *CTAB* cetyl trimethylammonium bromide, *SDBS* sodium dodecylbenenesulfonate, *PEO* polyethylene glycol, *PCL* polycaprolactone, *PGC* poly(glycerol monostearate-co-ε-caprolactone, *mPEG-PLGA* methoxypoly(ethyleneglycol)-block-poly(L-lactide), *PLGA* poly (lactic-co-glycolic acid), *PLLA* poly-l-lactic acid, *TMZ* temozolomide

Another study with cervical cancer focused on CT in combination with hyperthermia by producing PLLA NF loaded with DOX and multi-walled carbon nanotubes (MWCNT) [108]. During the in vitro drug release studies it was found that when near-infrared irradiation was applied, DOX was released in a burst rate in both acid and physiologic pH, however, when the radiation was stopped DOX release returned to slow mode, this behavior was explained by the relation between polymer mobility and the diffusion rate of DOX. By using Kunming mice implanted with U14 cells, this effect was also observed in vivo after 48 h of NF implantation into the tumor and irradiation treatment, moreover, it was detected that DOX accumulated in the tumor tissue more than in other vital organs. Finally, the in vivo antitumor effect of the nanocomposite was assessed by placing the material directly on the

Table 4 Overview of studies focused on the design and evaluation of fibrous bionanocomposite in vivo

System	Drug	Cancer	Outcome	References
Core-shell Core: DCA and Pt(IV)-micelles Shell: PVA NF	DCA, Pt(IV)	Cervical	*In vivo* release showed 58% of Pt released during 30 min, 90% of Pt released during 24 h and the tumor volume on day 15 was found to be half of the untreated control group tumor size	[107]
Core-shell Core: DOX and MWCNTs Shell: PLLA NF	DOX	Cervical	Enhanced DOX release after irradiation and preferable accumulation of DOX in the tumor than in vital organs	[108]
Core-shell Core: PVA micelles loaded with DOX and activefolate groups on the surface cross-linked with gelatin Shell: PCL/PEO	DOX	Breast	Drug concentration increased in the tumor site after 48 h (18 μg/g) when compared to free DOX (2 μg/g) and other organs; NF-treated animals presented a significate increased survival rate	[111]
Thixotropic silk NF hydrogels loaded with DOX	DOX	Breast	The system was capable of solidifying at a specific site and releasing to certain pH conditions. The *in vivo* studies: at the fifth week, showed significant differences in the volume and weight of the tumor treated with DOX-loaded hydrogels compared to free DOX	[112]

DCA sodium dichloroacetate, *Pt(IV)* platinum(IV), *NF* nanofiber, *DOX* dooxorubicin, *MWCNTs* multi-walled carbon nanotubes, *PLLA* poly-l-lactic acid, *PVA* polyvinyl acid, *PEO* polyethylene glycol, *PCL* polycaprolactone

tumor site and after 24 h of implantation the site was irradiated and as expected the MWCNT-DOX NF were effective against cancer cells, nevertheless, cells detected profound inside the tumor presented a certain resistance. Analyzing this study, it was possible to conclude that CT and PDT using MWCNT-DOX-loaded PLLA fibrous composite could have a synergic effect on antitumor activity [108].

A critical type of cancer in relation to mortality worldwide is breast cancer. This type of disease is the leading cause of women's deaths related to cancer, with more than half a million deaths [105] and nearly 2.5 million new cases in 2015 [109]. One important characteristic of this cancer is that the recurrence rate within 10 years can

reach 15% even after resection [110]. The standard therapy consists of mastectomy, RT, systemic CT, or hormonal blockade. However, systemic treatment is associated with severe cardiotoxic effects.

Aiming to diminish DOX side effects and reduce the risk of recurrence, Yang and colleagues [111] produced a complex active-targeting system. Firstly, DOX micelles were developed by using PCL-PEO copolymer with active folate groups on their surface. Then, core-shell NF were produced by coaxial electrospinning where the micelles were mixed with PVA to form the core phase and a cross-linked gelatin solution was used as the shell. After NF implantation, the micelles were released from the NF as a consequence of polymeric matrix degradation, then they were able to penetrate into the tumor cells and undergo endocytosis throughout binding to the folate receptors on the cells' membranes. To perform in vivo biodistribution and antitumor activity, 4T1 tumor-bearing Balbc nude mice were used. The distribution results showed a strong DOX accumulation at the tumor when compared to other organs such as heart, lung, kidney, and liver, suggesting that this bionanocomposite is an effective and safe system for DOX delivery. The antitumor activity evaluation compared two different deliveries: (a) intravenous injection of free DOX or micelles and (b) implantation of NF near the tumor. The NF exhibited a pronounced tumor volume reduction after 21 of implantation when compared to the other groups, in addition, the NF-treated mice presented increased body weights and survival rates, and therefore this system presents significant improvements to the breast cancer therapy [111].

It is known that most frequent actions for in vitro release studies embrace:

(a) cumulative release that occurs when a compound is released into the same amount of media volume and;
(b) non-cumulative release that occurs when there is a continuous replacement of the media mimicking a living organism where the drug concentration drops.

Related approaches are used in in vitro biological analysis focus on cytotoxic experiments on different cell lines. Though experimental conditions of in vitro evaluations simulate those of living organisms, for a system as bionanocomposites to be acknowledged as suitable and safe for local antitumor treatment, in vivo investigations are still essential. Unfortunately, only a few studies tested its bionanocomposites products in vivo, therefore the local application is still a challenge given the invasive procedures and the lack of in vivo evaluations.

4 Final Considerations

Bionanocomposites show great properties related to effective therapy of cancer such as increased thermal stability, enhanced tensile strength, reduced side effects and improved targeting. Drug delivery obtained by in situ approaches has the prospective to overcome the CT limitations by their capacity to increase drug concentration in the tumor and in the same instance decreasing the damage to the healthy tissue. Though

bionanocomposites may enable the efficacy of individualized drug delivery, issues such as the difficulty of testing the scaffolds and to develop surfaces that are able to maintain an appropriate environment may affect the drug distribution. It is necessary, in the future of bionanocomposites research, for a collaboration among researchers, regulatory agencies, and industry to ensure that novel approaches are safe and effective and will be provided to the society. Further development in bionanocomposite technology may prove potent, safe, controlled, and targeted drug delivery.

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3.5. Capítulo 5: Polymeric Nanocomposites for Cancer-Targeted Drug Delivery

Capítulo de livro publicado na revista Polymeric and Natural Composites.

Polymeric Nanocomposites for Cancer-Targeted Drug Delivery

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Abbreviations

α-CD	A-cyclodextrin
ADCs	Antibody-drug conjugates
Ag	Silver
ALK	Anaplastic lymphoma kinase
AuNR	Gold nanorod
BBB	Blood brain barrier
BD	Brownian dynamics
BRAF	V-raf murine sarcoma viral oncogene homolog B1
BRCA	Breast cancer gene
BRD4	Bromodomain containing 4
BTB	Brain tumor barrier
CDK4/6	Cyclin-dependent kinase
CDK7	Cyclin-dependent kinase 7

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CS	Chitosan
CSCs	Cancer stem cells
CT	Chemotherapy
CTS	Chondroitin sulphate
DDFT	Dynamic density functional theory
DOX	Doxorubicin
DPD	Dissipative particle dynamics
EGFR	Epidermal growth factor receptor
FEM	Finite element method
FDM	Finite difference method
FVM	Finite volume method
GO	Graphene-oxide
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
HDIs	Histone deacetylase inhibitors
HER2	Human epidermal growth factor receptor 2
IGF-1	Insulin-like growth factor-1
IONPs	Iron oxide NPs
LB	Lattice Boltzmann
LOI	Loss of imprinting
mTOR	Mammalian target of rapamycin
MET	Hepatocyte growth factor receptor
MD	Molecular dynamics
MC	Monte Carlo
MTX-PEG	Methotrexate-PEG
NIR	Near-infrared radiation
NK	Natural killer
NP	Nanoparticle
NSCLC	Non-small cell lung carcinoma
NTRK	Neurotrophic receptor tyrosine kinase
PAA	Poly (acrylic acid)
PARP	Poly (ADP-ribose) polymerase
PCL	Polycaprolactone
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death protein 1-ligand
PDMS	Poly(N-isopropylacrylamide)-metal NPs
PEG	Poly (ethyleneglycol)
PEI	Poly(ethylene imine)
PGA	Poly (glutamic acid)
PI3K	Phosphatidylinositol 3-kinase
PLGA	Poly (lactic-co-glycolic acid)
PLLA	Poly-l-lactic acid
PMMA	Poly (methyl methacrylate),
pNIPAM	Poly(N-isopropylacrylamide)
PS	Polystyrene

PTT	Photothermal therapy
PTX	Paclitaxel
PU	Polyurethane
PVA	Polyvinyl alcohol
PVDF	Polyvinylidene fluoride
QM	Quantum mechanics
RGD peptide	Arginylglycylaspartic
ROS	Reactive oxygen species
RT	Radiotherapy
SEs	Super-enhancers
SHH	Sonic hedgehog signalling molecule
TDGL	Time-dependent Ginzburg–Landau
VEGF	Vascular endothelial growth factor
WS_2-NT-CM-PEI	Tungsten disulphide nanotubes-ceric ammonium nitrate-PEI

1 Introduction

Cancer represents a multifaceted group of diseases that present unregulated cellular death and growth processes. Cancer is developed as a consequence of DNA mutations that can be triggered by the environment or/and DNA replication errors or can be hereditary [1]. This process is comprised by three stages, initiation, promotion and progression, and it is known as the carcinogenesis multistep model [2].

The conventional cancer management involves several different treatment options depending on different parameters and characteristics such as the tumour type and the grade, nevertheless, surgical resection, systemic chemotherapy (CT), radiotherapy (RT) and immunotherapy are the most common therapies. CT drugs are not specific, thus, they can affect healthy cells developing numerous side effects [3]. As an alternative to the CT conventional treatment, nanotechnological approaches that comprise targeted and controlled drug delivery systems can be used to improve the therapy outcomes including patients' quality of life and survival by enhancing drugs distribution, pharmacokinetics and stability [3–5].

Cancer-targeted drug delivery refers, quantitatively and selectively, to drug accumulation within the tumour site [6, 7]. Preferably, for CT formulations to be effective in cancer therapy, they must be biofunctionalized with suitable size and surface charge and must target specific biomolecules.

In the nanosystems field, polymeric nanocomposites have been shown promising features for cancer-targeted drug delivery [8–12], and thus, this chapter will discuss what is known about the use of nanocomposites for cancer management, what is been produced and tested, how these systems can bypass CT drawbacks and how multiscale molecular simulation for nanostructured polymer systems can guide the development of nanocomposites.

2 Cancer Overview

Cancer is currently recognized as a major disease estimating over 18 million of new cases, and resulting over 9,5 million deaths in 2018 worldwide [13]. The most common cancer types for both sexes and all ages in 2018 are lung, breast and colorectal cancer, which account for over 30 percentage of all cancer cases [13]. Taking into account the gender, the top five most common types of cancer are lung, prostate, colorectal, stomach and liver for men, and breast, colorectal, lung, cervix uteri and thyroid for women in 2018 [13].

Cancer is a collective term for diseases characterized by abnormal and uncontrolled cell growth affecting nearby tissues. Cancer cells are able to spread to other body parts (metastasis) using blood and lymph systems [14]. The mechanism of cancer formation is multifactorial and is initiated by environment as well as genetics, highlighting the combination of external factors and internal genetic modifications, which lead to cancer disease [15]. Several risk factors have been identified as crucial in tumorigenesis. These include lifestyle factors such as smoking, alcohol consumption, diet, obesity, cancer-causing substances and other external factors including age, radiation, infectious organisms, environmental pollution and sunlight [16, 17].

Among all risk factors, smoking is well documented [18, 19]. It has to be emphasized that not only regularly smokers belong to the group of increased risk of cancer, but also second-hand smokers are affected [18]. There are growing evidence that obesity is strongly associated with cancer risk and progression [20–22]. The excess of energy resulting from obesity is responsible for changes in insulin, insulin-like growth factor-1 (IGF-1), leptin and steroid hormones levels, what in turn leads to altering nutritional environment, and is able to support tumour initiation and progression [21]. Alcohol consumption is classified as carcinogenic due to its first product of metabolism: acetaldehyde. Acetaldehyde produces free radicals, promoting cancer development [23]. Additionally, ethanol is associated with reduction of inflammatory mediators increasing tumour formation [24]. Lifestyle factors required to be paid attention, as they are potentially changeable, and people are able to reduce or completely eliminate the impact of these factors on their life.

Next to environmental factors, landscape of cancer is created by genetic alterations as well as epigenetic abnormalities. Balance between tumour suppressor genes and oncogenes allows unrestricted cell growth leading to cancer progression. Activation of oncogenes and/or inactivation of tumour suppressors enhances division or inhibits cell apoptosis, as protein products of these genes regulate different cellular pathways that control cell proliferation, migration and apoptosis [25]. The development of malignant tumour occurs as a result of stepwise accumulation of alterations in both proto-oncogenes and tumour suppressor genes, which both are referred to as critical genes [25]. Genetic alteration includes genomic instability as well as genetic mutations affecting changes within nucleotide sequence. In turn, epigenetic modifications do not alter DNA sequence, wherein they are associated with histone modifications, DNA methylation, loss of imprinting (LOI) and regulation by miRNA [25, 26].

The initiation of the carcinogenesis process occurs as a result of either spontaneous mutations or mutations induced by chemical, physical and biological carcinogens.

Tumour suppressor genes encode molecules responsible for the regulation of many pathways and mechanisms involved in programmed cell death, cell division, differentiation and migration, repair of DNA damage, cell cycle checkpoints as well as inhibition of tumour metastasis [27]. Because the main function of tumour suppressors is to suppress the development of tumour, the appearance of mutations within these genes results in the onset of cancer development. By contrast, proto-oncogenes are normal genes that encode proteins involved in cell growth and cell differentiation, nonetheless, as a result of the mutation, the specific protein involved in the regulation is overexpressed and an oncogene is formed. Among the proto-oncogenes it should be distinguished: genes encoding growth factors, transcription factors, receptor and cytoplasmic tyrosine kinases as well as serine/threonine kinases [28, 29].

The traditional cancer treatment strategies include surgery, RT, CT and immunotherapy. Nowadays, innovative cancer therapies such as targeted therapies are being widely studied [30]. CT targets both, rapidly dividing cancer cells as well as normal cells, and is commonly used in combination with surgery or RT depending on type and stage of cancer [31]. The main limitation of CT is high number of serious side effects [32]. CT has short-term and long-term side effects. Short-time side effects are correlated with high toxicity of used drugs and occur during cancer treatment. In turn, long-term side effects include further complications proceeding after adjuvant CT. The side effects depend on the agents' specificity, dosage and duration of treatment [33]. According to The American Cancer Society, the most common CT side effects are: fatigue, loss of hair, infections, anaemia, vomiting, changes in appetite, obstipation, diarrhoea, pain with swallowing, destroying of nerve system as well as skin, nails, urine and bladder changes, and weight and sexual function changes [34]. Additionally, patients treated with CT may develop resistance to chemotherapeutics, leading to cancer recurrence and progression [35].

More accurate and advanced genetic and molecular characterization of cancer landscape significantly contributed to development of efficient immunotherapies. Immunotherapy supports the recognition of cancer cells as foreign to the host immune system and stimulates the immune system [36]. The main goal of this therapy is to improve antitumour response and decrease side effects commonly occurred during CT treatment. The mechanism of action is based on activation (or activation enhancement) of immune system to attack and destroy cancer cells using natural mechanism, which are often avoided in cancer progression [37]. There are growing number of immuno-drugs approved by US Food and Drug Administration [37], and many of immuno-drugs are currently under clinical trials [38]. Although the immunotherapy seems to be burdened with less side effects than CT, the serious adverse effects such as autoimmunity and non-specific inflammation have been observed [37].

Recently, the changes in genetic profile causing mutations and modifications of oncoproteins as well as tumour suppressor proteins have become promising targets in anticancer treatment [39]. These molecular targets are crucial for designing new drugs, which are able to directly affect the targets. In targeted therapy small molecules are used to enter inside the cell, and attack a specific protein or gene [40], as well as

monoclonal antibodies, which in turn are dedicated for targets placed outside the cells [41], and cancer vaccines as well [39]. The main limitation for molecular targeted therapy is its effectiveness only in cells that express a particular target. Moreover, as in CT, development of drug resistance can be expected [39].

Although the growing number of preclinical and clinical studies show satisfactory and promising results for patients at different types and stages of cancers [42–45] leading to decrease of overall cancer morbidity and mortality, the new strategies for cancer treatment are highly needed to reduce side effects and improve patient prognosis. Molecular biology combined with nanotechnology seems to be the key direction in searching for new cancer strategy assuring low-cost, non-invasive and more personalized oncological care [46].

3 Understanding Targeted Therapy

Targeted therapy is one category of cancer treatment that uses CT drugs as well as other compounds to precisely recognize and assault strictly defined types of cancer cells. Targeted therapy has an anticancer effect through numerous mechanisms, including: induction of apoptosis, inhibition of proliferation, suppression of metastasis, regulation of immune function as well as multidrug resistance reversal [47]. Trastuzumab, a recombinant monoclonal antibody, which can recognize the extracellular domain of human epidermal growth factor receptor 2 (HER2) transmembrane protein, was one of the first target-specific drugs that have been registered for clinical use of cancer. Recently, designing targeted drugs and incorporating them into treatment has become the therapeutic standard [48]. Modern targeted therapies include: monoclonal antibodies [48], antibody-drug conjugates [49], nanobodies [50], antiangiogenic agents [51], signal transduction inhibitors [52], immunotherapeutic agents [53], cancer stem cells targeted drugs [54], miRNAs [55] and complexes of super-enhancers targeted agents [56].

3.1 Antibody–Drug Conjugates (ADCs)

Antibody–drug conjugates (ADCs) are a category of innovative and promising therapeutic strategy for cancer therapy. ADCs are able to target highly expressed antigens on the surface of carcinoma cells and then selectively deliver cytotoxic active agents. The goal of this therapy is to reduce systemic cytotoxicity in comparison with classic CT drugs and optimizing tumour targeting. Researchers aim to improve the effectiveness of ADCs by using a cleavable linker, which allows the delivery of the toxic payload to surrounding cells that do not expressed the target protein. Thus, ADCs act not only on the heterogeneous tumour, but also on its microenvironment consisting of various cell populations [49].

3.2 Nanobodies

The development of targeted therapy significantly expanded the treatment options for oncological patients and sets new directions of research in the field of anti-cancer therapies. Monoclonal antibodies have become a treatment standard in recent years. Despite the popularity of this therapy, traditional monoclonal antibodies have a number of limitations related to their use, such as limited ability to penetrate the tumour, high ability to develop therapeutic resistance and high production costs. Recently discovered nanobodies are an alternative to monoclonal antibodies. By combining the therapeutic advantages of standard antibodies and the targeting potential of nanoscale delivery, nanobodies approach allows high translational potential in preclinical and clinical studies [50].

3.3 Antiangiogenic Agents

The vascular endothelial growth factor (VEGF) pathway is the key mediator of angiogenesis in cancer. Creating new blood vessels on the basis of an existing one is the key process for supplying nutrients and oxygen to proliferating cancer cells, which promotes tumour growth and formation of distant metastases. Therefore, many types of therapies, including tyrosine kinase inhibitors or monoclonal antibodies target this axis [51].

3.4 Immunotherapeutic Agents

Targeting innate checkpoint molecules on macrophages and natural killer (NK) cells has appeared as a new rational approach against tumours, which are resistant to T cell-mediated immunity. Because different monoclonal antibodies against carcinoma surface proteins have been clinically approved in haematological disorders, innate checkpoint blockade can play a pivotal role in augmenting phagocytosis and antibody-mediated cellular cytotoxicity [57]. Targeting the programmed cell death protein 1 (PD-1) and programmed cell death protein 1 - ligand (PD-L1) interactions is a relatively novel cancer therapeutic strategy. Inhibitors of PD-1/PD-L1 include small-molecule chemical compounds, peptides and antibodies [53].

3.5 Cancer Stem Cells (CSCs)-Targeted Therapy

Cancer stem cells (CSCs) are a population of cancer cells, which is responsible for tumour initiation, metastasis and relapse as well as drug and radiation resistance.

Therefore, targeting CSCs is considered a novel potential anticancer-targeted therapeutic strategy. CSCs play a key role in immune evasion, immunomodulation and effector immunity, which changes immune system balance. NOTCH, mammalian target of rapamycin (mTOR), sonic hedgehog signalling molecule (SHH) and Wnt/β-catenin are associated in CSCs targeted therapies due to the fact that they are involved in regulation the CSCs colonies progression and drug resistance. Understanding the signalling pathways regulating progression of CSCs and drug resistance is crucial in conducting effective targeted therapies [54].

3.6 MiRNAs-Targeted Therapy

MicroRNAs are able to regulate activity both oncogenes and tumour suppressor genes. Therefore, alteration in the expression of microRNAs can lead to tumorigenesis. Expression profiling of microRNAs has increased the possibilities of application of microRNAs as potential biomarkers and targeted therapeutic targets in cancers [55].

3.7 Complexes of Super-Enhancers Targeted Therapy

The overexpression and hyper activation of oncogenes commonly occur in many types of cancers. Latterly, the increased activation of oncogenes by super-enhancers (SEs) has attracted significant attention. Numerous studies indicate that the SEs and their associated complexes play an important role in the development of different types of malignant tumours. Clinical trials have demonstrated that small-molecule inhibitors, like bromodomain containing 4 (BRD4) and cyclin-dependent kinase 7 (CDK7) inhibitors are able to target the SEs resulting in considerable positive effect on cancer treatment [56].

3.8 Nanotechnology-Based Histone Deacetylase Inhibitors

Epigenetic reprogramming, including DNA histone modification and DNA methylation, regulates the expression of genes involved in immune checkpoints, cellular proliferation and the response to antineoplastic drugs [58]. Histone acetylation and deacetylation catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs) are the posttranslational epigenetic mechanisms of gene expression regulation. These epigenetic modifications of DNA structure affect the action of transcription factors, which can repress or induce gene transcription. Mutations and changes of the expression of HDAC genes can cause the aberrant transcription of key genes, which regulate many pivotal cancer pathways, such as cell-cycle regulation,

cell proliferation or apoptosis [59]. Histone deacetylase inhibitors (HDIs) have been accomplished therapeutic success in haematological diseases. Unfortunately, their application in solid tumours is hampered by the low treatment efficacy and confronts big challenges. Medicine with the use of nanotechnology could prolong the circulation half-life, improve drug stability and increase intratumoral drug accumulation. Hence, nanomedicine seems to be a promising approach to enhance HDIs therapy efficacy [60].

Targeted drugs used in the most common types of cancer (breast, colorectal, lung, prostate, skin) are summarized in Table 1.

Hence, since targeted medicine or targeted therapy means precise drug efficiency combine with minor side effects and interaction, at the molecular level, between a biomolecule and a drug, multifunctional drug delivery systems such as polymeric nanocomposites can be designed as biofunctionalized carriers that not only transport drugs but also, when combine to biomolecules such as membrane receptors, nucleic acids, antibodies and enzymes, are able to efficiently target cancerous cells.

4 Nanocomposites

Polymers demonstrate several advantages when it comes to develop drug delivery systems due to their ability to maintain a suitable stability and enhance mechanical and physical properties of compounds, however, depending on the polymer chose, if synthetic or natural, it can present some limitations for specific types of tumours and therefore, restrict its application. Combinations and composites of polymers can bypass these drawbacks and improve the quality of these systems, moreover, by combining nanosized materials while producing nanocomposites the drug delivery system can have multifaceted uses and purposes and it might be able to reach challenging areas such as the brain.

Amid the benefits of a nanocomposite system, this approach can avoid nanoparticle (NP) agglomeration by using a polymeric matrix where the NP can be dispersed [67], besides, the nanocomposite biodegradability increases after producing a composite with nanosized systems [68]. By rule, a nanocomposite is a two-phase system, where, at least one constituent must present a nanosized dimension up to 100 nm [69]. An important characteristic of nanocomposites is a large surface area, which results in higher interaction between its nanocomponents with the polymeric matrix [70].

Likewise, nanocomposite drug delivery systems theoretically are able to achieve requirements to deliver an effective cancer treatment owing to the following features:

a. Nanocomposites enhance drug pharmacodynamics and pharmacokinetics profiles.
b. Nanocomposites can selectively eradicate tumour cells without affecting healthy cells.
c. Nanocomposites prolong and control the release of drugs.

Table 1 Targeted drugs used in the most common types of cancer based on https://www.cancer.org

Type of the cancer	Subtype of the cancer	Type of targeted therapy	Drug	Reference
Breast cancer	Targeted therapy for HER2-positive breast cancer	Monoclonal antibodies	Trastuzumab–pertuzumab Hyaluronidase	[61]
		Antibody–drug conjugates	Ado-trastuzumab emtansine Fam-trastuzumab deruxtecan	
		Kinase inhibitors	Lapatinib, Neratinib Tucatinib	
	Targeted therapy for hormone receptor-positive breast cancer	CDK4/6 inhibitors	Palbociclib Ribociclib Abemaciclib	
		mTOR inhibitor	Everolimus	
		PI3K inhibitor	Alpelisib	
	Targeted therapy for women with *BRCA* mutations	PARP inhibitors	Olaparib Talazoparib	
	Targeted therapy for triple-negative breast cancer	Antibody–drug conjugate	Sacituzumab govitecan	
Colorectal cancer	Targeted therapy for colorectal cancer	Drugs that target blood vessel formation (VEGF)	Bevacizumab Ramucirumab Ziv-aflibercept	[62]
		Drugs that target cells with *EGFR* mutations	Cetuximab Panitumumab	
		Kinase inhibitor	Regorafenib	
Lung cancer	Targeted drug therapy for non-small cell lung cancer	Angiogenesis inhibitors	Bevacizumab Ramucirumab	[63]
		EGFR inhibitors used in NSCLC with *EGFR* mutations	Erlotinib Afatinib Gefitinib Osimertinib Dacomitinib	
		EGFR inhibitors that target cells with the *T790M* mutation	Osimertinib	
		EGFR inhibitors used for squamous cell NSCLC	Necitumumab	

(continued)

Table 1 (continued)

Type of the cancer	Subtype of the cancer	Type of targeted therapy	Drug	Reference
		Drugs that target cells with *ALK* mutations	Crizotinib Ceritinib Alectinib Brigatinib Lorlatinib	
		Drugs that target cells with *BRAF* changes	Dabrafenib Trametinib	
		RET inhibitors	Selpercatinib	
		MET inhibitors	Capmatinib	
		Drugs that target cells with *NTRK* mutations	Larotrectinib Entrectinib	
Prostate cancer	Targeted therapy for prostate cancer	PARP inhibitors	Rucaparib Olaparib	[64]
Skin cancer	Targeted therapy for basal and squamous cell skin cancers	Hedgehog pathway inhibitors	Vismodegib Sonidegib	[65]
		EGFR inhibitors	Cetuximab	
	Targeted therapy drugs for melanoma skin cancer	BRAF inhibitors	Vemurafenib Dabrafenib Encorafenib	[66]
		MEK inhibitors	Trametinib Cobimetinib Binimetinib	
		Drugs that target cells with *C-KIT* changes	Imatinib Nilotinib	

Abbreviations: ALK - anaplastic lymphoma kinase, BRAF—v-raf murine sarcoma viral oncogene homolog B1, BRCA—breast cancer gene, CDK4/6—cyclin-dependent kinase, EGFR—epidermal growth factor receptor, HER-2—human epidermal growth factor receptor 2, MET—hepatocyte growth factor receptor, mTOR—mammalian target of rapamycin, NSCLC—non-small-cell lung carcinoma, NTRK—neurotrophic receptor tyrosine kinase, PARP—poly (ADP-ribose) polymerase, PI3K—phosphatidylinositol 3-kinase, VEGF—vascular endothelial growth factor

d. Nanocomposites improve the cellular uptake of delivered drugs by a targeted approach.
e. Nanocomposites can diminish drugs dose decreasing its side effects [8, 11, 12, 71, 72].

5 Polymeric Nanocomposites

Recent advances in the biological, chemical and physical fields combined with the challenges and possibilities in nanomedicine have led to new developments in polymer-based nanocomposites for diverse biological applications.

Polymeric nanocomposites are very attractive structures with a dual assembly: one phase is called reinforcing (strong and low-density materials) and is embedded in the matrix phase (tough or ductile materials) [73] and consists of nanomaterials and polymers (synthetic or natural) that form a multiphase solid material [73, 74]. These complex materials generate an adjustable platform with different properties and functionalities, improving the overall features of the component materials used for their synthesis.

Polymeric nanocomposites present several advantages including the retaining, protecting and releasing of biological compounds such as drugs, genes, enzymes and fluorophores for treatment, imaging and diagnostics [75]. Nanocomposites also present advantages as enhanced chemical, electrical, thermal, magnetic, optical, catalytic and mechanical properties [76]. Therefore, polymeric nanocomposites promote enhanced solubility in aqueous medium, high stability in biological systems and increased biocompatibility [74, 75, 77]. Consequently, this multifaceted matrix has shown great potential in drug and gene delivery as suitable drug carriers due to improved features compared to pure NP and polymers.

NP addition into polymeric matrix changes the characteristics of polymers as drug carriers such as: decreases the burst release leading to slower and sustained release, improves drug stability, allows the encapsulation with two or more compounds and facilitates active targeting by functionalization with specific receptors [78]. The drug delivery behavior by polymeric nanocomposites has been evaluated in several studies due to unique features. Both organic and inorganic particles are silica, gold, carbon nanotubes, quantum dots, graphene, liposomes, dendrimers and with diverse forms are core-shell, tubes, sheets, spherical, cylindrical, bring great potential for polymeric nanocomposites on the biomedical field [79–81]. The NP have many advantages to the drug delivery system because of their adjustable particle size, charges and surface [82].

5.1 Types of Polymers Used for Nanocomposites Synthesis

Several polymers can be applied for biological purposes such as natural polymers including polysaccharides or proteins and synthetic polymers. The most common polymers used for nanocomposites synthesis are listed in Table 2.

The natural polymers present advantages as biological recognition, remarkable interactions with cells to promote proliferation, adhesion, non-immune response, and biodegradability [97]. However, they demonstrate poor mechanical strength, high speed of degradation and limited supply [98].

Table 2 Types of polymers (natural and synthetic) used for nanocomposites synthesis

Polymers (synthetic and natural)	Biomedical applications/ characteristics	Source	Reference
Polycaprolactone (PCL)	Drug carrier; implantable material	Synthetic	[83]
Poly (methyl methacrylate) (PMMA)	Drug carrier (high drug permeability); biocompatible	Synthetic	[84]
Poly ($_{L}$-lactic acid) (PLLA)	Drug carrier; scaffolds for tissue regeneration	Synthetic	[85]
Poly (lactic-co-glycolic) acid (PLGA)	Biocompatible; tailorable degradation rate; ease modifying the surface	Synthetic	[86]
Poly (ethylene glycol) (PEG)	Biocompatible; soluble in water; drug carrier	Synthetic	[87]
Polystyrene (PS)	Biocompatible; drug delivery	Synthetic	[88]
Polyvinylidene fluoride (PVDF)	Thermal stability; stimulus-responsive; tissue regeneration	Synthetic	[89]
Polyvinylalcohol (PVA)	Easy degradable; biocompatible decompose necrotic masses	Synthetic	[90]
Poly (glutamic acid) (PGA)	Biodegradable, biocompatible; water-soluble	Synthetic	[91]
Poly (acrylic acid) (PAA)	Biocide properties; biocompatible	Synthetic	[92]
Polyethyleneimine (PEI)	Drug delivery; attachment promoter	Synthetic	[93]
Alginate	Drug delivery; cell transplantation; biocompatible	Natural	[94]
Collagen	Cell attachment ability; biodegradation	Natural	[95]
Chitosan	Antibacterial activity; hydrophilicity; bone regeneration	Natural	[96]
Cellulose	Hydrophilicity; biofunctionality; biocompatible	Natural	[94]
Hyaluronic acid	Swelling capability; non-immunogenic	Natural	[95]
Starch	Biodegradable; biocompatible	Natural	[94]
Gellan gum	Bioadhesive; biocompatible	Natural	[94]

(continued)

Table 2 (continued)

Polymers (synthetic and natural)	Biomedical applications/ characteristics	Source	Reference
Chondroitin sulphate (CTS)	Biodegradable; water adsorbent	Natural	[95]

Abbreviations: CTS—Chondroitin sulphate,PAA—Poly (acrylic acid), PCL—Polycaprolactone, PEG—Poly (ethylene glycol), PGA—Poly (glutamic acid), PLGA—Poly (lactic-co-glycolic) acid, PLLA—Poly (L-lactic acid), PMMA—Poly (methyl methacrylate), PS—Polystyrene, PVA—Polyvinylalcohol, PVDF—Polyvinylidene Fluoride

In contrast, synthetic polymers present good mechanical properties, controllable degradability, adequate supply, and they are cheaper. However, some synthetic polymers can show uncontrollable shrinkage and possible local toxicity [99].

Nanocomposites are very interesting structures to overcome these disadvantages and expand the potential of polymers by the connection between them and NP, improving the overall characteristics of materials.

5.2 Polymeric Nanocomposites and the Advantages for Cancer-Targeted Therapy

Material science has been an important tool bringing innovations to the treatment, diagnosis, imaging, contrast agent, photothermal ablation agents and magnetic resonance imaging (MRI) in cancer through the materials composition [100]. Due to their complex structure with NP and specific matrix carriers, polymeric nanocomposites improve a set of factors increasing the drug effectiveness in the biological system [101, 102]. CT drugs can affect the healthy tissues and not just the tumor; therefore, these nanomaterials have shown great potential for target-specificity drugs reducing the side effects and increasing the treatment effectiveness [103, 104].

Polymeric nanocomposites achieve many advantages on their use for cancer treatment, such as:

a. Increases the lifetime of chemotherapeutics;
b. Improves the solubility of hydrophobic drugs;
c. Allows the controlled and sustained drug delivery;
d. Enhances the bioavailability due to accumulation of nanosystems in the tumor tissues;
e. Protects the drugs against degradation mechanism of the body;
f. Avoids immunological recognition by surface functionalization;
g. Permits site-specific active targeting through the use of ligands as antibodies, peptides, growth factors;
h. Avoids multiple-drug resistance due to passive targeting;
i. Allows multimodal system acting, concerning different therapeutic approaches (such as hyperthermia) and diagnosis (such as bioimaging).

These advantages make polymeric nanocomposites a potential system for improved treatment if compared to traditional therapies [93, 105–107]. Different types of nanosystems-based nanocomposites with their formulation techniques are summarized in Table 3.

Polymeric nanocomposites have wide applications in controlled, sustained and targeted drug delivery. As shown in the table above, these systems present a set of advantages for cancer therapy as pH-dependant behavior, infrared-light sensitivity, multitargeting and specific targeting as well. The multifunctional features of nanocomposites bring new alternatives in many fields, such as molecular medicine, mostly cancer diagnostics, therapeutics, theranostics and imaging.

5.3 Localized Treatment of Solid Tumors with Polymeric Nanocomposite Systems

Worldwide, large efforts have been made in order to develop nanocomposites systems for localized treatment of tumors, owing to the systemic side effects associated with CT-based approaches. Another important fact is the major percentage of patients with cancer suffer from metastasis [131, 132]. Moreover, some drugs, biomolecules or nanocarriers cannot penetrate the body barriers including membranes, the brain blood barrier (BBB) and the brain tumor barrier (BTB), which suggests the necessity of targeted drug delivery systems for solid tumors therapy.

5.3.1 Injectable Hydrogels

The use of composite nanosystems also could be beneficial for the treatment of solid tumors prior or after surgical procedures guaranteeing suitable drug concentrations in the tumor site and affected regions. Localized treatment can be achieved mainly by using two platforms: intratumoral injection or direct implantation into the tumor site. Even though surgical resection is the standard procedure to treat solid tumors, the complete resection is often impossible and the tumor recurrence incidence is a still a challenge [133]. Thus, with the benefits that the mechanical properties of polymers provide, polymeric hydrogels can be injected into tumors greatly improving the stability of common used drugs.

Recently, Cacicedo et al. produced a nanocomposite by combining cellulose hydrogel with DOX-loaded lipid nanocarriers. It was observed that its intratumor administration in vivo in an orthotropic breast cancer mouse model significantly reduced tumor size, metastasis incidence and side effects associated with DOX application [134], suggesting that is possible to achieve better responses with lower doses of CT drugs. Another possibility for injectable polymeric nanocomposites is the delivery of photothermal therapeutic agents which provide an in situ thermal effect and the drug release can be controlled by local light-radiation heating. Hence, an

Table 3 Polymeric nanocomposites and their formulation techniques, which have conferred targeting ability and efficacy for anticancer therapeutics

Polymeric nanocomposites	Fabrication technique	Therapy and experimental model used and outcome	Reference
PMMA-Si–Gd NPs functionalized with folic acid	Self-assembly	DOX; MCF-7 (breast cancer cell line); pH-responsive; prevent drug leakage, targeting effect in breast cancer cells	[108]
Tungsten disulphide nanotubes-ceric ammonium nitrate-PEI (WS_2-NT-CM-PEI)	Focused ultrasound in an emulsion solvent diffusion	PTT; MCF-7 and HeLa (cervical cancer cell line) cells; improved PTT activity in the functionalized group	[109]
Poly(N-isopropylacrylamide)-metal nanoparticles (PDMS)	Soft lithography	MDA-MB-231 (Breast cancer cell line); device multimodal implantable	[9]
Gold nanorod-attached PEGylated graphene-oxide (AuNR-PEG-GO)	Self-assembly	PTT; A431(epidermoid carcinoma cell line) and xenograft mice were used for testing; improved PTT activity	[110]
PLGA polymeric vesicles-Quantum dots	Emulsion evaporation	Busulfan; J774A (macrophage) cells and rats were used for testing; enhanced drug delivery and improved imaging	[111]
PLGA-PEG-nanoparticles	Double emulsion method	Curcumin; MCF-7 cells; enhanced cytotoxic effects	[112]
PEG-phospholipids-graphene oxide	Hydration method	Resveratrol; Mice; chemo-PTT; eradicated xenografted tumor	[113]
PEG-PCL micelles functionalized with cyclic RGD peptide	Core-crosslinked; Solvent exchange	DOX; U87-MG (glioblastoma) cells; specific target	[114]
PLGA-Silver (Ag) nanofibres	Electrospinning	Hep-G2 (Liver carcinoma cell line); enhanced antitumor activity	[115]
PVA-graphene oxide-organoclay (PVA-ODA-MMT)	Electrospinning;	Osteocarcinoma cells; enhanced antitumor activity	[116]
PCL-polyurethane-Au nanoparticles (PCL-Diol-PU/Au)	Sol–gel	Temozolomide; U87-MG cells; lower burst release; gold coating enhanced the cytotoxicity	[117]

(continued)

Table 3 (continued)

Polymeric nanocomposites	Fabrication technique	Therapy and experimental model used and outcome	Reference
Polyethylene glycolated methotrexate-PEG-chitosan-iron oxide nanoparticles (MTX-PEG-CS-IONPs)	Electrospinning	Prodrug: MTX-PEG; Cy5.5; HeLa cells and xenograft mice; improved anticancer activity	[118]
Organic frameworks-cyanine-Cis-aconityl-DOX (COF-IR783-CAD)	Non-solvent-aided coacervation followed by a chemical crosslinking	DOX; 4T1 (breast cancer cell line) and xenograft mice; chemo-PTT; significant tumor ablation	[119]
Sodium alginate-Poly dopamine-Polyvinylpyrrolidone (ALG/PDA-PVP)	Reversible condensation	DOX; HT29 (human colon cancer cell line); enhanced therapeutic effect	[120]
Polypyrrole/chitosan shell Ag (AgCl/PPC)	One-step electrostatic spraying	3-amino-2-phenyl-4(3H)-quinazolinone; Ehrlich ascites carcinoma cells (EAC); increased cell death in group treated compared to empty formulation and pH-dependant release	[121]
Iron oxide-polyethylene-glycol NPs (Fe3O4-PEG-NPS)	Magnetic stirring and dialysis	DOX or paclitaxel; A2780 and OVCAR-3 (human epithelial ovarian cancer cell lines); apoptosis activation through NF-kB and BAX overexpression and Bcl-2/survivin inhibition; DOX-loaded was more efficient than paclitaxel-loaded in vivo	[122]
PMAA-grafted Chitosan NPs with Fe3O4 (DOX@Fe3O4@CS/MAA)	Coprecipitation	DOX; MDA-MB-231 and MCF-7 (breast cancer cell lines); cell viability reduction and DNA fragmentation; pH-dependant release	[123]
Gold nanorods dopamine and PEG-coated (AuNR-PDA)	Graft-polymerization by crosslinking	DOX or Methylene blue; HeLa cells and xenograft mice; ROS generation under laser irradiation and apoptosis induction; PTT combined therapy led to improved tumor reduction	[124]

(continued)

Table 3 (continued)

Polymeric nanocomposites	Fabrication technique	Therapy and experimental model used and outcome	Reference
N-naphthyl-O-dimethymaleoyl chitosan micelles with nanocrystals of iron(III) and manganese(II) (NCS-DMNPs)	Pegylation and self-polymerization	DOX; NIH3T6.7 (fibrobasts) cells; bioimaging (diagnosis) and drug delivery	[125]
Fe3O4 PLGA-PVP-coated nanoparticles anchored with Herceptin and tamoxifen	Reductive amination and dialysis	Tamoxifen; MCF-7 and HeLa cells; hemocompatible and pH-dependant release; apoptosis induction and selective uptake by MCF-7 (HER2 +); PTT combined therapy with tumor reduction	[126]
N-isopropylacrylamide (NIPAm) and methacrylated β-cyclodextrin-based macromer (MβCD) with incorporated Au-NRs and AD-DOX (NIPAm- MβCD-AuNRs-AD-DOX)	Precipitation and dialysis	DOX and PTT; MCF-7, HeLa cells and S180 (murine sarcoma) cells; slow 1-month release from implant; PTT combined therapy with drug release rate increase; biocompatible and pH-dependant system	[127]
Pluronic F127 system with iron oxide (PF127-Fe3O4-DOX)	Self-crosslinking copolymerization	DOX; BE–2–M17 (neuroblastoma cell line) cells; pH-dependant behavior; effective in vitro	[128]
Lanthanide upconverted NPs co-doped with YB + 3 and TM + 3, mesoporous silica coated with a folate-conjugate and copolymerized MAPEG (MUCNPs@C18@PSMN-FA)	Emulsion/solvent evaporation technique	DOX; Human KB cell lines with folic acid receptor, A549 (human alveolar adenocarcinoma cell line) without folic acid receptor and Beas2B (Human bronchial epithelial cells); NIR mediating release and folate-receptor targeting; bioimaging	[129]
Dopamine loaded poly(N-isopropylacrylamide)-propylacrylamide copolymer (pNIPAm-co-pAm/DP)	Free radical polymerization and self-assembly	PTT and Bortezomib or DOX; MC3T3-E1 (osteoblast precursor cell line) and CT26 (colon cancer cell line) cells; PTT combined therapy with apoptosis and irreversible changes in cell morphology; pH-dependant and NIR-dependant release	[130]

Abbreviations:Ag—Silver, AuNR—Gold nanorod, CS—chitosan, DOX—doxorubicin, DP—dopamine, GO—graphene oxide, UONPs—iron oxide nanoparticles, MAPEG—Poly (ethylene glycol) methacrylate, MTX-PEG—methotrexate-PEG, PCL—Polycaprolactone, pAM: propylacrylamide, pNIPAm—poly(N-isopropylacrylamide), PDNH—poly(DBAM-co-NASco–HEMA),PDMS—Poly(N-isopropylacrylamide), PEG—Poly (ethylene glycol), PLGA—Poly (lactic-co-glycolic) acid, PMAA—Poly(methacrylic acid), PMMA—Poly (methyl methacrylate), PTT—Photothermal therapy,PU—polyurethane, PVA—Polyvinylalcohol, RGD peptide—Arginylglycylaspartic acid, WS2-NT-CM-PEI—Tungsten disulphide nanotubes-ceric ammonium nitrate-PEI, NIPAM—N-isopropylacrylamide, NIR—Near-infrared irradiation, PTT—Photothermal therapy and ROS—reactive oxygen species

ideal system must be multifaceted and biocompatible at the same time, and it should control the release of sufficient CT drugs and other agents such as photothermal agents for extended periods and preferentially target only cancerous cells. In view of this, Liu et al. designed and produced a very elegant injectable nanocomposite hydrogel by using methoxy poly(ethylene glycol)-b-poly(ε-caprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone) (mPECT) diblock copolymer with gold nanorods (AuNR-PECT) combined with paclitaxel-loaded mPECT NP (PTX/mPECT NP) and α-cyclodextrin (α-CD) for local chemo-photothermal synergetic cancer therapy by applying near-infrared radiation (NIR). The authors tested this system in breast cancer models (in vitro and in vivo) and observed that it was capable of delivering a synergetic treatment and inhibiting tumor growth and recurrence [133]. What makes this system incredibly promising is the fact that it can be applied for different combined and multidrug approaches for targeting different types of tumors by changing the target ligands.

In this context, Xu et al. synthesized a core-shell nanocomposite with PEGylated (polyethylene glycol modified) magnetic Prussian blue (PB) NP for the controlled release of DOX, targeted photothermal ablation and pH-triggered CT of tumor cells. The authors observed a significant growth inhibition in vitro in HeLa cells [135]. However, more experiments are necessary to prove these effects. Similarly, Fan et al. produced cyclo (Arg-Gly-Asp-d-Phe-Cys) [c(RGD)] conjugated DOX-loaded PEG Fe3O4@polydopamine (PDA) NP to achieve an integrated tumor diagnosis and treatment [136]. It was shown that this system was able to target tumor cells and it presented a suitable stability, moreover, by using xenograft tumor nude mouse injected with HCT-116 cells it was possible to detect clear contrast signals therefore, this nanocomposite demonstrated effective CT-photothermal therapy under NIR irradiation.

Magnetic NP have been considerably studied to treat cancer cells and more recently, they attracted attention to be used as a multimodal therapy owing to the possibility to deliver drugs and heat locally [137–144]. From this perspective, Hervault et al. developed magnetic nanocomposites by combining a thermo and pH responsive polymeric shell (PEG methyl ether methacrylate—PEGMA, di(ethylene glycol) methyl ether methacrylate—DEGMA, 3-(trimethoxysilyl)propyl methacrylate—TMSPMA and 3-vinylbenzaldehyde) with an iron oxide core [145] for the delivery of DOX. This system demonstrated suitable physical and chemical properties and showed potential for in vitro and in vivo testing. Taken together, these studies provide a in situ strategy for the clinical application of nanocomposites in cancer CT-phototherapy.

6 Future Challenges in Cancer Therapy

One of the biggest challenges related to the applicability of polymeric nanocomposites is the translation of an in vitro study to an in vivo study. It is known that most frequent actions for in vitro release studies embrace: cumulative release that

occurs when a compound is released into the same amount of media volume and non-cumulative release that takes place when there is a continuous replacement of the media mimicking a living organism where the drug concentration drops.

These methods are commonly used in in vitro biological experiments where cytotoxic analysis is performed by using cancer cell lines. Although experimental conditions of in vitro evaluations mimic those of living organisms, for complex drug delivery system as nanocomposites to be recognized as appropriate and safe for antitumor treatment, in vivo studies are still indispensable. Unfortunately, only a few studies tested nanocomposites systems in vivo, therefore their applicability is still uncertain.

In all assurance, to reach a successful therapy without harming healthy tissues has been the key effort of targeting approaches. It is estimated the enhancement of drug delivery to cancer cells with an improved well-developed nanoproduct [6, 7], however, even with intense research developing in the nanocomposites field, no significant clinical results have been described. It is a challenge to bypass drug delivery issues such as early drug degradation, bloodstream circulation time, tissue membranes and systemic toxicity, therefore, for some cancers, the main promising approach to achieve suitable drug delivery is a biofunctionalized nanocarrier. In fact, several nanocarriers have an initial burst release that can cause acute toxicity or release the total amount of a therapeutic compound before reaching the tumor cells, fortunately, both of these issues can be addressed by using targeted drug delivery with polymeric nanocomposites.

7 Multiscale Molecular Simulation for Nanostructured Polymer Systems

Nanostructured polymer systems present particular features which range from the angstrom level of an individual bond between atoms, to nanometres of the polymer chain, micrometres, millimetres, larger in solutions and polymeric nanostructured (Fig. 1).

The different time scales for each material properties may range from femtoseconds to seconds or even hours. On the literature, there are many examples of multiscale nature of nanostructured polymer systems [147–155]. Because of that, several computational methods were developed in order to address these issues [156–164]. These new methods present novel options to design, optimize and predict polymeric structures and properties.

Until the present moment, no computational method is able to cover different size scales of polymers [165] systems. Therefore, the multiscale simulation framework is considered one of the best choices to deal with this issue. The multiscale approach combines various methods and, in the computational chemistry field, it is considered one of the key topics. In order to perform a multiscale simulation, different theories

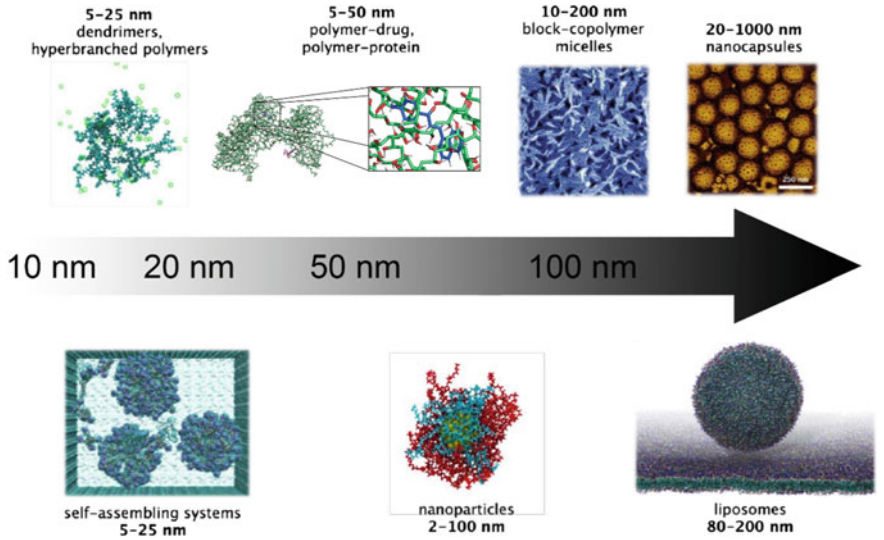

Fig. 1 Nanostructured polymer systems of potential interest for biomedical applications. Increasing structural complexity the dimensions of polymeric systems change from few nanometres to hundreds of nanometres Adapted from Laurini et al. [146]

and models from four characteristics length and time scales are combined. These features can be divided into the following scales:

I. The quantum scale: In this method, the nuclei and electrons are part of the calculation, and quantum mechanics (QM) calculations are used to model their state. This approach allows to investigate several phenomena related to chemical reaction, such as rupture and formation of chemical bonds between atoms, the transitions in electrons configurations and other important phenomena on polymers material, that need to be modelled at quantum scale.

II. The atomistic scale: In the atomistic calculations, all atoms are explicitly represented and treated by a single spheres. The force field, typical interactions in the system, is responsible for the potential energy of the system. These interactions include the bonded interactions that are the bond length, the bond angle and the dihedral angle potentials between atoms. In addition to the bonded interactions, force fields also comprise non-bonded interactions. Non-bonded interactions act between atoms in the same molecule and those in other molecules. Force fields typically distribute non-bonded interactions into two: electrostatic interactions and van der Waals interactions. Molecular dynamics (MD) and Monte Carlo (MC) simulations are examples used at this level to model molecular processes comprising a larger group of atoms, such as proteins, membranes and nucleic acids.

III. The mesoscopic scale: At this scale, a molecule is defined as a microscopic particle, identified as a bead. In this method, some details of the system are

presented indirectly which offers the opportunity to simulate the phenomena on time scales barely accessible by atomistic simulations. An interesting example is coarse-grained model, where the particles are accumulated in beads. During the calculation, interactions between the beads are used to characterize the system. There are a several methods that were developed to investigate the mesoscopic structures of polymers, including: Brownian dynamics (BD), lattice Boltzmann (LB), dissipative particle dynamics (DPD), dynamic density functional theory (DDFT) and time-dependent Ginzburg–Landau (TDGL).

IV. The macroscale: In this methodology, the characteristics of atoms and molecules are disregarded, and the system is considered as a continuous. The constitutive laws are responsible for the behaviour of the system. These laws are associated with conservation laws to simulate several phenomena. The main functions, such as velocity and stress components, are continuous. On the other hand, a finite amount of locations which separate continuity regions is considered discontinuous on these calculations. The central supposition at this scale is in substituting a heterogeneous material with a corresponding homogeneous model. To perform molecular simulations at this scale, there are used the following methods: finite volume method (FVM), finite element method (FEM) and finite difference method (FDM).

The development of nanostructured polymer systems requires a comprehensive knowledge of the phenomena at different time and length scales. Because of that, the theoretical and computational methods present great progress, allowing the study of these systems. Finally, given the peculiarities of the polymeric systems, no single method can be used for their simulation. Consequently, it is advantageous to rely on a multiscale molecular modelling approach that has been presented in this chapter. The methodology discussed is an overall design approach for complex nanostructured systems to be effectively interpreted during the experiments and for the design of active nanocomposites and nanosystems.

Final Considerations

Polymeric nanocomposites have been shown suitable mechanical and physicochemical properties to be use as drug delivery systems for cancer therapy. These properties including increased thermal stability, enhanced tensile strength and decreased side effects can be improved by using ligands to target cancer cells. Cancer-drug delivery obtained by targeted approaches has the potential to overcome CT limitations by increasing drug concentrations in the tumor cells and at the same time diminishing the side effects to the normal cells.

Although polymeric nanocomposites are able to provide an efficient drug delivery, it has been observed a lack of in vivo testing, which makes difficult to verify their efficacy. Thus, it is essential, for polymeric nanocomposites research, a collaboration among researchers, regulatory agencies and industry to ensure that these innovative approaches are effective, non-toxic and can be used in the treatment of patients. Further development in nanocomposite technology may prove potent, safe and efficient cancer-targeted drug delivery approaches.

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3.6. Capítulo 6: Nek1-inhibitor and temozolomide-loaded microfibers as a co-therapy strategy for glioblastoma treatment

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Nek1-inhibitor and temozolomide-loaded microfibers as a co-therapy strategy for glioblastoma treatment

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ABSTRACT

Malignant glioblastoma (GB) is the predominant primary brain tumour in adults, but despite the efforts towards novel therapies, the median survival of GB patients has not significantly improved in the last decades. Therefore, localised approaches that treat GB straight into the tumour site provide an alternative to enhance chemotherapy bioavailability and efficacy, reducing systemic toxicity. Likewise, the discovery of protein targets, such as the NIMA-related kinase 1 (Nek1), which was previously shown to be associated with temozolomide (TMZ) resistance in GB, has stimulated the clinical development of target therapy approaches to treat GB patients. In this study, we report an electrospun polyvinyl alcohol (PVA) microfiber (MF) brain-implant prepared for the controlled release of Nek1 protein inhibitor (iNek1) and TMZ or TMZ-loaded nanoparticles. The formulations revealed adequate stability and drug loading, which prolonged the drugs' release allowing a sustained exposure of the GB cells to the treatment and enhancing the drugs' therapeutic effects. TMZ-loaded MF provided the highest concentration of TMZ within the brain of tumour-bearing rats, and it was statistically significant when compared to TMZ via intraperitoneal (IP). All animals treated with either co-therapy formulation (TMZ + iNek1 MF or TMZ nanoparticles + iNek1 MF) survived until the endpoint (60 days), whereas the Blank MF (drug-unloaded), TMZ MF and TMZ IP-treated rats' median survival was found to be 16, 31 and 25 days, respectively. The tumour/brain area ratio of the rats implanted with either MF co-therapy was found to be reduced by 5-fold when compared to Blank MF-implanted rats. Taken together, our results strongly suggest that Nek1 is an

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; AST, aspartate aminotransferase; BBB, blood-brain barrier; CK, creatine kinase; CN, coordination number; DMEM, Dulbecco's Modified Eagle Medium; DDR, DNA damage response; DDS, drug delivery systems; DSB, double-strand breaks; EE%, percentage of entrapment efficiency; FBS, foetal bovine serum; GB, malignant glioblastoma; GGT, gamma-glutamyl transferase; H2AX, histone 2AX; iNek1, Nek1 inhibitor; JNK2, c-Jun NH (2)-terminal kinase 2; MGMT, O^6-methylguanine-DNA methyltransferase; MF, microfiber; Mre11, double strand break repair nuclease; MTT, methylthiazolyldiphenyl-tetrazolium bromide; Nek1, NIMA-related kinase 1; NMR, nuclear magnetic resonance; NP, nanoparticles; PBS, phosphate-buffered saline; PVA, polyvinyl alcohol; SASA, solvent-accessible surface area; SD, standard deviation; TMZ, temozolomide; UV, ultraviolet; VDAC1, mitochondrial voltage-dependent anion channel.

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important GB oncotarget and the inhibition of Nek1′s activity significantly decreases GB cells' viability and tumour size when combined with TMZ treatment.

1. Introduction

Malignant glioblastoma (GB) is the predominant primary brain tumour in adults, with a median survival of < 2 years (Norouzi et al., 2016; Tseng et al., 2016; Brodbelt et al., 2015; Rape et al., 2014; Tan et al., 2020). Currently, surgical resection followed by radiotherapy and adjuvant chemotherapy is considered the standard therapy for GB patients (Tseng et al., 2015; Huang et al., 2016). However, GB exhibits a high capacity of infiltrating normal tissue, which makes it practically impossible the complete tumour resection through surgery, and even after resection, the recurrent GB invasive cells can form tumours within few centimetres of the original site (Tseng et al., 2015; Huang et al., 2016; Weller et al., 2013; Sawyer et al., 2006).

Amid the obstacles of GB therapy, the blood–brain barrier (BBB) and its tortuous angiogenesis restrict effective drug delivery to the brain, contributing to chemotherapy inefficacy (Sun et al., 2017; Irani et al., 2017; Wei et al., 2014; Fan et al., 2015; Park et al., 2017). Several drugs fail to achieve therapeutic concentrations at the tumour site, even at toxic systemic level concentrations (Tseng et al., 2015; Tseng et al., 2013). Therefore, temozolomide (TMZ) is still being used as the standard chemotherapeutical drug by oral administration (Denny et al., 1994), since the use of this alkylating agent as adjuvant therapy after surgery has been widely accepted as the most effective and well-tolerated option (Akbar et al., 2009).

The discovery of potent genetic and protein targets has stimulated the clinical development of novel therapeutic approaches to treat patients with GB (Zhu et al., 2016). However, several of these approaches, including O^6-benzylguanine (Quinn et al., 20092009) and AZD0156 (Abida et al., 2018), which inhibit O^6-methylguanine-DNA methyltransferase (MGMT) and ataxia telangiectasia mutated (ATM) respectively, fail in offering improvements in patients' prognostics. One of the reasons that justify the clinic failure of these approaches may be the incapacity of conventional treatment modalities to reach the tumour site using tolerable doses of chemotherapeutical agents throughout highly disseminated tumours and to target oncotargets that are contributing to tumour resistance (Westphal and Lamszus, 2011). Nek1 (NIMA-related Kinase 1) is a member of the Nek family kinases (Patil et al., 2013; White and Quarmby, 2008), acting in mitosis (Patil et al., 2013; White and Quarmby, 2008) and DNA damage repair (Liu et al., 2013; Spies et al., 2016). Nek1 is required for activating DNA damage response (DDR) pathways through ATR (Liu et al., 2013); moreover, its upregulation has an anti-apoptotic effect through phosphorylation and deactivation of the mitochondrial voltage-dependent anion channel (VDAC1) (Chen et al., 2009; Chen et al., 2010; Chen et al., 2014). In addition, other DNA damage-related proteins including Rad54 and Mre11, are associated with Nek1 (Spies et al., 2016), and recently, Higelin et al. demonstrated that compromised DDR machinery caused by *NEK1* mutation in motoneurons of amyotrophic lateral sclerosis patients lead to accumulation of DNA damage and increased cell death (Higelin et al., 2018), highlighting the role of this protein in DDR. In a cancer context, Nek1 has been associated with prostate cancer progression (Singh et al., 2019; Singh et al., 2019; Singh et al., 2020), decreased sensitivity to DNA-damaging therapy in renal cell carcinoma (Chen et al., 2014), reduced disease-free survival in cervical cancer (Freund et al., 2020), malignancy and aggressiveness of thyroid cancers (Melo-Hanchuk et al., 2020), and TMZ-resistance in GB cells (Zhu et al., 2016). In glioma cells, Zhu et al. suggested that Nek1 may be a novel and important oncotarget since its role in GB malignancy is related to cell growth promotion and chemoresistance (Zhu et al., 2016). Our unpublished data suggest that Nek1 knockout in U87MG GB cells increases the cells sensitivity to DNA-damaging agents such as TMZ and a radiomimetic drug, and modulates the DDR. Taken together, these studies imply the importance of inhibiting the activity of Nek1 during therapies that activate the DDR.

To improve the therapeutic effectiveness of TMZ and decrease its side effects such as hematologic toxicity (Stupp et al., 2005; Corsa et al., 2006; Mutter and Stupp, 2006; Vera et al., 2004); *in situ* drug delivery systems (DDS) can be administered to prevent tumour recurrence. Localised and controlled approaches that treat GB directly into the tumour site provide an alternative to enhance chemotherapy efficacy and reduce systemic toxicity (Ranganath and Wang, 2008; Han et al., 2017; Kuramitsu et al., 2014; Hirschberg et al., 2013). Due to their biocompatibility, polymers are one of the most promising resources for DDS development (Bei et al., 2009). Several DDS, such as micro- and nanosystems, and wafers have been developed to provide adequate drug release into the GB site (Irani et al., 2017; Ranganath and Wang, 2008; Ahmed et al., 2006; Kumarnaraharisetti et al., 2007; Hernán Pérez de la Ossa et al., 2013; Scott et al., 2011; Kim et al., 2007; Hua et al., 2011; Ramachandran et al., 2017; Ramachandran et al., 2014; Játiva and Ceña, 2017; Cheng et al., 2014; Mangraviti et al., 2016). Nevertheless, given the high interstitial pressure in the brain, micro- and nanoparticles (NP) can be easily expelled from the target site, and these formulations frequently present burst release of loaded drugs causing neurotoxicity (Irani et al., 2017, Fernandes et al). Additionally, hydrogels and wafers have low surface areas resulting in inappropriate and inconsistent drug dissolution (Norouzi et al., 2016, Ranganath and Wang, 2008).

Our research group has been working with polyvinyl alcohol (PVA), which is a noteworthy choice for designing DDS since it is biodegradable and presents very low toxicity (Ranganath and Wang, 2008, Ahmed et al., 2006, Pourgholi et al., 2016, Steffens et al, 2020, Reinhardt et al, 2021, Seba et al., 2021). Recently, we produced dacarbazine-PVA nanofibers to treat GB and this formulation showed great mechanical and delivery properties improving the *in vitro* efficacy of dacarbazine (Steffens et al., 2020). Hence, in this study, we aimed the development of electrospun PVA microfibers (MF) to be used as brain implants for the controlled release of a co-therapy of TMZ and Nek1 inhibitor (iNek1). Moreover, this study aimed the evaluation of Nek1 as a GB oncotarget. Our results strongly indicate that Nek1 inhibition could improve therapy outcome *in vivo* when combined with TMZ treatment, and the use of implantable DDS could enhance therapy efficacy and facilitate the delivery of compounds to the brain.

2. Material and methods

2.1. In silico evaluations

2.1.1. Molecular modelling

Nek1 complex was obtained through molecular modelling of missing loops of PDB ID 4APC model. The process was performed on Modeller v.9.19 (Webb and Sali, 2016). For each model was build 1000 models and selected the one with the best stereo chemical quality with DOPE assessment method evaluation. The quality of the model was checked with Procheck (Laskowski, 2001), Verify 3D (Bowie et al., 1991; Lüthy et al., 1992) and MolProbity (Chen et al., 2010) software.

2.1.2. Molecular docking protocol and validation

Molecular docking assays were performed using AutoDock Vina (Trott and Olson, 2010). AutoDock Vina employs a gradient-based conformational search method and outlines the search space by a grid box defined by the box centre coordinates and its dimensions of x, y and z in grid resolution internally assigned to 1 Ã… (Trott and Olson, 2010). The number of binding modes was set to 1000 and exhaustiveness set to 200 to control how many times the calculations are repeated. The grid

dimensions were 40 × 40 × 40 (x, y, z) points centre on the binding site −26.22 × 11.13 × 16.78 (x, y, z). The scoring of the generated docking poses and ranking of the ligands were based on the Vina empirical scoring function. All rotatable dihedral angles of iNek1 were considered as flexible. The orientations of the iNek1 molecule at the binding sites on Nek1 and JNK2 (PDB ID 3NPC) proteins were chosen from docked conformations as representative of the lower energy clusters generated by Autodock Vina.

2.1.3. Nomenclature and software

For nomenclature and symbols, the IUPAC recommendations were applied in the present work. Concerning MD simulations, the GROMACS 2019 simulation suite (Pronk et al., 2013) was used; along with the GROMOS 53A6 force field (Oostenbrink et al., 2004) and the GROMOS 53A6GLYC force field (Pol-Fachin et al., 2012; Pol-Fachin et al., 2014). For iNek1 and TMZ molecules; the parameters of previous work were selected (Polêto et al., 2018). For the manipulation and generation of the entire PVA molecule; the Assemble! (Degiacomi et al., 2016) tool was used. This tool makes easier the simulation of polymeric systems. Assemble! permits the creation of polymers from monomer building blocks with a user-defined force field. For the manipulation and visualisation of structures; the software VMD (Humphrey et al, 1996) and PyMOL (Delano) were used.

2.1.4. Molecular dynamics simulations

For the construction of the system, the same approach of our previous paper has been utilised (Steffens et al., 2020). The system was generated by applying the Assemble! tool (Degiacomi et al., 2016) under the following experimental data: 1 molecule of PVA (1765 units) was set as 5%, the iNek1 as 0.05% and the TMZ as 0.05%, resulting on 8 molecules of the drugs (4 iNek1 and 4 TMZ) for 1 PVA molecule. Two systems were constructed, PVA with four molecules of iNek1 and four molecules of TMZ and eight drugs (iNek1 and TMZ) molecules in water. To randomise the structure and relax local stresses, PVA chain was exposed to the steepest descent energy minimisation and 1 ns of molecular dynamics pre-equilibration of the polymer around the drug, as previously described (Steffens et al., 2020; Kyrychenko et al., 2017; Tallury and Pasquinelli, 2010). Subsequent to the pre-equilibration steps, the dodecahedron box was solvated with SPC water model (Berendsen et al., 1987) and periodic boundary conditions. Prior to this process, to retain the physiological ionic strength 0.15 M NaCl was added to the aqueous solution. The LINCS algorithm (Hess et al., 1997) was selected to constrain covalent bond lengths. This way, an integration step of 2 fs was applied. As for the electrostatic interactions, calculations were performed by the particle mesh Ewald (PME) method (Darden et al., 1993). The pressure barostat chosen was Parrinello–Rahman (Parrinello and Rahman, 1981; Nosé and Klein, 1983), with a 2.0 ps coupling constant, while the temperature thermostats chosen were V-rescale (NVT step) (Bussi et al., 2007) and Nosé–Hoover (NPT equilibration and production MD) (Nosé, 1984; Hoover, 1985), with a coupling constant of $\tau = 0.5$. Constant temperature of 298 K and constant pressure of 1 atm were also applied. Steepest Descent algorithm was used in the energy minimisations performed. First, two simulations of equilibration were carried out with position restraints: an NVT and an NPT of 2 ns and 5 ns, respectively. Not only that, 500 ns of unrestrained NPT MD simulations were carried out for each system (iNek1-TMZ and PVA-iNek1-TMZ), creating the production run from which data were collected. Each system was simulated in three independent runs, with distinct starting velocities, to filter conformational events of low probability (Nemec and Hoffmann, 2017; Perez et al., 2016). For analysis, coordination number is the total number of neighbours of a nitrogen atom on the imidazole rings, obtained from optimal binding distance calculated by radial distribution function.

2.2. Preparation of temozolomide-loaded nanocarriers

Temozolomide (TMZ; Merck, Darmstadt, Germany)-loaded stearic acid (Merck) nanocarriers (TMZ NP) were produced by solvent diffusion method according to Hafeez et al. (Hafeez and Kazmi, 2017) with minor modifications. TMZ (10% w/w) and stearic acid were suspended in an organic phase prepared using acetone:ethanol (Merck) mixture (1:1). Then, the aqueous phase was prepared by heating up to 70 °C distilled water (dH_2O) on a continuous stirring. Finally, the organic phase was poured and mixed into the aqueous phase during 30 min. After cooling at room temperature, the mixture was sonicated for 5 min and frozen overnight. Later, the mixture was lyophilised in a freeze dryer (Heto, LyoLab 3000, Thermo Fisher Scientific, MA, United States). For comparison purposes, blank nanocarriers (Blank NP) were prepared by using stearic acid only.

2.3. Particle size and zeta potential

Particle size and zeta potential analyses were carried out on the Beckman Coulter Delsa Nano C (Beckman Coulter, CA, United States). For particle sizing, the sample was run 9 times to ensure that results were statistically relevant. Analysis was carried out on the measured autocorrelation functions using the non-negative least square algorithm. Very large aggregate results (>5 μm) were removed from the analysis as the fitting and algorithm cannot provide accurate results at this size. For zeta potential, the sample was run 3 times (as each run is repeated 10 times and averaged to provide the result). The applied voltage on the electrodes of the cell was 60 mV.

2.4. Preparation of microfibers

Polyvinyl alcohol (PVA; Merck) hydrogel solutions were produced by dissolving PVA at 10% (w/v) in dH_2O at 90 °C, under continuous stirring until its whole solubilisation. After the solutions were colder, ethanol (10% v/v) was added. Then, different solutions were prepared by adding known amounts of TMZ (0.01% w/w) or TMZ and Nek1 inhibitor (0.01% w/w; iNek1; JNK inhibitor II; Merck) solutions prepared in DMSO or TMZ NP (10% w/w; TMZ final concentration in nanocarriers: 0.1% w/w) or TMZ NP and iNek1 solution into the PVA solution. The formulations were electrospun (Spraybase, Co. Kildare, Ireland) through a blunt-end 20-gauge needle, with a flow rate of 0.5 mL/h, a conductivity of 9 kV, and a distance of 5 cm between the needle tip and the collector plate. For comparison purposes, Blank MF were prepared by using only PVA or by adding Blank NP into a PVA solution prior electrospinning. For visualisation of fluorescent fibers, curcumin (Merck) MF were prepared by adding Blank NP into a curcumin (0.01% w/w)-PVA solution prior electrospinning.

2.5. Microfiber sterilisation and sterility validation

Isolated colonies of *Escherichia coli* (*E. coli*) (NC 12241) and *Staphylococcus aureus* (*S. aureus*) (NC 12981) from stock strains cryogenised at −80 °C were obtained through isolation seeding and grown overnight. Each colony of each bacterium was separately suspended in 5 mL of sterile PBS (Thermo Fisher Scientific) and standardised with spectrophotometer by 0.5 McFarland scale (1.5×10^8 CFU/mL in 600 nm wavelength). Subsequently, 50 μL of standardised bacterial suspension were added into each polymer sample (1 cm^2 each sample) on 3 cm^2 diameter sterile plates. Contaminated samples were sterilised by total immersion in 500 μL of isopropanol (IPA; Merck) for ten seconds, and subsequently drained. Serial dilution of samples was conducted in a 96-well sterile microplate previously filled with 270 μL of sterile PBS until the fifth well. Each sample was dispersed into 1 mL of sterile PBS and briefly mixed. From this dilution, 30 μL were taken out and transferred to the first well in the microplate, briefly mixed, being now the 10^{-1} dilution, and this went through until the 10^{-5} dilution. This procedure

was repeated for each sample separately. From each dilution 100 μL were taken and inoculated through spread plate method in agar plate with 20 mL of solid nutrient agar (NA; Merck) with a bent rod at once. Other three replicates were made with micro drop technique (10 μL) in NA plates. Plates were then incubated at 37 °C for 24 h. After the first reading, the plates were left on incubator for 2 weeks to analyse the growth possibility and no bacterial growth was observed on sterilised plates.

2.6. Microfiber size and morphology analysis

The fibers' and particles' morphologies were observed via scanning electron microscope (SEM; Tescan Mira XMU SEM, TESCAN, Brno, Czech Republic). Back-scattered electron mode was used with magnifications ranging from 10 kX to 30 kX after the fibers were sputtered with gold. For fiber size analysis, ImageJ software (ImageJ 1.53v, National Institute of Health, Bethesda, MD, United States) was used and the mean diameter was extracted by analysing>300 fibers. Fluorescence images of the fibers were acquired using a Leica DM 2000 confocal microscope with an x40 oil lens (Leica Microsystems, Ashbourne, Ireland). Image acquisition was achieved by using the LAS V3.8 software (Leica Microsystems, Ashbourne, Ireland).

2.7. Solid-state NMR

The stability of the drugs and the polymer after formulation preparation was evaluated by nuclear magnetic resonance (NMR) spectroscopy. The analysis was executed using a Bruker 400 MHz Avance III HD (Bruker, MA, United States) equipped with a 3.2 mm H/X CPMAS probe.

2.8. Determination of the encapsulation efficiency of TMZ and iNek1

To measure the percent encapsulation efficiency (EE%) and the total amount of loaded TMZ and iNek1, MF or NP were entirely dissolved in DMSO (Merck) and the amount of released drug was measured by ultraviolet (UV) light on a Shimadzu UV 1280 spectrometer (Shimadzu, Kyoto, Japan) at 328 and 407 nm, respectively. Loaded formulations were used to generate standard curves for each drug. Empty (blank) formulations were also dissolved in DMSO and used as the blank for the spectrometer reading. The EE% was determined as follows:

$$EE\% = \frac{actualamountofdrugrelease}{theoreticalamountofloadeddrug} X100$$

2.9. In vitro drug release studies

Drug dissolution studies were performed using a Distek Model 2500 Dissolution System (Distek Inc., NJ, United States). The samples were tested in PBS (Thermo Fisher Scientific) buffers (pH 7.4 or 6.8) at 37 °C. Each vessel contained 300 mL of dissolution media and a stir rate of 50 rpm was used. Samples were taken at set intervals and measured by UV light on a Shimadzu UV 1280 spectrometer (Shimadzu). Loaded formulations were used to generate standard curves for each drug. Empty (blank) formulations were used as the blank for the spectrometer reading.

2.10. Nek1 protein expression

U87MG GB cells were maintained in Dulbeco's Modified Eagle Medium (DMEM), supplemented with 10% foetal bovine serum (FBS), penicillin, streptomycin and L-glutamine (Thermo Fisher Scientific) at 37 °C in a 5% CO_2 humidified atmosphere. Cells were treated with TMZ (75 μM) for 24 h. After treatment, the cells were trypsinised (Thermo Fisher Scientific), 15 min fixed with 3.7% paraformaldehyde (v/v) (Merck), permeabilised with 0.5% Triton X-100 (v/v) (Merck) in PBS (Thermo Fisher Scientific) buffer for 15 min and blocked with 10% FBS in PBS buffer (v/v) for 1 h. In the next step, the samples were incubated overnight at 4 °C with the following diluted antibodies: 1:100 Alexa Fluor 488 Mouse anti- γH2AX (pS-139) (BD Biosciences, CA, United States), 1:50 anti-vinculin-FITC (Merck) or 1:100 anti-Nek1 (Merck). Anti-rabbit Alexa Fluor 594 (Life Technologies) at a dilution of 1:500 was used as the secondary antibody for Nek1 staining. The samples were placed in a glass slide and each slide was dropwise stained with Hoechst 33,258 (Thermo Fisher Scientific) for cells' nuclei visualisation. The slides were observed in a cell imaging system (IN Cell Analyser 2200, GE Healthcare Life Sciences, NJ, United States). Nek1 protein expression was also evaluated by western blot. Briefly, U87MG, A172, M059J, T98G and U138MG cells were treated with TMZ (75 μM) for 24 h and protein extracts were prepared by using lysis buffer and separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. The membranes were blocked with 5% skim milk in tris-buffered saline with 0.05% of Tween 20 (Merck) (v/v) for 1 h and incubated overnight at 4 °C with following antibodies at a dilution of 1:500 anti-Nek1 (Merck) or 1:500 anti-β-actin (Santa Cruz Biotechnology, CA, United States). The secondary antibodies used were 1:3000 mouse-anti-rabbit or 1:3000 goat-anti-mouse (Santa Cruz Biotechnology). The membranes were incubated with Luminol-based Enhanced Chemiluminescent mix and exposed to films to develop.

2.11. Cell viability evaluation

Two different media (pH 6.8 or 7.4) were used to perform this evaluation. The drugs screening and the formulations cytotoxicity potential were determined by using methylthiazolyldiphenyl-tetrazolium bromide (MTT; Merck) colorimetric assay. Firstly, TMZ and iNek1 stock solutions prepared in DMSO (stock solution TMZ: 21 mM and iNek1: 13.6 mM) were further diluted in 10% FBS DMEM and tested aiming to select the IC_{50} used in the following experiments. Briefly, U87MG cells were seeded in 96-well plates at $1x10^5$ cells/well, following 24 h, cells were treated with increasing concentrations of TMZ (25–200 μM) or iNek1 (15–240 μM) for 48 h. Following treatment, cells were incubated with MTT for 3 h at 37 °C. Then, formazan crystals were dissolved in DMSO, and the absorbance was recorded at 540 nm in a microplate reader (BioTek Synergy HT, Swindon, United Kingdom). The cell viability was assessed by using the negative control (cells treated with vehicle: 0.1% DMSO) as 100%. After this screening, U87MG cells were treated with several formulations at the IC_{50} previously determined or with the neat drugs during 2, 5 or 5 (+2 days of recovery = media replenish) days. C6 cells were also treated with TMZ (75 μM) or the co-treatment (TMZ 75 μM + iNek1 50 μM) during 5 days. For the 3D cell culture assessment, cells were incubated with magnetic NP (NanoShuttle™-PL, Greiner Bio-One, Frickenhausen, Germany) for 24 h at 37 °C, thereafter disposed into a cell-repellent 96-well plate at $1x10^5$ cells/well and incubated for 24 h over a magnetic drive. Then, cells were exposed to complete media (negative control), neat drugs or the formulations for 5 (+2 day of recovery) days. Spheroid size was analysed by using an EVOS FL Auto 2, Imaging System microscope (Thermo Fisher Scientific) and calculated by using ImageJ software.

2.12. Animals

All experimental procedures were reviewed, approved, and performed in accordance with the Federal University of Health Sciences from Porto Alegre's Ethics Committee guidelines (approval number: 603/18). Sixty days-old and around 300 g male Wistar rats were obtained from animal housing facility of UFCSPA and were maintained in the laboratory at 22 ± 2 °C, with water and food *ad libitum*, and under a 12:12 h light and dark photoperiod.

2.13. In vivo evaluation

C6 glioma cells were cultured in DMEM supplied with 10% FBS,

penicillin, streptomycin and L-glutamine at 37 °C in a 5% CO_2-humidified atmosphere incubator to approximately 70% confluence. Then, cells were trypsinised, counted with trypan blue staining and a total of $1x10^6$ cells were suspended in 3 μL of free-FBS DMEM. The animals were anesthetised using ketamine/xylazine (Agener Uniao, SP, Brazil), and the cell suspension was implanted by stereotaxic surgery into the right striatum of the brain of the rats at a depth of 6.0 mm (coordinates with regard from bregma: 0.5 mm posterior and 3.0 mm lateral) with injection flow of 1 μL/min (Braganhol et al., 2009). On the tenth day from tumour inoculation, the animals were sedated using ketamine/xylazine and a circular incision (around 5 mm of diameter) was made in the brain cap using stereotaxic equipment (following the same coordinates as previously mentioned) to implant the MF (15 mg/MF) (Fig. 5A). The animals were randomly divided into six groups with three to eight animals each: Blank MF (control formulation), TMZ MF, TMZ + iNek1 MF, TMZ NP + Blank MF, TMZ NP + iNek1 MF or intraperitoneal (IP) TMZ (5 mg/kg; once a day for five days). Post-operative pain was evaluated for the first five hours, and then daily using the grimace scale (Sotocina et al., 2011). Three animals/group of Blank MF, TMZ MF or TMZ NP + Blank MF were followed up for two days (for TMZ quantification within the brain). The other animals were followed up until they reached endpoint criteria (moribund animal and/or loss of > 15% of initial body weight) up to sixty days. Clinical and behavioural changes were monitored daily, and signs of pain were treated by IP opioid administration (tramadol 12.5 mg/kg; every eight hours up to five days). Body weight was recorded biweekly with a digital weighing balance for body weight change evaluation and at the end of treatment. All animals were euthanised by overdose of ketamine and xylazine. After euthanasia, the brain tissue was rapidly removed. The brains were excised and preserved in 3.7% formaldehyde solution (v/v; pH 7.4) or were washed in PBS (Thermo Fisher Scientific) and kept in −80 °C. The samples processing and hematoxylin and eosin slides preparation were performed by UFCSPA's Pathology Laboratory according to established protocols. The slides were scanned by using an EVOS FL Auto 2, Imaging System microscope (Thermo Fisher Scientific) and measured by using ImageJ software. The brain and the tumour areas were measured and the tumour/brain area ratio was calculated. Kidney and lung tissues were collected for histological evaluation and preserved in formaldehyde solution. Blood samples were collected from the abdominal aorta artery by puncture in a test tube without anticoagulant substances to obtain serum samples, followed by immediate centrifugation at 3000 rpm for 15 min. The collected serum was preserved at −70 °C for biochemical analysis.

2.14. Biochemical analysis

Urea, creatinine, amylase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein, lactate, and creatine kinase (CK) (Bioclin, MG, Brazil) analyses were performed by the chemistry analyser BS-120 (Mindray, SP, Brazil) at UFCSPA's Clinical Analysis Laboratory.

2.15. TMZ extraction and quantification from the brain tissue

The samples' preparation was based on previous studies with minor modifications (Khosa et al., 2018). Briefly, 100 mg of brain tissue were used to extract TMZ. The samples were acidified by using 200 μL of acetic acid (0.1% v/v; Merck) and homogenized. The protein amount was precipitated by adding 200 μL of cold acetonitrile (Merck), the samples were vortexed and centrifuged for 10 min at 10,000 rpm. The supernatants were collected and filtered by using a 0.45 μm filter. For the TMZ standard curve, brain tissue from control rats were used and spiked with a range of TMZ solutions (0.05 – 5 μg/mL) in acetic acid. Control samples (without TMZ) were also evaluated to assess possible interferences in the composition. The HPLC-DAD method was based on previous studies (Gilant et al., 2012; Michels et al., 2019) and was carried out in a Shimadzu Prominence (Shimadzu, Tokyo, Japan) chromatograph equipped with a quaternary, low-pressure mixing pump and inline vacuum degassing, controlled by a CBM-20^A interface module, an automatic injector (SIL-20A) and Diode Array Detector (SPD-M20A). The separation was performed using a reverse-phase Phenomenex – Luna C18 (5 μm × 150 mm × 4.6 mm) column. The mobile phases consisted of acetic acid (2% v/v) – phase A and acetonitrile – phase B. The injection volume was set at 20 μL with a flow rate of 1.1 mL/min and the samples were monitored at 330 nm. The running time was set at 10 min and the retention time of TMZ was found to be 1.6 min (Supplementary Fig. 1).

2.16. Statistical analysis

Quantitative data were expressed as the mean ± standard deviation (SD) and statistical analysis was achieved by unpaired *t*-test or one-way ANOVA followed by Tukey post-test. Kaplan–Meier survival curves were generated to compare the different mice treatment groups (GraphPad Prism 8.0, La Jolla, CA, USA). P value < 0.05 was assumed as statistically significant.

3. Results and discussion

3.1. Nek1 expression in GB cells

It is known that Nek1 plays a vital role in DNA damage signalling and that the genetic inhibition of this protein increases apoptosis in glioma cells followed by TMZ treatment (Zhu et al., 2016; Liu et al., 2013; Spies et al., 2016). Thus, aiming to verify if Nek1 responds to stress induced by TMZ we analysed the expression of Nek1 in different GB cell lines after treatment with TMZ. Firstly, a screening of TMZ concentrations was performed to find the IC_{50}, which was used for the *in vitro* experiments (a dose–response curve of TMZ is shown in Supplementary Fig. 2A; the IC_{50} was found to be 76.3 μM, thus, 75 μM was chosen for the further experiments). Among the different cell lines (U87MG, A172, M059J, T98G and U138MG), the highest expression of Nek1 was observed in the U87MG cell line (Supplementary Fig. 3A), thus, we selected this cell line to perform the following *in vitro* experiments. U87MG cells were treated with TMZ, then fixed and co-stained with vinculin and Nek1 antibodies and the localisation and expression of Nek1 was evaluated. Results showed a significant increase ($p < 0.05$) of Nek1 expression in the nucleus (Supplementary Fig. 3B, C) following treatment when compared to non-treated cells, suggesting that Nek1 translocated to the nucleus to act during the DDR signalling (Liu et al., 2013).

During the DNA damage repair triggered by TMZ-induced lesions, there is a formation of double-strand breaks (DSB) and when DSB establish in DNA, the histone 2AX (H2AX) flanks the damage site and it is phosphorylated (γ). Owing to the fact that γH2AX foci usually increases after DSB formation (Nakamura et al., 2006), its expression was also verified after TMZ treatment. The intensity of fluorescence of γH2AX and Nek1 increased and they were correlated ($p < 0.05$) (Supplementary Fig. 3B-E).

3.2. JNK2 inhibitor in silico interaction with Nek1

Given Nek1′s role in the DDR and its increased expression after TMZ exposure, we hypothesised that by inhibiting Nek1 and combining it with TMZ treatment, the GB therapy efficacy would improve. Aiming to inhibit Nek1 activity, an ATP-mimetic inhibitor was used, which has been previously found to inhibit Nek1′s activity in almost 30% (to 71.5% ± 0.1%) at a 50 μM concentration (Moraes et al., 2015). Since the inhibitor was produced as a c-Jun NH (2)-terminal kinase 2 (JNK2) inhibitor, its *in silico* interaction with Nek1 and JNK2 was evaluated (Fig. 1).

Although Nek1 has two structures deposited in the Protein Data Bank (PDB ID 4APC and PDB ID 4B9D), it was necessary to carry out the

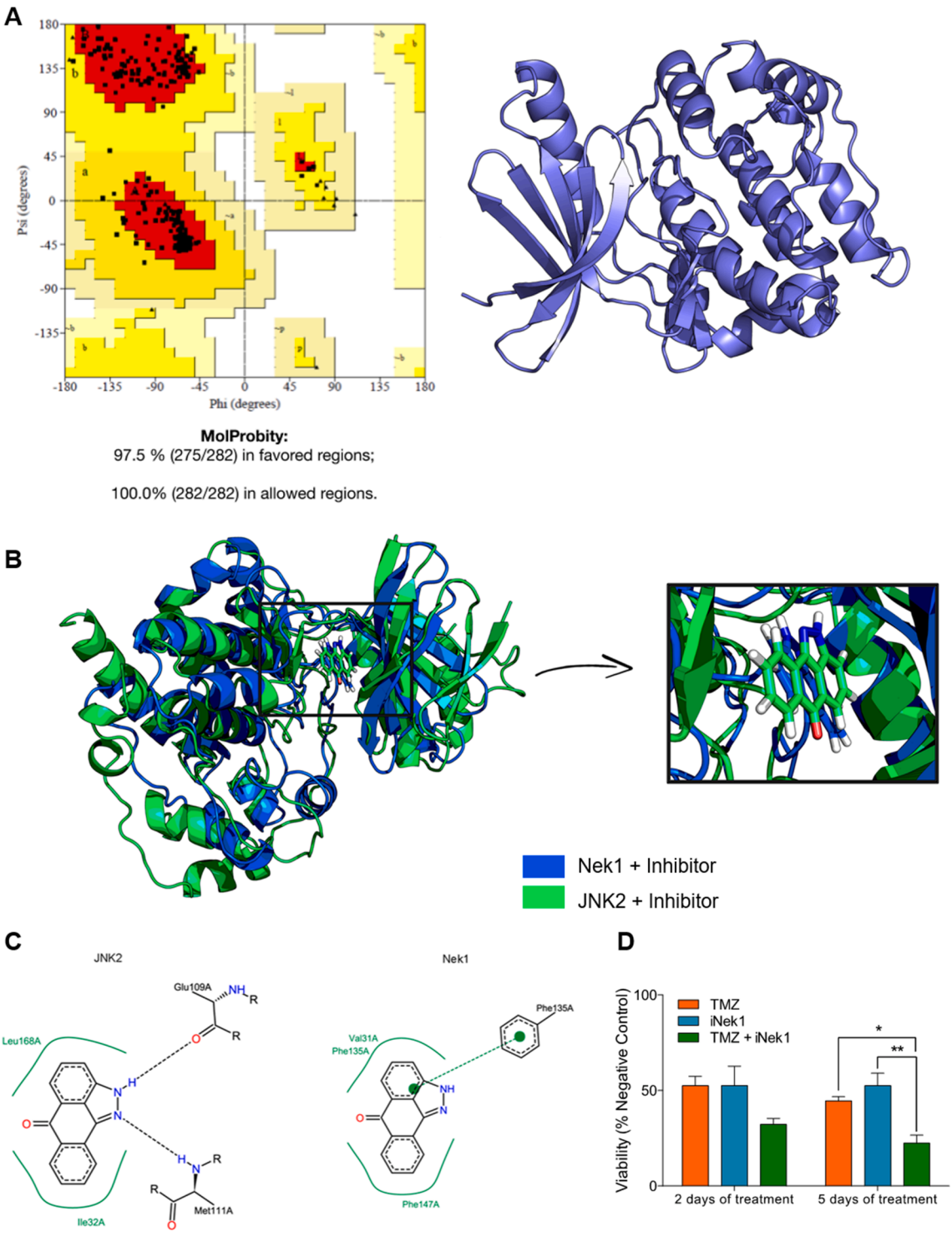

Fig. 1. JNK2 inhibitor II (referred to as iNek1) interacts with Nek1 and decreases cells viability. (A) Ramachandran plot for the new Nek1 model. In blue, the complete structure of Nek1 built in the present study. (B) Results of molecular docking calculations between Nek1 (blue) and JNK2 (green) proteins with iNek1 inhibitor. The iNek1 orientations on both structures are similar and binding energy values are practically the same for both proteins: JNK2-iNek1 = − 9.5 kcal/mol and Nek1-iNek1 = − 9.9 kcal/mol. (C) Main interactions between iNek1 and JNK2 or Nek1 proteins. Both systems have shown hydrophobic interactions (green lines). Hydrogen bonds between the inhibitor and JNK2 are shown as dashed lines, and Nek1-iNek1 pi-pi (π - π) stacking interactions is shown as a dashed green line. (D) Cell viability analysis of TMZ, iNek1 or the co-treatment of TMZ + iNek1. Statistical analysis was performed using one-way ANOVA and Tukey post-test. Data were considered significant different at $p < 0.05$ (*) and $p < 0.01$ (**). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

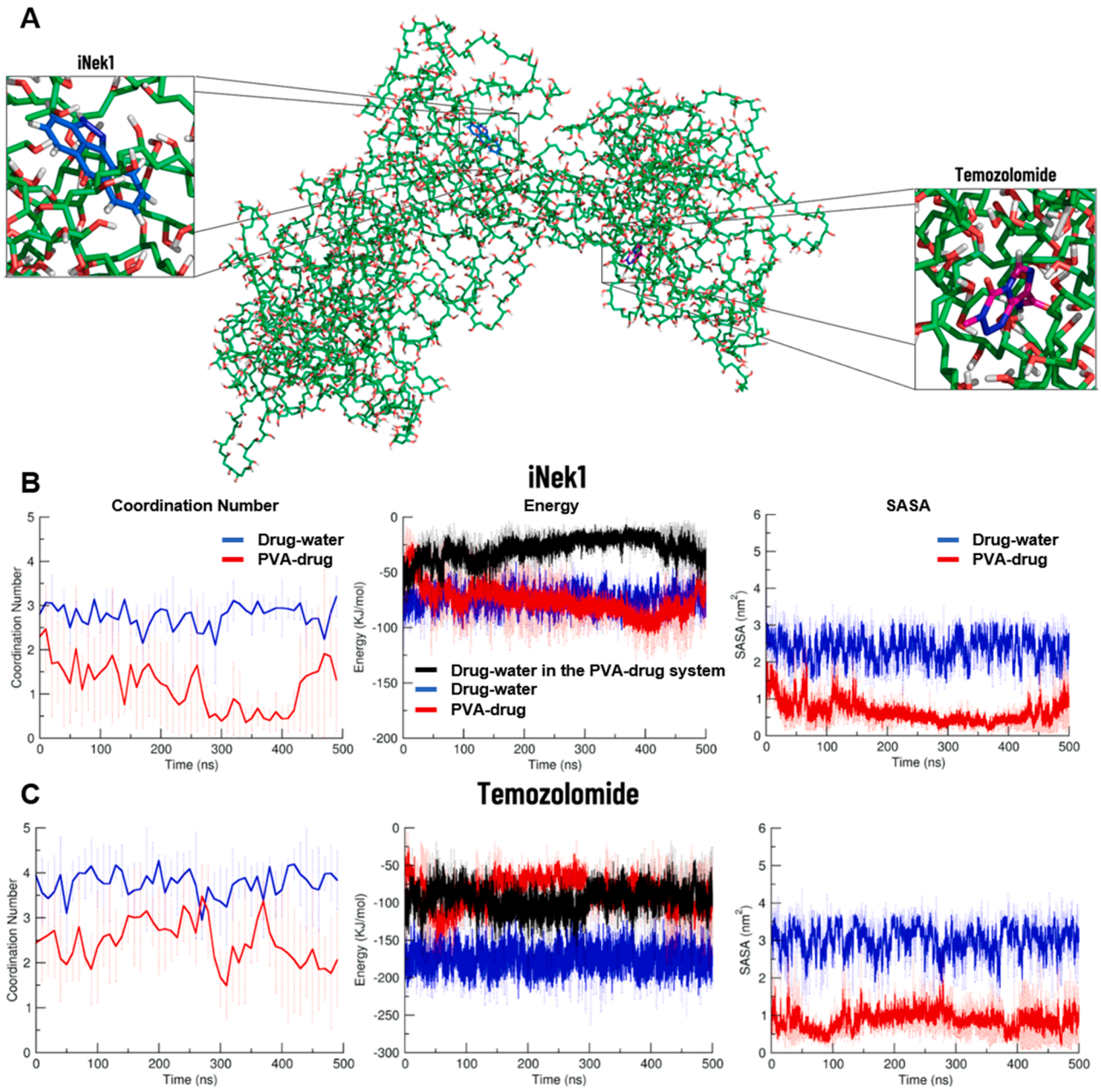

Fig. 2. Interaction between the polymer, iNek1 and TMZ. (A) PVA, iNek1 and TMZ are presented as sticks highlighted in green, blue and pink, respectively. The frame was retrieved at 400 ns of MD simulations. (B, C) Coordination number (CN) of water molecules on nitrogen atom of imidazole rings, total interaction energy between PVA-drugs and water-drugs and average of solvent accessible surface area (SASA) on drugs during the molecular dynamics simulations. (B) Left: CN calculation of the system of PVA-iNek1 (red) and iNek1-water (blue). Middle: the total interaction energy between PVA-iNek1 (red), and the total interaction energy between water-iNek1 in each system, PVA-iNek1 (black) and iNek1-water (blue). Right: SASA of the system of PVA-iNek1 (red) and iNek1-water (blue). (C) Left: CN calculation of the system PVA-TMZ (red) and TMZ-water (blue). Middle: the total interaction energy between PVA-TMZ (red), and the total interaction energy between water-TMZ in each system, PVA-TMZ (black) and TMZ-water (blue). Right: SASA of the system PVA-TMZ (red) and TMZ-water (blue). Each point in the graphs represents the average of three simulations for each system. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

construction of a model by comparative modelling. The Nek1 structures available in the database presented problems, probably due to the resolution of the crystal, where parts of the sequence were not elucidated. These gaps in the structure were solved with the construction of a new model. The protein was modelled using the structure found under the PDB ID 4B9D. MODELLER9v17 (Webb and Sali, 2016) software was used, using as input an alignment of the two amino acid sequences carried out by the Clustal Omega (Sievers et al., 2011) software. The sequences were: complete Nek1 to be modelled, the crystal sequence of PDB ID 4B9D. The routine used in the production of the models was *model* and 1000 models were built. Thus, among these built structures, the selected model was the one that had the best score in the evaluation of stereochemistry (analysed with the PDB sum server and the PROCHECK (Laskowski, 2001) program). The quality of the Nek1 model was checked with Procheck, Verify 3D and MolProbity. MolProbity revealed through the Ramachandran plot that 97.5% (275/282) of all residues were in favoured (98%) regions and 100.0% (282/282) of all residues were in allowed (>99.8%) regions. The results of the Ramachandran plot (Fig. 1A), and analysis of the dihedral angles ϕ and ψ for the protein amino acids showed that the model presents acceptable results. It is clear

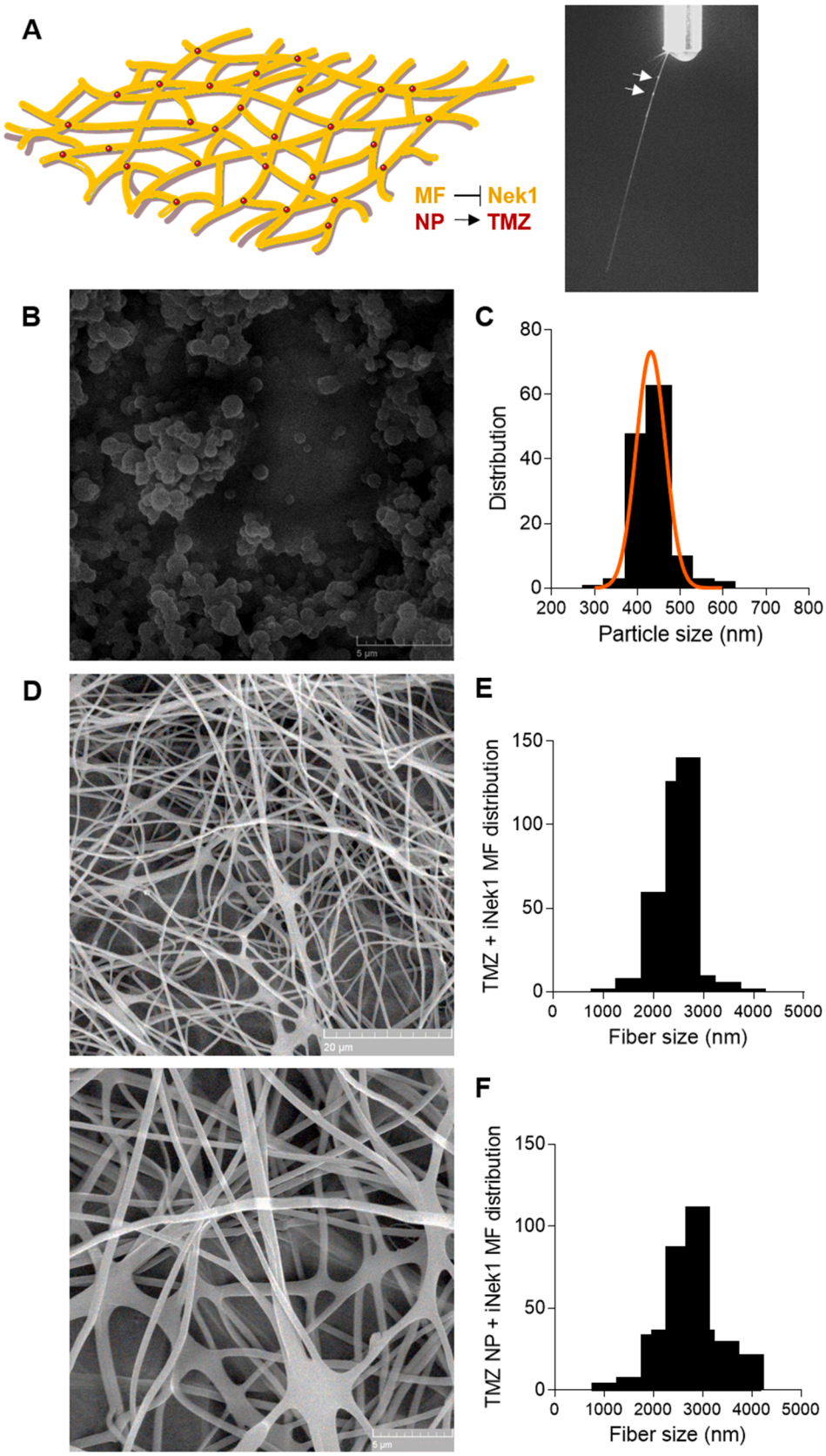

Fig. 3. Characterisation of the formulations. (A) Left: schematic representation of TMZ NP inside electrospun iNek1 MF. Right: representative image of the PVA solution with NP during electrospinning (white arrows demonstrate the NP inside of the MF). (B) TMZ NP SEM characterisation and (C) particle size distribution (the experiment was repeated 9 times). (D) TMZ + iNek1 MF morphology by SEM and (E) TMZ + iNek1 MF fiber size distribution. (F) TMZ NP + iNek1 MF fiber size distribution.

that there is a high number of residues in favourable regions in the model, and waste in unfavourable regions has an extremely low value in the model obtained, confirming a robust model. With these results, it is possible to infer that the model obtained has a suitable resolution compared to the Nek1 crystal, allowing us to obtain the complete three-dimensional structure of Nek1, which was used in the following steps of the work for molecular docking calculations.

It was possible to observe that iNek1 orientations on both structures are similar and binding energy values are practically the same for both proteins: JNK2-iNek1 = -9.5 kcal/mol and Nek1-iNek1 = -9.9 kcal/mol (Fig. 1B). Both systems have shown hydrophobic interactions (green lines), however, when compared both proteins, clearly, the JNK2 protein presents hydrogen bonds and Nek1 pi-pi (π - π) stacking interactions (Fig. 1C), suggesting that the inhibitor not only is capable of inhibiting Nek1, supporting previous studies (Moraes et al., 2015), but possibly the interaction of Nek1-iNek1 is stronger than iNek1-JNK2. It is important to state that since this inhibitor is an ATP-mimetic, it is capable of inhibiting other mitotic kinases not tested in this work, including other Neks (Moraes et al., 2015). Nevertheless, this extensive inhibition capacity could contribute to the results found here.

3.3. Nek1 inhibitor treatment decreases GB cells viability

Aiming to evaluate the efficacy of iNek1 in decreasing the viability of GB cells, a screening of iNek1 concentrations was performed (Supplementary Fig. 1B). The IC_{50} was found to be 54.4 μM, thus, 50 μM was chosen for the further experiments. Subsequently, U87MG and C6 cells were treated with the combination treatment (TMZ + iNek1) (Fig. 1D, Supplementary Fig. 4). It was observed that the co-therapy significantly decreased U87MG cells' viability after 5 days of treatment when compared to TMZ ($p < 0.05$) or iNek1 ($p < 0.01$) (Fig. 1D). Similarly, C6 cells' viability decreased following TMZ + iNek1 treatment when compared to TMZ ($p < 0.05$) (Supplementary Fig. 4), indicating that inhibiting Nek1′s activity enhances TMZ efficacy in killing GB cells.

3.4. In silico interaction between PVA and iNek1 or TMZ

TMZ presents poor solubility in water and a short half-life in blood circulation along with severe side effects (Stupp et al., 2005; Corsa et al., 2006; Mutter and Stupp, 2006; Vera et al., 2004), considering these features, the current study aimed at the controlled release of TMZ. Moreover, given the difficulties of delivering drugs to the brain, a polymeric DDS for local application was developed. Prior to the production of the DDS, in order to assess the main interactions between PVA and iNek1 or TMZ, two systems were constructed: (1) iNek1 and TMZ in water and (2) PVA-iNek1 and TMZ in water. Each system was simulated in three independent runs to filter low probability conformational events (Nemec and Hoffmann, 2017; Perez et al., 2016) and the obtained data are shown as an average of these three simulations. With regards to the possibility of PVA interaction with the iNek1 and TMZ, the exposition of all molecules to solvent was investigated using different approaches as demonstrated in our previous study (Steffens et al., 2020). The analyses include the coordination number (CN) of water molecules, the total energetic contribution for the interaction between PVA-iNek1-TMZ and solvent-accessible surface area (SASA) surrounding the drugs (Fig. 2B, C), as well as the visual examination of drugs during the simulations (Fig. 2A). Accordingly, when complexed to the polymer, both drugs do not expose to solvent (Fig. 2A-C, Supplementary Fig. 5, 6). The CN of water molecules agreed with the interaction energy and SASA results, reinforcing the drugs' complexation with PVA. However, when comparing iNek1 and TMZ molecules, clearly iNek1 has more interaction with PVA than TMZ. The interaction energy data (Fig. 2B, C) have shown a higher interaction between iNek1 and PVA. In fact, the interaction of these molecules is in the same range of interaction energy of iNek1 with water. On the other hand, the interaction energy between the PVA and TMZ is lower when compared to the interaction of TMZ and water. The CN of waters is higher on TMZ than iNek1, reinforcing this data.

The visual examination (Fig. 2A) confirmed the interaction of the polymer with the drugs, probably through electrostatic interactions. It was possible to observe during the simulations that not all drugs'

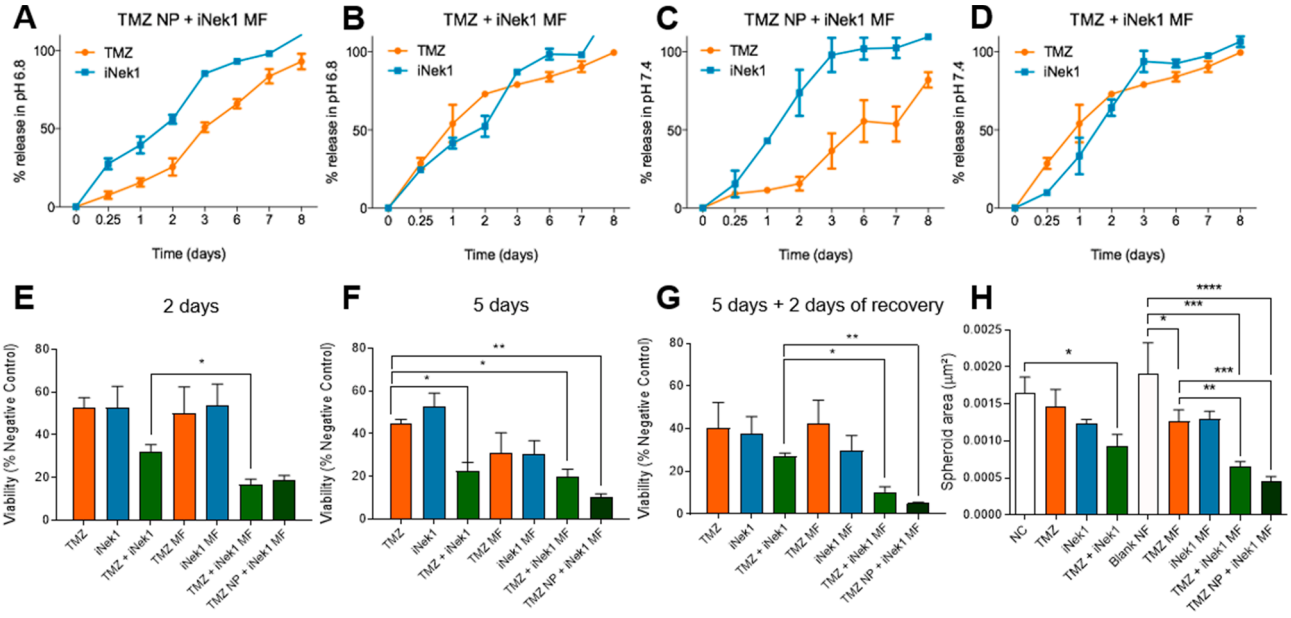

Fig. 4. Drug release studies and *in vitro* efficacy analysis of the formulations. (A) TMZ NP + iNek1 MF and (B) TMZ + iNek1 MF using pH 6.8 buffer. (C) TMZ NP + iNek1 MF (D) TMZ + iNek1 MF using pH 7.4 buffer. (E) Cell viability analysis using the U87MG cell line of all formulations after 2 days of treatment, (F) 5 days of treatment or (G) 5 days of treatment + 2 days of recovery (media replenish). Results are expressed as mean in treated cells compared to vehicle-treated cells (negative control) ± SD. (H) Cell spheroids size after 5 days of treatment + 2 days of recovery with or without the formulations. Data shown represent three independent experiments. Statistical analysis was performed using one-way ANOVA and Tukey post-test. Data were considered significant different at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****).

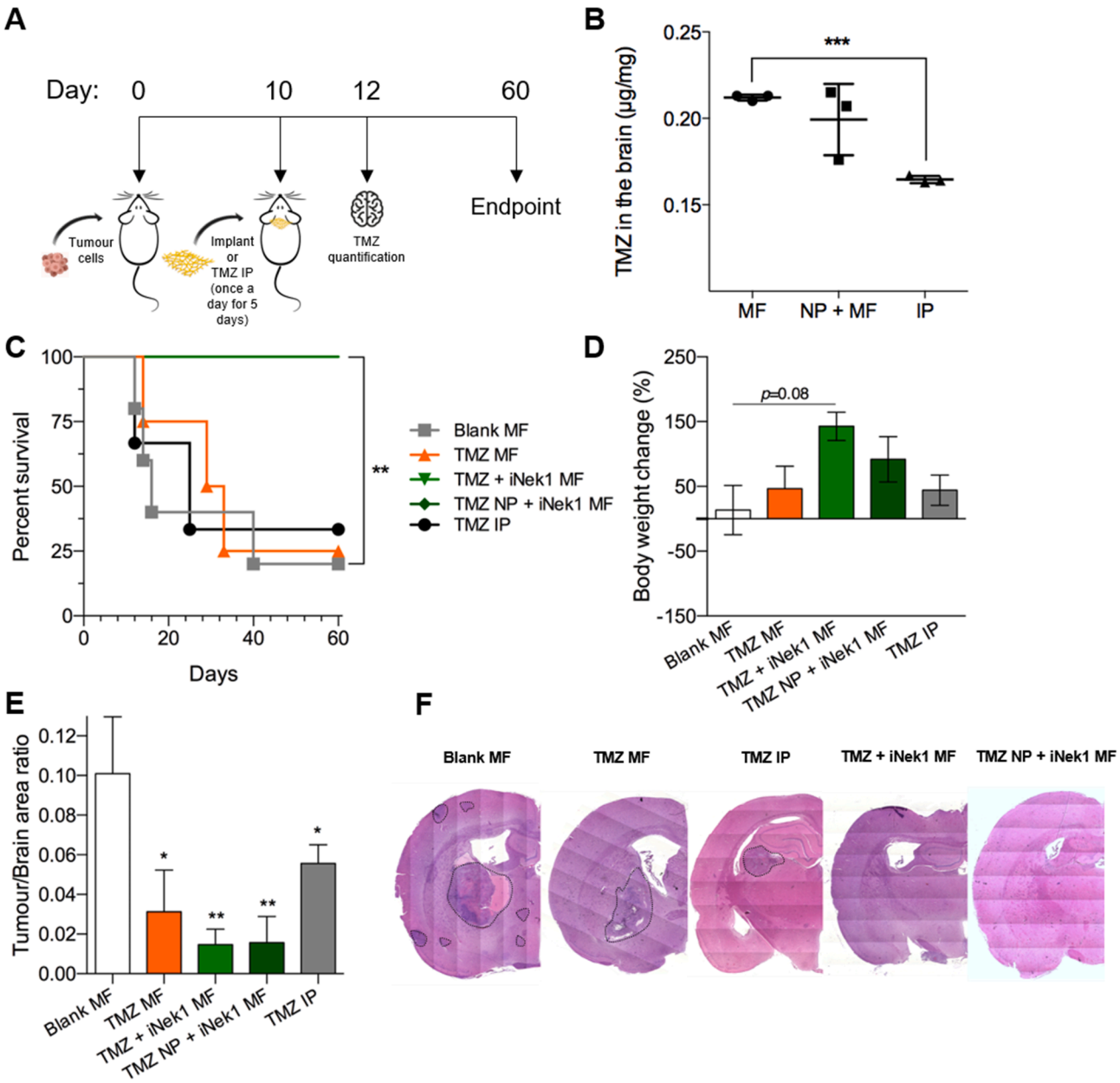

Fig. 5. *In vivo* efficacy analysis of the formulations. (A) Schematic representation of the *in vivo* treatment: on day 0, the animals were orthotopic injected with C6 cells, following ten days, the animals were implanted with Blank MF, TMZ MF, TMZ + iNek1 MF, or TMZ NP + iNek1 MF, or treated with TMZ IP once a day for five days. The animals were monitored until end-point criteria or until the sixth day. In a separated experiment, three animals/group were implanted with TMZ MF or TMZ NP + blank MF or treated with TMZ IP, and on the twelfth day, the animals were euthanised for drug quantification within the brain. (B) TMZ quantification in the brain of treated rats. (C) Kaplan-Meier curve of the survival of the treated groups. (D) Body weight was measured weekly and the body weight change (%) was calculated at the end of the experiment. (E) Tumour/brain area ratio was measured by using ImageJ software. (F) Histological representation of the brains of the animals (tumour location is shown in dashed lines). Statistical analysis was performed using one-way ANOVA and Tukey post-test. Data were considered significant different at $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p < 0.0001$ (****).

molecules interact with PVA (Supplementary Fig. 5, 6). This result is in agreement with our previous work (Steffens et al., 2020), where we have been shown the controlled release of a TMZ analogue, dacarbazine, in PVA systems. Furthermore, the higher interaction of PVA with iNek1 when compared to TMZ helped to design the next steps of the work. The molecular dynamics simulations data were an important step in the present study to decide to produce TMZ-loaded NP to protect the drug from the solvent (Fig. 3A). As previously demonstrated, the TMZ has less affinity for PVA resulting in higher interaction with the solvent.

3.5. Formulations preparation and sterility validation

PVA electrospun MF were produced loaded with TMZ and iNek1 (TMZ + iNek1 MF), or MF loaded with iNek1 and TMZ NP (TMZ NP + iNek1 MF) (Fig. 3A – white arrows point to NP inside the MF during the electrospinning), which aimed the release of iNek1 prior to TMZ targeting Nek1′s activity and then releasing TMZ. MF loaded with only TMZ or iNek1 were also produced for comparison reasons. To generate TMZ NP, TMZ was incorporated in nanostructured lipid NP by using an emulsion method. Prior to characterisation and efficacy testing, it was necessary to ensure that the implant samples are absolutely sterilised due to the further *in vitro* and *in vivo* analyses. In order to evaluate the

efficiency of the sterilisation process used for the MF, the samples were contaminated with *E. coli* and *S. aureus* and IPA was used for decontamination. There was no observable growth in any of the plates of the sterilisation groups, including those containing the MF. These results show that the IPA sterilisation method was suitable for eradicating bacterial contaminants from PVA MF (Supplementary Fig. 7). Even though this method has proven efficient, for future applications of PVA MF, sterilisation methods more related with pharmaceutical industry reality and clinical application must be established.

3.6. Formulations characterisation

Morphology, size and distribution of NP are essential in the evaluation of nanocarrier formulations (Zeng et al., 2016), with particle size showing a significant effect on drug release rates. In our experiments, TMZ NP presented a circular shape analysed by SEM (Fig. 3B) and a mean size of 419.5 ± 52 nm and (Fig. 3C). Additionally, the particle surface charge determines its interaction with biological environments and its interaction with biologically active compounds (Giri et al., 2014). In general, optimum colloidal stability, where there is no particle aggregation, is improved when zeta potential is more positive or negative, around ± 45 mV (Krai et al., 2017). The zeta potential of the TMZ NP was found to be −31.6 ± 0.7 mV, therefore, we anticipated high stability and efficacy of the produced nanocarriers.

The MF were produced by electrospinning and were characterised according to their morphology and size using SEM (Fig. 3D-F). MF loaded with TMZ and iNek1 mixture presented a distribution size of 2723 ± 684 nm (Fig. 3D, E, Supplementary Fig. 8), while TMZ NP + iNek1 MF showed a distribution size of 2900 ± 1142 nm (Fig. 3F, Supplementary Fig. 8). Supplementary Fig. 8 shows TMZ + iNek1 MF after 2 h of incubation in PBS where the MF size increased only 2-fold indicating no significant water uptake, which would avoid possible side effects associated with sample swelling during the application *in situ*. Supplementary Fig. 8 shows curcumin MF with Blank NP inside, which could be used in future localised cell uptake *in vivo* studies.

The stability of the samples after drug encapsulation was examined by using NMR. In the ^{13}C analysis (Supplementary Fig. 9A), stearic acid Blank NP presented peaks in 14.7 ppm (C11), 32.5 ppm (C3) and 182.1 ppm (C1), the other carbon peaks were not obtained. TMZ NP presented these three peaks corresponding to the stearic acid and peaks related to TMZ: 36.8, 128.1, 137.1, 140.3 and 164.9 ppm (Łaszcz et al., 2013). PVA presents four NMR fingerprints peaks: a sharp peak related to CH_2 (around 45 ppm) and three CH-OH typical peaks (between 60 and 80 ppm). It was possible to observe these peaks in all MF samples (Supplementary Fig. 9B). No significant peaks related to the drugs are visible given that the drug concentrations are very low in the formulations when compared to the polymeric concentration, however, the TMZ NP + iNek1 MF sample presented peaks related to stearic acid. No shifting peaks were observed; therefore, these results suggested that the formulations were stable and because of that, TMZ and iNek1 bioactivities were preserved in the formulations (Vashisth et al., 2015).

To investigate the total percentage of encapsulated drugs into the formulations, the samples were totally dissolved in DMSO and the EE% was measured by using the absorbance values. The EE% of TMZ from the TMZ NP formulation was found to be 79.8 ± 6.8, whereas the EE% of TMZ from the MF formulation presented an EE% of 75.2 ± 5.9. The EE% of iNek1 from the MF formulation was 79.2 ± 7.3. Finally, TMZ + iNek1 MF were incubated in PBS buffer and the sample's weight was recorded daily to assess the MF weight loss. The results showed that the MF lost 50% of their initial weight after 30 days and 80% after 40 days (Supplementary Fig. 10).

3.7. MF drug release studies

Given that the GB site presents an acidic extracellular pH around 6.8 (Honasoge and Sontheimer, 2013), we decided to investigate the drug release using two different pH: 6.8 and 7.4. GB cells usually change their metabolic route from oxidative phosphorylation to glycolysis and when this happens the cells release lactate and H + generating an acid microenvironment, which has been associated with tumour progression and resistance (Honasoge and Sontheimer, 2013; Estrella et al., 2013).

The resulting drug release from the formulations provided a continuous release profile of TMZ and iNek1 over 8 days (Fig. 4A-D). The TMZ NP + iNek1 MF samples presented 50% release of TMZ after 3 days in pH 6.8 (Fig. 4A) and 6 days in pH 7.4 (Fig. 4C), whereas, in the TMZ + iNek1 MF formulation, TMZ was 50% released after 1 day in both pH: 6.8 (Fig. 4B) and 7.4 (Fig. 4D). This observation indicates the interesting approach of nanocarriers inside fibers to slow the release of drugs such as TMZ. iNek1 was 50% released after ~ 2 days in pH 6.8 (Fig. 4A, B) and pH 7.4 (Fig. 4B, D).

3.8. In vitro efficacy of formulations

To evaluate the efficacy of the formulations in decreasing GB cells viability, U87MG cells were used and tested in pH 6.8 media at three different treatment points: 2 days, 5 days or 5 (+2 days of recovery = media replenish) days and compared to the previously found IC_{50} of the drugs. For the control samples, the results (Supplementary Fig. 11) visibly shown a lack of cytotoxicity of the unloaded formulations, since the percentage of viable cells was >90% after all treatments (Blank MF, Blank NP, or Blank NP-loaded Blank MF). However, the MF might not facilitate cell attachment, and this can explain the slight decrease in cell viability.

All treatment strategies (with and without formulations) significantly decreased cell viability when compared to the non-treated cells at all treatment points ($p < 0.01$) (data not shown). After 2 days of treatment, the TMZ + iNek1 MF was more efficient in decreasing cell viability than the co-therapy without formulation (Fig. 4E), however, after 5 days of treatment no differences were found between the co-therapies (MF versus no formulation), nevertheless, the three co-therapy treatment groups (TMZ + iNek1, TMZ + iNek1 MF and TMZ NP + iNek1 MF) were more effective reducing cell viability when compared to TMZ alone (Fig. 4F). Interestingly, after 5 (+2 days of recovery) days, both TMZ + iNek1 MF and TMZ NP + iNek1 MF were more effective than the co-therapy without formulation (Fig. 4G). To confirm these results, the formulations were tested in 3D cell spheroids for 5 (+2 days of recovery) days (Fig. 4H). It was possible to observe that the co-therapy of TMZ + iNek1 significantly decreased spheroid size when compared to the vehicle-treated spheroids (Fig. 4H), however, no differences were found when compared to the drugs alone. Contrasting, both formulations, TMZ + iNek1 MF and TMZ NP + iNek1 MF, were able to significantly reduce spheroid size when compared to Blank MF, TMZ MF and iNek1 MF (Fig. 4H). This result suggests the importance of inhibiting Nek1 in GB to improve the response to TMZ, moreover, the prolonged and sustained treatment provided by the MF could significantly maintain the therapeutical effect of TMZ and iNek1.

3.9. In vivo efficacy in glioblastoma therapy

Next, it was evaluated if the formulations could provide higher concentrations of TMZ within the brain when compared to TMZ delivered via IP (Fig. 5A, B). Three animals/group were euthanised after 2 days of TMZ MF or TMZ NP + Blank MF implantation or TMZ IP treatment and their brains were collected for TMZ quantification. MF provided the highest concentration of TMZ in the brain, and it was statistically significant when compared to TMZ IP ($p < 0.001$) (Fig. 5B). Moreover, it was possible to observe a higher variation among the animals treated with TMZ NP + Blank MF, suggesting that this formulation provides less TMZ release uniformity, but still, the mean concentration was higher than for the TMZ IP-treated animals.

For the *in vivo* efficacy evaluation of the formulations, the tumour-bearing animals were implanted with Blank MF, TMZ MF, TMZ +

iNek1 MF, TMZ NP + iNek1 MF or treated with TMZ IP (once a day for five days; (Cancer), and were followed up for 60 days or until they reached end-point criteria (Fig. 5A). The survival analysis (Fig. 5C) demonstrated no statistical differences for the Blank MF when compared to TMZ MF or IP, where the Blank MF-treated rats' median survival was found to be 16 days, and 25 and 31 days for the TMZ IP and TMZ MF-treated groups, respectively. However, all animals treated with either co-therapy formulation (TMZ + iNek1 MF or TMZ NP + iNek1 MF) survived until the 60th day (Fig. 5C). With exception of the Blank MF-treated rats, all animals presented a positive body weight change after treatment (Fig. 5D), demonstrating that the treated rats were able to maintain or increase their weights. The lack of side effects observed was supported by the biochemical analysis (Supplementary Fig. 12), as we did not detect any significant sign of renal and pancreatic toxicity. Moreover, macroscopic analysis of kidneys and lungs revealed no signs of organ-specific toxicity (data not shown). Regarding liver function, ALT, which is a specific marker of liver damage, showed a significant decrease in all treatment groups, even so remaining within the reference values (21–52 IU/L). Similar results were observed in AST, which can also be found in muscles (reference values: 96–200 IU/L) (Palmeiro et al., 2003). Blank MF-treated rats showed increased values for both ALT and AST, however, due to the high SD of these groups, and the absence of significant differences in other markers, such as GGT and ALP, this set of results may be of no clinical relevance, suggesting that there was no collateral toxicity associated with the implants.

The tumour/brain area ratio of the rats implanted with either co-therapy MF was found to be reduced by 5-fold when compared to Blank MF-implanted rats ($p < 0.001$) (Fig. 5E, F), furthermore, both delivery strategies for TMZ (MF or IP) were able to significantly ($p < 0.05$) reduce tumour size when compared to the control (Fig. 5E, F). Other studies have shown promising results associated with the local implantation of fibers and wafers (Reviewed by (Norouzi, 2018), nevertheless, only a few studies have compared the formulations with the standard treatment using TMZ and/or other antitumour drugs, therefore, it is a challenge to predict the real efficacy associated with the implant formulations and if the risks associated with the implantation are worthwhile. Even though our study demonstrates great potential, TMZ and iNek1 release rates must be improved to decrease the probability of tumour recurrence.

Despite the fact that brain implants and wafers are effective in controlling tumour growth locally, several side effects have been previously reported, hence, this management strategy might be a double-edged sword because of the chance of severe symptomatic oedema within the brain (Kuramitsu et al., 2014), nonetheless, these side effects are strongly related to high concentrations of cytotoxic drugs. The results shown in this manuscript revealed that it is possible to achieve a successful treatment outcome with reduced drug concentration combined with a DDR-related kinase inhibitor by using a versatile and straightforward technique. Finally, the NP inside MF strategy could be used for a plethora of protein inhibitors, including DDR-related targets, and/or gene therapy such as RNA interference technologies that might need extra protection during delivery.

4. Conclusion

GB presents a high capacity of tumour recurrence, consequently, localised and controlled therapy approaches could provide an alternative to enhance chemotherapy efficacy and reduce systemic toxicity. In addition, GB resistance is related to oncotargets upregulation, and the impairment of their cellular activities could improve treatment efficacy. Nek1, one of these possible oncotargets, was previously related to cell proliferation and TMZ-resistance. Our results support previous findings (Zhu et al., 2016) and strongly suggest that Nek1 is an important target in GB cells and its inhibition significantly decreases cell viability when combined with TMZ. Furthermore, in this study, polymeric brain-implants prepared using TMZ and iNek1 were effectively produced, characterised and their anticancer efficacy was determined. The formulations revealed a high drug loading, which prolonged the drug's release improving the antitumor effects of iNek1 and TMZ. The produced brain-implants may be promising approaches for innovative *in situ* therapies, however, further research aiming to improve and prolong the drug release rate must be engaged.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Statement of authors' contributions to manuscript

L.S.R. conceived and planned the experiments, produced and characterised the formulations. L.S.R. and A.M.M. carried out cell experiments and statistical analysis. L.S.R., A.M.M., J.G.H., M.B.F, E.B. and P. O.S performed the *in vivo* experiments. P.R.A developed the *in silico* simulations and analysis. J.O.M, G.R.B. and C.S.D. contributed to sample preparation and drug quantification. M.C.H.B. conducted the sterilisation of the formulations. L.S.R. wrote the original draft. L.S.R. wrote the manuscript in consultation with A.M.M., J.G.H., M.N. and D.J.M. D. J.M. and M.N. acquired the funding used in this project. All authors reviewed the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2022.121584.

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4. DISCUSSÃO

Os DDS permitiram o desenvolvimento de muitos produtos farmacêuticos, que resultam na melhora da saúde de pacientes, tornando mais eficiente a entrega de fármacos a tecidos-alvo, minimizando o acúmulo de fármacos fora de tecidos-alvo, acarretando na diminuição de efeitos colaterais e facilitando a adesão de pacientes. À medida que as modalidades terapêuticas para determinadas doenças se expandiram além de pequenas moléculas, para incluir ácidos nucleicos, peptídeos, proteínas e anticorpos, os DDS foram adaptados para enfrentar os desafios que surgiram, convertendo modalidades terapêuticas promissoras em terapias eficazes (8). Por exemplo, a utilização de formas de dosagem oral de várias vezes ao dia para uma vez ao dia, proporciona conveniência aos pacientes e menor exposição a picos de alta concentração plasmática, que eventualmente poderiam causar efeitos colaterais.

Alguns desafios relacionados à entrega de fármacos, tais como, direcionamento, barreiras biológicas que limitam a entrega de moléculas e liberação controlada, podem ser totalmente ou parcialmente resolvidos com o uso de DDS. Por outro lado, além de várias vantagens, os DDS também podem apresentar algumas desvantagens, tais como: instabilidade, reações imunes e, em alguns casos, alta dosagem. Por isso, nano e microfibras podem melhorar a entrega de fármacos localmente e são uma das opções de DDS mais promissoras tanto para o uso como curativos no tratamento de feridas, como sistemas de entrega de quimioterápicos no local do tumor durante a ressecção tumoral.

Outros sistemas disponíveis podem apresentar desvantagens como, por exemplo, hidrogéis que apresentam absorção excessiva de fluidos e dificuldade de aplicação como observado nos hidrogéis de PVA contendo o extrato de *P. australis* (Capítulo 2). Outro exemplo são as nanopartículas não-biofuncionalizadas que podem ser degradadas rapidamente antes de chegar ao tecido-alvo e podem estar associadas com surgimento de tumores secundários ou metástases, tendo em vista que podem induzir um extravazamento de células cancerígenas (do inglês, *cell leakiness*), como foi revisado em (36), e observado nas nanopartículas produzidas em (36) (apêndice B). Portanto, esta tese propõe o uso de fibras poliméricas para diferentes aplicações biomédicas. Embora os desafios relacionados à implementação de DDS possam ainda afetar a funcionalidade de fibras, é possível afirmar que as

fibras poliméricas produzidas neste trabalho demonstraram resultados promissores *in vivo* e foram eficazes na entrega de fármacos.

As nanofibras e microfibras de PVA produzidas pela técnica de eletrofiação apresentaram características vantajosas e necessárias, incluindo propriedades mecânicas, perfil de liberação contínua e fácil aplicação. As nanofibras contendo o extrato de *P. australis* (Capítulo 2) são uma abordagem farmacológica diferenciada para o uso no tratamento de feridas, tendo em vista que o extrato foi obtido por meio de uma planta que já é utilizada na medicina popular, portanto, pode ser aplicada clinicamente. Uma vez que o PVA já é aprovado pelo FDA para uso humano, esta formulação possui um potencial translacional significativo.

No contexto do tratamento de GB, o presente estudo desenvolveu e caracterizou microfibras de PVA para entrega de temozolomida e um inibidor de Nek1 (Capítulo 6). O GB é uma doença extremamente agressiva, no entanto, a atual terapia para tratar GB não sugere resultados eficientes ao longo prazo e a cura é uma realidade distante. O uso de DDS pode ser visto como uma abordagem promissora para a terapia GB, no entanto, existem várias respostas desconhecidas que precisam ser mais investigadas. O GB pode recorrer e invadir áreas funcionais do cérebro, porém, a segunda ressecção cirúrgica é arriscada. Portanto, a implantação de sistemas após o ato cirúrgico é uma opção de tratamento interessante e pode preencher a lacuna de tratamento entre a ressecção cirúrgica e o início da radioterapia e quimioterapia convencionais, possivelmente diminuindo os riscos de recorrências, uma vez que estudos de autópsia sugerem que os GB recorrentes são principalmente locais e aparecem dentro de 2 centímetros do local inicial do tumor. Assim, um tratamento *in situ* com DDS implantáveis possibilitaria a resolução de alguns problemas relacionados à terapia convencional de GB.

Apesar dos resultados apresentados neste trabalho e na literatura serem promissores, poucos implantes cerebrais atingem ensaios clínicos. Atualmente, as pastilhas de Gliadel® são o único DDS implantável com autorização de comercialização e indicado no tratamento de GB recém-diagnosticado ou recorrente (55). O implante consiste em um copolímero associado à carmustina (também conhecida como BCNU) em forma de discos de 1,4 cm de largura e 1 mm de espessura. No entanto, após a implantação dessas pastilhas, vários efeitos colaterais foram observados, incluindo convulsões, hipertensão intracraniana, meningite, e edemas cerebrais (55) (FDA, referência: 3358686. Disponível em: https://www.fda

.gov/). Algumas dessas complicações podem estar ligadas às características rígidas do implante que podem causar micro-rasgos no local na implantação (55). Portanto, é necessária uma adequação destes implantes para evitar efeitos adversos. Ainda, visando uma aplicação clínica, as microfibras produzidas neste trabalho devem ser aprimoradas para prolongar a liberação dos fármacos.

É importante ressaltar que apesar das melhorias nas técnicas neurocirúrgicas, nem todos os GB são necessariamente operáveis. Assim, a terapia de GB baseada em implantes não é uma opção para todos pacientes (56). Outras estratégias, visando contornar a BHE e a barreira sangue-líquido cefalorraquidiano, como o uso da via de administração intranasal, devem ser implementadas concomitantemente.

De maneira geral, como perspectivas na área de produção de DDS, ensaios pré-clínicos e clínicos devem ser realizados para analisar as características farmacológicas das formulações e a biodistribuição, e eventualmente explorar novas rotas e vias de administração que superem barreiras biológicas e fisiológicas. Somente com estes tipos de avaliações será possível obter terapias otimizadas. Ainda, também é essencial desenvolver e patronizar métodos de avaliação de DDS. Isso é extremamente necessário para entender, não apenas a variação entre formulações e resultados entre laboratórios, mas também, para compreender as propriedades físico-químicas dos polímeros, a resposta imune que os sistemas podem desencadear em diferentes aplicações, as mudanças no microambiente tumoral quanto utilizados neste contexto, e a dinâmica entre a matriz polímerica e os fármacos. Neste contexto, análises de dinâmica molecular, *docking* computacional e modelagem molecular foram extremamente importantes na construção deste trabalho, tendo em vista que são estratégias úteis para avaliar o comportamento dos sistemas e podem ser utilizadas para otimizar formulações.

Em síntese, modalidades terapêuticas baseadas em DDS poliméricos podem ser usadas para melhorar e direcionar a entrega de fármacos conhecidos e novos compostos para o tratamento de diversas doenças. É importante destacar que a entrega local de fármacos com o uso de implantes poliméricos pode ser considerada para diversas patologias que requerem este esquema terapêutico. Por fim, para o progresso imediato e de longo prazo no campo de desenvolvimento de DDS deve-se utilizar tempo e recursos para ideias de pesquisa básica, translacional e clínica de maneira unificada a fim de produzir sistemas com objetivos realistas e aplicáveis clinicamente.

5. CONCLUSÕES

Este trabalho objetivou a produção de formulações baseadas em sistemas poliméricos para o uso no tratamento de cicatrizes e de glioblastomas. Foi possível concluir que as formulações apresentam resultados promissores para o uso *in vivo*. As formulações contendo o extrato hidroetanólico de *P. australis* foram eficazes *in vitro* e *in vivo* no tratamento de feridas. As nanofibras de PVA produzidas podem eventualmente serem utilizadas como curativos capazes de proteger feridas e acelerar o processo de cicatrização, devido à liberação prolongada do extrato. No contexto do tratamento de glioblastomas, o uso das microfibras de PVA contendo temozolomida e o inibidor da proteína Nek1 melhorou a eficácia do tratamento *in vivo* quando comparado com a quimioterapia convencional. Ainda, o uso de implantes baseados em sistemas poliméricos possibilita uma nova modalidade de tratamento para cânceres cerebrais e abre uma oportunidade para o uso de uma série de moléculas que são promissoras para o tratamento mas apresentam limitações em relação a suas estruturas químicas e estabilidade.

Por fim, a elaboração de revisões da literatura e capítulos de livro é essencial em qualquer área da ciência uma vez que possibilita a construção do conhecimento em determinada área de pesquisa. Tendo em vista que o uso de sistemas poliméricos no tratamento de doenças é uma área relativamente nova mas em rápido crescimento, os capítulos apresentados nesta tese podem contribuir com a compreensão das pesquisas existentes e podem ajudar a consolidar o que já se sabe sobre os DDS, permitindo a identificação de eventuais lacunas de conhecimento.

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APÊNDICE A

Natural Polysaccharides for the Delivery of Anticancer Therapeutics

Capítulo de livro publicado no livro Natural Polysaccharides in Drug Delivery and Biomedical Applications.

CHAPTER 19

Natural polysaccharides for the delivery of anticancer therapeutics

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Chapter Outline

List of abbreviations

AE-CS-CP-Fe-bLf	Alginate-enclosed chitosan—calcium phosphate iron—loaded bovine lactoferrin nanocapsules
ANP	Atrial natriuretic peptide
bFGF	Human basic fibroblast growth factor
bLf	Bovine lactoferrin
BMP-4	Bone morphogenetic protein
BoHc/A	*Clostridium botulinum* type-A neurotoxin
BSA	Bovine serum albumin
CC	Cytochrome C
CD	Cyclodextrins
CMC	Carboxymethylcellulose

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CMCS-FA	Carboxymethyl chitosan−folate
CS NPs	Chitosan-based nanoparticles
CS	Chitosan
DDS	Drug delivery system
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
DTX	Docetaxel
EPR	Enhanced permeability and retention effect
FA	Folic acid
Fe-bLf	Iron-saturated bovine lactoferrin
FR (−)	Folate receptor overexpression negative tumor
FR (+)	Folate receptor overexpression positive tumor
GA	Gambogic acid
GE-11, E3 and K3	Peptides
GrB	Granzyme B
HA	Hyaluronic acid
HA-TCA	Hyaluronic acid−taurocholic acid
HIV	Human immunodeficiency virus
miRNA	Microinterfering RNAs
NABDs	Nucleic acids−based drugs
NK	Natural killer cells
NPs	Nanoparticles
PAMAM	Poly(amidoamine)
PDS	Polysaccharides-based delivery systems
PEG	Poly(ethylene glycol)
PEI	Poly(ethyleneimine)
Pep/pro	peptide and protein
PLGA	Poly(lactide-co-glycolide)
PLLD	poly(L-lysine) dendrons
PSC	Polysaccharide
PTX	Paclitaxel
RISC	RNA-inducing silencing complex
RNA	Ribonucleic acid
siRNAs	Small interfering RNAs
TAT	Trans-activator of transcription protein
TRAIL	Tumor necrosis factor−related apoptosis-inducing ligand
VEGF	Vascular endothelial growth factor

List of cell lines

1205Lu	Metastatic melanoma
143B	Bone osteosarcoma
4T1	Murine mammary cancer
9L	Gliosarcoma rat
A2780	Ovary carcinoma
A375	Malignant melanoma
A549	Lung adenocarcinoma
AA8	Chinese hamster ovary
AGS	Stomach gastric adenocarcinoma
B16F10	Mouse melanoma
Caco-2	Colon cancer

Cal-27	Oral cancer
Chago	Bronchogenic carcinoma
CNE-2	Nasopharyngeal carcinoma
CT26	Murine colon carcinoma
DLD1	Colon cancer
Gl261	Murine glioma
H460	Non-small cell lung cancer
H9	Lymphoma
H9C2	Rat cardiac
HaCat	Keratinocytes
HCT116	Colon carcinoma
HeLa	Cervical cancer
HepG2	Liver cancer
HeyA8	Ovarian serous adenocarcinoma
HNE-1	Epithelial tumor
HT1080	Fibrosarcoma
HT29	Colon adenocarcinoma
HUVEC	Umbilical vein endothelial
IPA220	Signet ring cell gastric adenocarcinoma
J774A	*Mus musculus* ascites reticulum
Jurkat	Lymphoma
KB	Mouth epidermal carcinoma
LLC	Lewis lung carcinoma
MCF7	Breast cancer
MDA-MB-231	Breast cancer
MDA-MB-468NL	Breast cancer
MDCK	Madin−Darby canine kidney
MHCC-97H	Hepatocellular carcinoma
MOEC	Mouse endothelial
Namalwa	Lymphoma
NCI-H358	Non−small cell lung carcinoma
NCI-N87	Gastric carcinoma
OVCAR-8/TR	Ovarian cancer
PC3	Prostate cancer
RH7777 HCC	*Rattus norvegicus* liver hepatoma
RPMI 8226	Myeloma
SCC7	Squamous carcinoma
SK-LU1	Lung adenocarcinoma
SK-MM-1	Multiple myeloma
SKOV3	Ovarian carcinoma
SU-DHL-4	Lymphoma
U343MG	Brain glioblastoma
U87MG	Brain glioblastoma

1. Cancer overview

Cancer is a multifaceted disease that is one of the predominant causes of human morbidity and mortality [1]. GLOBOCAN 2012 estimates that the new cases projection for 2025 will reach approximately 20 million cases [1]. This disorder comprises a group of more than a

hundred diseases that include the uncontrolled cell division [2]. The main approaches to treat cancer are surgery, radiation therapy (RT), chemotherapy (CT), and immunotherapy [3]. The two first approaches can eradicate localized tumors; however, for cancers that have metastasis, only CT and immunotherapy are efficient against them [4]. Despite CT being the most commonly applied treatment, it has several limitations, such as high systemic toxicity, insufficient quantity of delivered therapeutics, water insolubility of the drugs, nonspecific biodistribution, and delivery of drugs to healthy cells [3,5]. Under these circumstances, smart, harmless, and efficient drug delivery systems (DDSs) are necessary to improve cancer treatment [1]. Recently, there have been significant efforts in achieving effective approaches to treat cancer, including bioactive molecules, such as nucleic acids, peptides, and proteins. To accomplish the ideal delivery of these novel molecules and common CT drugs, it is important to use appropriate DDS; one of the most promising candidates is polysaccharide-based delivery system (PDS) [6].

2. Importance of polysaccharides-based delivery systems (PDSs) in cancer therapy

The necessity for more targeted and controlled drug delivery has been the central reason for the development and design of PDS. The main benefit related to the PDS utilization is its easy modification and manipulation to achieve adequate delivery. The clinical effectiveness of CT and functional biological macromolecules is frequently restricted by several obstacles, as pointed out by Miao et al. [7] and Ranjbari et al. [6], such as

a. insolubility, insufficient cell uptake;
b. loss of bioactivity before reaching the tumor site;
c. short half-life owing to enzymatic degradation and rapid renal clearance;
d. CT resistance owing to the overexpression of proteins including efflux transporters; and
e. side effects in view of the systemic delivery.

The PDS development has become one important method to address difficulties in the treatment of complex diseases and to develop personalized medicine, especially in cancer treatment [7]. Polysaccharides are natural polymeric molecules that can undergo several chemical modifications, owing to the presence of various functional groups such as amine, carboxyl, and hydroxyl. These groups provide hydrophilicity and support bioadhesion between the PDS and the biological tissue [8–11]. The significant biocompatibility of PDS and the option of multifunctional modification in the PSC structure made this system a unique DDS for cancer therapy [7]. PDS bioproducts can achieve favorable properties such as optimal size and solubility in the same fashion. They can be designed to improve the drug circulation time, and if they were designed with adequate biomarkers, they can reach the tumor site and target the cancer cells. Additionally, PDSs have increased permeability, and because of the modification with ligands, they have enhanced

permeability and retention effect (EPR) [12] because they are able to accumulate in cancer cells while delivering CT or biocompounds, and this can increase the targeting efficacy of common delivery systems. Modification with ligands on the surface of PDS products also aids the delivery and decreases the common unwanted effects of CT drugs [13].

3. PDS for cancer treatment

PDS represents noteworthy DDS that can be easily developed into several pharmaceutical structures, including hydrogels, micelles, nanogels, nanocarriers, and nanoparticles (NPs) [6]. Because of different physiochemical features, each structure can encapsulate agents in different ways, modifying the release and effectiveness of the final product [14]. Moreover, PDS can be prepared as complexes or conjugates with bioactive compounds such as peptides and proteins (pep/pro) and traditional CT drugs [15]. Hence, the main strategies to design the PDS with targeted and controlled release are as follows:

a. Delivery to tumor site according to its environmental features, such as ion concentration, pH, redox potential, temperature, and specific molecules [16].
b. Delivery to tumor site using specific receptors or targeting agents (protein, peptides, aptamers, and folate) to improve targeting and cell uptake [17,18].

These features of PDS make them promising materials to produce smart systems that are able to deliver the appropriate amount of drugs at specific sites in response to determined physiological stimuli [19]. Additionally, agent-conjugated polysaccharides are one interesting approach to produce biocompatible formulations that can diminish the undesirable effects of conventional therapy [6]. The next sections will focus on PDS for the delivery of relevant antitumor drugs, nucleic acids, peptides, and proteins.

3.1 Delivery of CT drugs

Most conventional CT drugs are distributed systemically with the possibility of inducing several secondary effects. Consequently, the use of PDS for cancer treatment can improve therapy outcome, with less amount of the drug. The PDS has been extensively studied in several tumor cells combined with different CT drugs. Some studies are mentioned in Table 19.1.

Chitosan (CS) is a polysaccharide produced from chitin, a component of crustacean exoskeletons. It has been extensively investigated for tumor targeting because of some important features such as mucoadhesive, absorption enhancer, and controlled release facilitator [62]. Some recent studies [27,28] using CS as delivery systems suggested that this PDS is an important vehicle for drug delivery specially in colon-targeted cancer therapy. In this regard, Liang et al. [28] produced pH-responsive hydrogels for delivery of doxorubicin (DOX). These hydrogels effectively killed HCT116 colon tumor cells and

Table 19.1: Polysaccharides-based delivery system of CT drugs.

System	Drug or agent	Cancer type	Evaluation model	Outcomes	References
Chitosan					
Chitosan–alginate multilayer microcapsules	Doxorubicin	Hepatoma	HepG2 and xenograft mice (balb/c/nu mice)	Induced apoptosis of tumor cells both in vitro and in vivo	[20]
N-trimethyl chitosan nanoparticles	Cisplatin–alginate complex	Human ovarian and lung cancer	Human A2780 and A549 cells	Induced apoptosis	[21]
Nanoparticles	Doxorubicin–dextran complex	Several tumors	Xenograft mice	Induced apoptosis and decreased tumor size	[22]
Nanoparticles	Paclitaxel	Melanoma and non–small cell lung carcinoma	Xenograft mice	Strong antitumor activity	[23]
Nanoparticles	Docetaxel	Glioblastoma and non–small cell lung carcinoma	NCI–H358 and U87MG cells, BALB/c mice, and xenograft mice	Enhanced cell uptake in vitro and decreased side effects in vivo	[24]
Nanoparticles	Paclitaxel and Cy5.5	Squamous cell carcinoma	SCC7 cells and xenograft mice	Enhanced stability and cell uptake and decreased tumor size	[25]
Nanoparticles	Doxorubicin and Cy5.5	Fibrosarcoma	HT1080 cells	The pH-responsive NPs improved drug accumulation into the cells	[26]
Nanoparticles	Chlorine e6	Squamous cell carcinoma and adenocarcinoma	SCC7, HT-29 cells, and xenograft mice	Enhanced accumulation in the tumor site	[27]
Hydrogels	Doxorubicin	Colon cancer	HCT116 cells	pH-responsive hydrogels exhibited good mucosal adhesion and efficacy	[28]
Nanocarrier	Doxorubicin	Breast cancer	MCF-7 cells	pH-responsive carrier enhanced cell uptake and apoptosis	[29]
Nanogels	5-Fluorouracil	Melanoma	Porcine tissue (ex vivo), HaCat cells, and swiss albino male mice	Improved tumor inhibition	[30]
Nanocarriers	Methotrexate and pemetrexed	Lung cancer	A549 and LLC cells and xenograft mice	Improved cytotoxicity and presented a good synergistic anticancer efficacy	[31]

Nanoparticles	Doxorubicin and rose Bengal	Oral cancer	Cal-27 cells	Improved photodynamic therapy efficacy	[32]
Hyaluronic acid					
Nanoparticles	Cy5.5	Xenograft subcutaneous dorsa of athymic nude mice	Xenograft mice	NPs accumulated in the tumor site with a combination of passive and active targeting mechanism	[33]
Nanoparticles	Cisplatin	Human malignant gliomas	U343MG and U87MG cells	Induced apoptosis	[34]
Nanoparticles	Cisplatin, siRNA, and indocyanine green	Lung cancer	Xenograft mice	Overcome multidrug resistance in xenograft model and induced apoptosis	[35]
Nanoparticles	Doxorubicin	Breast cancer	MDA-MB-231 cells	Decreased cell viability more effectively than the drug alone	[36]
Nanocarriers	Doxorubicin	Breast cancer	MDA-MB-468NL cells and xenograft mice	pH-sensitive NPs reduced tumor growth more efficiently than the drug alone and increased mice survival rate	[37]
Nanocarriers	Cisplatin	Lung cancer	A549 cells and xenograft mice	Increased drug efficacy and decreased side effects	[38]
Nanoparticles	Paclitaxel	Head and neck cancer	SCC7 cells and xenograft mice	Increased anticancer effectivity, accumulation in the tumor site, and decreased tumor growth	[39]
Gold nanocluster	Doxorubicin and several other drugs	Breast cancer	MCF7 cells	Decreased cell viability	[40]

Continued

Table 19.1: Polysaccharides-based delivery system of CT drugs.—cont'd

System	Drug or agent	Cancer type	Evaluation model	Outcomes	References
Nanoparticles	Docetaxel and Disulfonate tetraphenyl chlorin	Cervical cancer and breast cancer	HeLa and MDA-MB-231 cells	Produced a strong synergism between chemo- and photodynamic therapies	[41]
Nanoparticles	Paclitaxel and indocyanine green	Breast cancer	4T1 cells and mice-bearing orthotopic 4T1	Increased tumor targeting and chemophotothermal therapies	[42]
Nanocarriers	Doxorubicin and 5-aminolevulinic acid	Breast cancer	MCF7 cells	Enhanced cytotoxic effect	[43]
Dextran					
Microcapsules	Doxorubicin	Cervical cancer	HeLa cells	pH-sensitive capsules increased cell uptake in acid pH and reduced cell viability	[44]
Nanocarriers	Doxorubicin and irinotecan	Breast cancer and colon cancer	MCF7 and DLD1 cells	Combined administration improved anticancer effect	[45]
Nanogels	Doxorubicin	Breast cancer	MCF-7 cells	pH-responsive gel improved cell uptake	[46]
Nanocarriers	Doxorubicin	Lymphoma	Jurkat, H9, Namalwa, and SU-DHL-4, H9C2 cells and xenograft mice	Improved antilymphoma activity and decreased cardiac toxicity	[47]
Pullulan					
Nanoparticles	Paclitaxel	Colon carcinoma	HCT116 cells and xenograft mice	Increased effectiveness and reduced side effects	[48]
Nanoparticles	IR780 and paclitaxel	Hepatocellular carcinoma	MHCC-97H cells and xenograft mice	Inhibited tumor growth and tumor angiogenesis	[49]
Gold nanoparticles	Doxorubicin	Bronchogenic carcinoma	Chago cells	Improved doxorubicin cytotoxic and exhibited less toxicity to normal cells	[50]

Cellulose					
Microspheres	Daunorubicin	Cervical cancer	HeLa cells and mouse	Decreased cell viability	[51]
Nanocarriers	Tetrahydrocurcumin	Colon cancer	HT-29 cells	Increased toxicity in cancer cells compared with normal cells	[52]
Nanogels	Temozolomide and CdSE quantum dots	Melanoma	B16F10 cells	Did not alter the potency of the drug	[53]
Hydrogels	Doxorubicin	Melanoma	A375 cells	Showed efficacy against skin melanoma cells	[54]
Nanoparticles	Coumarin and curcumin	Breast cancer	MCF7 cells	Improved therapy to cancer cells	[55]
Alginate					
Nanoparticles	Doxorubicin	Liver tumor	Kunming mice	Tumor necrosis without affecting normal cells	[56]
Micelles	Doxorubicin and FCR-675	Squamous cell carcinoma	Xenograft mice	Significant anticancer activity in vivo without any side effects	[57]
Liposomes	Cisplatin and Cy5.5	Human caucasian ovary adenocarcinoma	EGFR-positive SKOV3 cells and xenograft mice	Enhanced delivery and antitumor efficacy, while reducing side effects (e.g., nephrotoxicity)	[58]
Nanocarriers	6-Gingerol and doxorubicin	Breast and liver cancer	MCF7 and HepG2 cells	Exhibited ability to selectively kill cancerous cells	[59]
Others					
Mauran/chitosan nanoparticles	5-Fluoracil	Breast cancer	MCF7 cells	Enhanced antiproliferative activity and increased cell uptake	[60]
Arabinogalactan/folic acid nanoparticles	Methotrexate	FA overexpressed cells	AA8 cells	Increased cytotoxic activity	[61]

showed effective antibacterial properties. In addition, after subcutaneous injections of the formulations, the hydrogels exhibited strong adhesion to the tissue, showing that these formulations are an interesting approach for localized drug delivery. In another study, using a pH-responsive nanocarrier for the delivery of DOX as well, Abazari et al. [29] produced bio−metal−organic−CS nanostructures and tested them against MCF-7 breast cancer cells. The results suggested that this carrier greatly enhances cell uptake and the rate of apoptosis. Another promising pH-responsive nanogel was produced by Sahu et al. [30]. They loaded 5-fluorouracil into the CS nanogels for topical treatment of aggressive melanoma. The ex vivo skin permeation assay was done using porcine skin, and the formulation presented good penetration potential. Moreover, dimethyl benzene anthracene was used to chemically induce melanoma formation in Swiss albino mice model, and the nanogels exhibited significant tumor inhibition.

It is known that combined CT is important to achieve synergic anticancer effects in the clinic, improving outcomes and suppressing drug resistance [31]. Using this approach, Chen et al. [31] developed methotrexate plus pemetrexed CS-methoxy poly(ethylene glycol) (mPEG) NPs and evaluated it using A549 human lung adenocarcinoma epithelial and LLC Lewis lung carcinoma cell lines, revealing the anticancer efficacy of the formulation. Using a lung cancer mouse model, NPs treatment revealed a significantly sustained drug circulation in the body and drug accumulation in the tumor site. This codelivery strategy provides a prospective treatment against lung cancer. Using a similar approach, Zhang et al. [32] designed Rose Bengal and DOX-loaded CS NPs for combination of photodynamic and CT treatments. The authors observed an inhibitory effect on Cal-27 oral cancer cells. Thus, the NPs featured strong efficacy on photodynamic therapy and excellent photosensitizer features.

Recently, several authors have been studying photodynamic and CT cotherapies [41−43]. Using an endogenous polysaccharide that exists in the extracellular matrix [63] called hyaluronic acid (HA), Gaio et al. [41] produced HA double-layered NPs for the delivery of docetaxel (DTX) and the photosensitizer disulfonate tetraphenyl chlorin (TPCS2a). The codelivery was tested against HeLa cells (resistant to CT and nonresistant) and MDA-MB-231 breast cancer cells. The results suggested that the combination of drugs induced high synergism effect; furthermore, DTX dose could be decreased by ∼2.6- and 10.7-fold in HeLa (nonresistant) and MDA-MB-231, respectively. Additionally, codelivery had great efficacy killing resistant HeLa cells with overexpression of P-glycoprotein 1.

To achieve a synergistic CT-photothermal therapeutic effect and to deliver paclitaxel (PTX) and indocyanine green to mice-bearing orthotopic 4T1 breast tumor, Zhao et al. [42] produced HA NPs that showed enhanced photothermal effect and cytotoxicity in cancer cells. In a different study, novel multifunctional HA-based nanocomplexes with pH-responsive surface were prepared to deliver DOX and 5-aminolevulinic acid.

The authors added anti-HER2 antibody onto the NPs surface to achieve active targeting. This strategy significantly improved cell uptake and cytotoxicity in MCF-7 cells, suggesting that the produced NPs are an excellent approach for targeted codeliver therapy to treat breast cancer [43]. Therefore, it seems that the combination of CT and photodynamic approaches is emerging as promising anticancer strategies.

3.2 Delivery of nucleic acids–based drugs (NABDs)

The development of molecular biology and chemistry techniques promoted an increase in new technologies aimed for therapeutic applications. Gene therapy is an outstanding technology to treat genetic disorders and complex diseases. This approach restores deficient protein production, modulates gene expression, and can silence an oncogene [64]. The delivery possibilities include short sequences of DNA and RNA, as antisense oligonucleotides, small interfering RNAs (siRNAs), decoy oligonucleotides, microinterfering RNAs (miRNA), aptamers, triple helix–forming oligonucleotides, ribozymes, and DNAzymes [19]. The majority of the research in this area has focused on delivery of a single nucleic acid strategy (e.g., siRNA) against a single gene target.

One of the most commonly used strategies to specifically target cancer cells is the use of siRNA, in which a synthetic siRNA triggers mRNA degradation in a sequence-dependent form of overexpressed genes that contribute to uncontrolled growth of the malignant cells [65,66]. SiRNA filaments associate with a multiprotein RNA-inducing silencing complex (RISC) bond to target mRNA by complementarity and trigger its cleavage by Argonaute-2, an enzyme residing within the RISC complex [67]. The siRNA can be easily synthesized owing to its short length and it has the ability to knock down a large number of different genes overexpressed in malignant cells [19].

A key limitation of clinical perspectives on the use of these strategies has been problems associated with delivery of the nucleic acids [68]. Several problems are related to pure form administrations. Before reaching the target tissue, there are many possibilities of eliminations such as nuclease activity, elimination by kidney filtration, and activation of the immune system [19]. In addition, negative charges of NABDs and the hydrophilicity profile can hinder cell membrane crossing. In the cells, pure form of NABDs can be degraded by nucleases or be sequestrated into endosomes without the possibility of reaching the target. Consequently, successful NABDs delivery will depend on appropriate encapsulating systems. Many advanced PDSs have been developed and applied in combination of cancer therapies to achieve a treatment that is less toxic and more effective [19].

Approaches combining natural polysaccharides and NABDs can be used to enable efficient carrying and selective intracellular delivery. Table 19.2 describes some carrier-mediated polysaccharides used to deliver RNA and DNA.

Table 19.2: Polysaccharide polymers to delivery of nucleic acids–based drugs.

Polysaccharide	NABD	System	Evaluation model	References
Hyaluronic acid	siRNA/miRNA	DPA-polymeric NPs	HCT116 cells and xenograft mice	[68]
	MDR1 siRNA	HA-PEI/HA-PEG NPs	OVCAR8TR cells and xenograft mice	[69]
	PKM2 and *MDR1* siRNA		SKOV-3 cells and xenograft mice	[70]
	siRNA	HA-modified PLGA–PEG copolymer NPs	U87 and HepG2 cells	[71]
	siRNA VEGF	Lipid–polycation–HA (PolyMetformin) NPs	H460 and 1205Lu cells	[72]
	*PLXDC1*siRNA	CS NPs coated with HA	A2780, HeyA8, SKOV3, HUVEC, and MOEC cells	[73]
	AKT siRNA	siRNA/protamine nanocomplex–protected HA-TCA conjugate to oral administration	MDCK, HepG2, and HCT-116 cells and CT-26 xenograft mice	[74]
	Plasmid DNA expressing wt *P53* and microRNA-125b	HA-PEI/PEG	SK-LU-1 and J774 cells and KP mice	[75]
	Plasmid of recombinant methioninase	HA-G5 PAMAM-Au	GC cells and xenograft mice	[76]
Chitosan	miRNA34a	CS-poloxamer 188 and PLGA nanocomplexes	RPMI8226 and SKMM1 cells and xenograft mice	[77]
			PC3 cells and xenograft mice	[78]
	CDX2 siRNA	Imidazole–CH NPs	AGS and IPA220 cells and mice	[79]
	miR-281 and AEG-1 siRNA	CS–folic acid nanogel	U87MG cells and xenograft mice	[80]
			143B cells and xenograft mice	[81]
	siRNA against Luc	NP-siRNA-GPC3 Ab NPs	RH7777 HCC cells and xenograft mice	[82]
	GAL1 siRNA	Intranasal formulation of CS NPs	GL261 cells, human primary culture glioblastoma cells, and xenograft mice	[83]
Cyclodextrin	Plasmid DNA encoding *P53*	FA-modified CD core with PEI arm	KB and A549 cells	[84]
	MMP-9 siRNA	FA-poly(L-lysine)	HNE-1 cells and BALB/c mice	[85]
			HNE-1 and CNE-2 cells and xenograft mice	[86]
	PKM2 siRNA	Mesoporous silica NPs (MSNPs)	MDA-MB-231 cells and xenograft mice	[87]
	siRNA targeting M2 subunit of ribonucleotide reductase	Cyclodextrin complexed with PEG and a targeting agent	*Clinical trial*	[88,89]

HA is an endogenous polysaccharide that binds specifically to Cluster Determinant 44 (CD44) receptors [63]. The adhesion molecule CD44 is a transmembrane glycoprotein and the major cell surface receptor, being related to cell−matrix and cell−cell adhesion [90]. Normal cells express low levels of this receptor; however, malignant cells have overexpression of a CD44 variant [91]. The integration triggers extracellular conditions favorable to alteration of cellular form for proliferation and migration of cancerous cells, being associated with mesenchymal epithelial transition and metastasis [91,92]. There are several studies that explore this, particularly in cancer research, because it is possible to devise strategies for a more specific tumor therapy, exploring the affinity of the HA with CD44 [93].

To allow a clinically viable therapeutic application, negatively charged NABDs are complexed with a polycation system by electrostatic interactions, chemically modified or complexed with engineered RNA receptor. These complexes can be used in combination with DDS, allowing the target of tumor cells [68]. Choi et al. [68] combined an artificial RNA receptor Zn(II)-dipicolylamine (DPA/Zn) that holds a high affinity to RNA molecules, with HA-based polymeric NPs to allow efficient RNA delivery and provide tumor target ability. Yang et al. [69] chemically conjugated NPs of HA with PEG (HA-PEG) or poly(ethyleneimine) (HA-PEI) to encapsulate *MDR1* siRNA. The authors demonstrated that NPs efficiently downregulated expression of *MDR1* and the protein product of this gene, P-glycoprotein. This ATP-dependent transporter was associated with drug resistance, thereupon its downregulation increased cell sensitivity to PTX in ovarian cancer both in vitro and in vivo [69]. The same formulation of HA-PEG and HA-PEI was used by Talekar et al. [75] to cosilencing *PKM-2* and *MDR-1*. It was proved that downregulation of these genes in resistant ovarian cancer cells improves the efficacy of PTX. Lin and Lee [71] used a similar strategy of NPs engineering, when they synthesized a copolymer of HA with poly(lactide-co-glycolide) (PLGA) and PEG, showing that both copolymers exhibited a pH-dependent release and had a faster release in acid environment. In virtue of acid extracellular pH of the tumor tissue, pH-responsive RNA nanoformulation can increase the efficacy of treatment with a decrease of side effects [94]. Besides that, the authors observed a significant transfection capacity higher in overexpressed CD44 cells. Kim et al. [73] developed PLXDC1 siRNA-incorporated CS NPs (CS NPs/siRNA) coated with HA to target the CD44 receptor on endothelial tumor cells. It is known that PLXDC1 promotes cancer cell migration and invasion; therefore the inhibition using this approach resulted in significant decrease of cell proliferation, reduced microvessel density, and an increased cell apoptosis mainly in CD44+ tumor endothelial cells, showing a useful application for antiangiogenic tumor therapy. Inspired by enterohepatic recycling of bile acids, which allows reabsorption of steroidal amphiphilic molecules, and the CD44-mediated selective uptake of HA conjugates by cancer cells, Hyun et al. [74] developed a siRNA/protamine nanocomplex protected by a multifunctional

HA−taurocholic acid (HA-TCA) conjugate to oral administration. This system was used to silence AKT, a serine threonine kinase that plays central functions in cell signaling pathways, modulating survival and death in colorectal cancer.

Multidrug resistance impacts negatively in clinical practice of several tumors. Thus, the use of PDS NPs can provide an enhancement of nucleic acid molecules delivery as well as an increase of cancer cells sensitivity to drug treatments. An interesting codelivery strategy was developed by Zhao et al. [72]. They synthesized a PolyMetformin through conjugation of PEI with dicyandiamide. In this system, the cationic charges delocalization of PolyMetformin reduces the toxicity of PEI and permits entrapment of siRNA core membrane−structured lipid−polycation−HA NPs for systemic gene delivery. The system applied shows anticancer efficacy of Metformin and successfully enhances tumor suppressive efficacy through knockdown of vascular endothelial growth factor (VEGF) siRNA.

Significant understanding of genetics of cancer allows the use of strategies based on plasmid DNA to supplement downregulation, replace mutated genes, or regulate substrate levels necessary for cancer growth. Talekar et al. [70] formulated HA-PEI/PEG encapsulating plasmid DNA expressing wild-type *P*53 and microRNA-125b in SK-LU1 cells as well as mouse models of lung cancer. The authors demonstrated an increase of apoptosis in vitro and a growth inhibition and apoptotic induction in vivo, indicating that gene therapy using dual HA CP vector is a promising option for lung cancer treatment. Li et al. [76] synthesized NPs of HA−poly(amidoamine) (PAMAM) and encapsulated in gold NPs. The objective was to deliver a plasmid of recombinant methioninase, knowing that cancer cells normally require high levels of this amino acid to self-growth. Targeting cancer stem cells, which are related to therapy resistance and have CD44 as a surface marker, the transfection decreased methionine levels and inhibited proliferation of NCI-N87 gastric carcinoma cells and reduced tumor growth by targeting mitochondrial functions. These results indicated HA efficiency on stabilizing nucleic acids and penetrating cell membranes through endocytosis mediated by interaction with CD44. The improvement of specificity increases therapeutic efficacy and decreases side effects [95].

CS has been widely used as NPs carrier agent mainly because of its cationic nature that permits electrostatic interactions with negatively charged molecules. CS is known to be biocompatible, minimally toxic, nonimmunogenic, degradable by enzymes, and very stable [96]. Besides that, CS is able to attach to bladder urothelium and its high viscosity impairs its excretion. CS-based DDS increases penetration of the bladder barrier and it has the ability to deliver substantial amounts of siRNA across urothelium and tumor site, improving therapeutic response [97].

Gaur et al. [78] demonstrated CS NPs-mediated delivery of miRNA34a, a tumor suppressive miRNA, which downregulates genes involved of prostate cancer and induces apoptosis and autophagy. The delivery of mRi-34a was explored in another strategy using

CS, poloxamer 188, and PLGA, and this system was able to efficiently encapsulate, protect, and deliver genetic material and it showed interesting physicochemical parameters to multiple myeloma treatment [77].

However, gene delivery mediated by CS is nonspecific. Thus, some works attempted to chemically modifying its structure to improve the specificity and transgenic capacity. Sadio et al. [79] introduced imidazole moieties into CS backbone to enhance siRNA delivery into gastric mucosa and concluded that these NPs were able to penetrate gastric mucus. Fan et al. [80] conjugated folic acid (FA) to CS in a nanogel formulation with focus on tumor tissue-targeting ligand overexpressed in several epithelial malignancies. This system was used to codeliver miR-281 and temozolomide in U87MG cells and it exhibited a significant antitumor efficacy mediated by targeting delivery tumor site and an increase of intracellular uptake. FA was also coupled with CS conjugated with propargyl focal point poly(L-lysine) dendrons (PLLDs) to deliver AEG-1 siRNA in 143B osteosarcoma cells [81]. Still aiming to produce CS-based NPs (CS NPs) specific for tumors cells, Wang et al. [82] designed an iron oxide core coated with CS-PEI and conjugated with a monoclonal antibody against human glypican-3 receptor, which is overexpressed in tumor cells. CS-PEI coated with iron oxide demonstrated appropriate physicochemical properties that are required to protect and deliver siRNA. Moreover, CS-based formulations allow alternative administration forms to point out that intranasal formulation of CS NPs to deliver siRNA targeting Gal-1 was used to treat GL261 glioblastoma cells [83].

Cyclodextrins (CDs) are potential candidates for drug delivery because CDs are easily induced to form supramolecular structure and could be cross-linked with polymers. The codelivery strategy became therapeutically interesting because it provides the possibility of a synergic effect improving target selectivity and impairs the development of drug resistance. Copolymers based on a CD core and cationic arms could be conjugated with hydrophobic drugs and NABDs simultaneously. Zhao et al. [84] developed an FA-modified CD core with PEI arms to codeliver plasmid DNA encoding p-53 and PTX specifically in tumor cells through overexpression of folate receptors. This simple multifunctional codelivery was also used to codeliver DOX and BCL2 siRNA in breast cancer cell line improving therapeutic effect [98]. FA was also explored to improve the efficacy of a CD core with poly(L-lysine) arms to codeliver DOX and siRNA targeting MMP-9, a metalloproteinase involved in extracellular matrix remodeling and angiogenesis, in nasopharyngeal cancer models [85,86]. Thus, drug, DNA, and siRNA delivery systems based on folate-appended CD have been explored in cancer research [99].

Ma et al. [100] developed a redox-responsive formulation based on mesoporous silica NPs capped by ethylenediamine-modified CD rings to codeliver siRNA and DOX. In this system, glutathione cleaves disulfide bonds and triggers drug/siRNA release, resulting in

enhanced CT efficacy in vitro. A similar chemical strategy was used with PKM2 siRNA in human breast cancer cell lines, resulting in an effective inhibition of tumor cell growth, invasion, and migration [87].

The first clinical trial to deliver siRNA targeting M2 subunit of ribonucleotide reductase (R2) used CD complexed with PEG and a targeting agent [88]. In this case, a human transferrin protein was used, because transferrin receptors are upregulated in cancer cells, providing a specific siRNA delivery. These NPs were administered intravenously to patients with relapsed or refractory cancer. More details can be found at https://clinicaltrials.gov/ct2/show/NCT00689065 [89].

3.3 Delivery of peptides and proteins (P-PDS)

Most of the CT anticancer drugs have a narrow therapeutic index, slow DDS, and high hydrophobicity, showing toxicity in human organism. Therefore, there is a constant search into new therapies that can provide high specificity, lower immunogenicity, and toxicity to overcome negative points of current cancer therapy [7].

Peptides are short linear chains of amino acids (AAs) often stabilized by disulfide bonds. When the chain is 50 > AA, they are called proteins. Owing to their natural existence in the human body, both proteins and peptides have low toxicity, well-known pharmacology (distribution, metabolization, and elimination), and biological abundance, generally being usually biologically safe [12]. Overall, peptides are designed and synthesized to bind and modulate proteins and their interactions involved with the carcinogenic process, and proteins are commonly involved in the immune system as antibodies, cytokines, or interferons [101].

Recently, Marqus et al. [101] reviewed therapeutic peptides for cancer treatment and classified them into four categories according to their mechanism of action: targeting signal transduction pathways, targeting the cell cycle inducing cell death, targeting tumor suppressor protein, and targeting transcription factors. Peptides have high specificity of targeting when compared with proteins, as well as easy cell penetration and interaction with cell receptors, and also are more accessible to synthesize (Fig. 19.1).

Some peptides are already available to cancer therapy clinical use [102]. To enumerate, somatostatin and its analogs as growth inhibitors [103], endostatin as an antiangiogenic drug [104], and well-known proteins as monoclonal antibodies [105] are used in cancer therapeutics, leading to cell apoptosis through direct tumor cell killing, targeting and delivering drug into the cells, or recruiting the immune system. For example, Herceptin, a widely used drug that targets HER-2 receptors overexpressed in some types of breast cancer [106]; Rituxan, a humanized antibody is effective against non-Hodgkin lymphoma once it targets the B-cell-specific antigen CD20 [107]; L-asparaginase, an important component of multiagent CT schemes for the treatment of acute lymphoblastic leukemia,

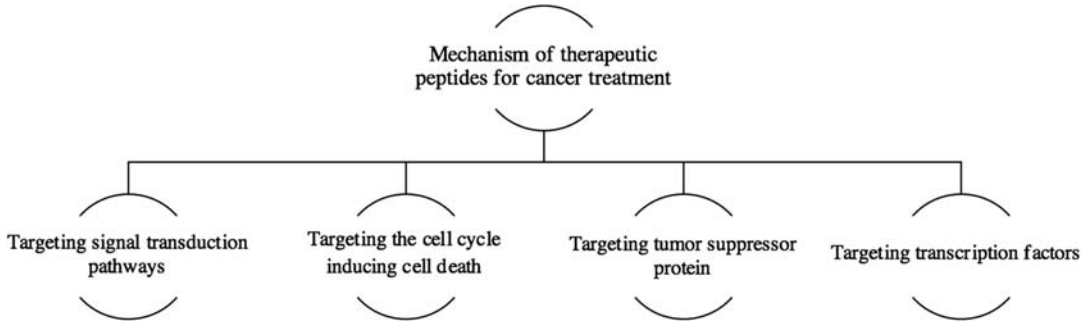

Figure 19.1

Mechanism of therapeutic peptides for cancer treatment. According to Marqus et al. [101].

is an enzyme that prevents tumor development by breaking down asparagine, a peptide that specific cancer cells require in higher amounts when compared to healthy cells [108]. Recently, derivatives of the human immunodeficiency virus (HIV) trans-activator of transcription protein (TAT) were patented for use as an anticancer targeting agent (US 207/0274,040 A1), as well as some recombinant cytokines in combined treatments to amplify the NK-mediated antitumor response [109].

In spite of all those benefits and already proved activity, the use of pep/pro in cancer therapeutics remains a challenge due to some drawbacks summarized in Table 19.3. Macromolecules as proteins display low stability in the organism—this instability is majorly related to the presence of various functional fractions susceptible to chemical degradation and their high hydrophilic character [110]. Given these points, their adequate delivery to target tissues has been considerably limited in vivo because these molecules can hardly pass through various hydrophobic biological barriers, and on the other hand, peptides are more manageable to synthesize and have better cell penetrability.

In the past decades, nanosized systems have been studied in function of improving pep/pro therapeutics efficacy in reaching the disease site exploiting the EPR characteristic of pathological angiogenic vasculature in cancer [6,12,19,104,110]. To summarize, studies

Table 19.3: Peptide and proteins use drawbacks in cancer therapeutics.

Drawbacks
Chemical degradation in the organism owing to enzymatic degradation and pH instability [101]
Accumulation in nontargeted organs and tissues [102]
Rapid elimination owing to renal clearance [102]
Production and manufacturing challenges [101]
Low permeability of cell membranes [102]
Poor oral bioavailability in function of gastric proteases degradation [101]

involving protein delivery by a PSC-derived matrix tested in cancer cell lines in vitro and in vivo are exposed in Table 19.4.

Bovine lactoferrin (bLF) is a well-known protein for its anticancer and antiinflammatory properties [115]. The effects of lactoferrin-loaded NPs on breast cancer [111] and colon cancer [115] were evaluated in alginate-enclosed CS−calcium phosphate iron-loaded bovine lactoferrin nanocapsules (AE-CS-CP-Fe-bLf), which proved to enhance bioavailability and activity of this protein, with gastric protection by the alginate gel encapsulation and nanosizing with the CS. AE-CS-CP-Fe-bLf showed better blood half-life and increased antitumor activity against MDA-MB-231 cells in vitro and in vivo, orally administered. Additionally, it not only killed cancer cells but also downregulated cancer stem cells [111]. Another study demonstrated AE-CS-CP-Fe-bLf activity against Caco-2 colon cancer cells and cancer stem cells, in vitro and in vivo, with a remarkable reduction in angiogenesis markers [115]. Both studies cited above concluded that when the NPs were uptaken, they modified the expression of specific miRNAs which intensified their uptake by the cells and improved their effectiveness, with an increase in body iron and calcium levels. This feature can be valuable for cancer patients and perform as a supporting therapy, lowering conventional therapy doses [111,115].

Moreover, some pep/pro complexed with polysaccharides as nanocarriers were evaluated, aiming to improve cell targeting [114,116−118]. Antoniraj et al. [117] applied atrial natriuretic peptide (ANP, a cell-specific ligand) conjugated with CS-hydrazone-methoxy PEG copolymer for intracellular delivery of prednisone. It could be observed that conjugation with ANP enhanced the cellular uptake of the polymeric NPs by the A549 cells, with selective delivery based on the pH cleavability of the polymer in function of the acid-cleavable hydrazine linkage. On a complex strategy, Chen et al. [114] applied the dual-targeted concept to kill SKOV-3 ovarian cancer cells and MDA-MB-231 cells with granzyme B (GrB)-loaded and peptide-targeted nanogel with an HA matrix and GE-11 outer signalization. GrB is a protease secreted by NK cells and T cytotoxic cells to eliminate infected or cancerous cells, and it has multiple uses in anticancer therapy. To add, HA has a known property to target CD44+ cells [119], and GE-11 peptide has the ability to connect EGFR+ cells. These two combined enhanced cellular uptake of the complex and cytoplasmic release of GrB henceforth stimulated caspase cascade activation and cell death by apoptosis. The authors tested both in vitro and in vivo separately cytochrome C (CC) and GrB loading in nanogel, but GrB displayed better antitumor activity probably in function of overexpression of Bax and gtBid proteins, which combined together can generate CC and therefore improve cancer therapy.

Table 19.4: Polysaccharide polymers encapsulating proteins/peptides tested for anticancer activity in vitro and in vivo.

PSC	System	Pep/Pro	Structure PDS	Evaluation model	Outcome	References
Alginate and chitosan	Nanocapsules	Fe-bLf	AEC-CP-Fe-bLf	MDA-MB-231 cells and C57 Balb/C xenograft mice	Active orally as prevention, treatment after tumor growth (4.8-fold decrease in tumor size), and inhibited tumor recurrence	[111]
				Caco-2 cells and xenograft mice	Ability to induce apoptosis in cancer cells and cancer stem cells, when given orally in diet	[112]
Chitosan	Nanoparticles complex	TRAIL	CMCS-FA-PEI-BSA-TRAIL-GA	MCF-7 (FR+) and A549 (FR-) cells and Balb/C mice	pH-dependent and surface charge-switchable NPs loading GA and TRAIL achieved a precise release in specific sites, which resulted in improved anticancer efficacy and reduced undesirable side effects	[113]
Hyaluronic acid	Nanogel	GrB	HA-GE11-GrB	SKOV-3 and mda-mb-231 cells and xenograft nude mice	Tumor cell death by apoptosis owing to CD44 and EGFR-specific internalization of granzyme B and improved anticancer activity than cytochrome C	[114]

Recently, Ding et al. [120] have exploited HA-based nanogel activity against overexpressing MCF-7 cells in vitro with a pH-dependant coiled-coil E3 and K3 peptide cross-linked structure loading saporin as a therapeutic protein model or CC as a protein release model. The peptides provided an α-helicoidal configuration that enabled better structural stability in blood with neutral conditions and caused unfolding in acidic pH-dependent manner, which in fusion with endosomal membrane helped proteins to escape from endosomal entrapment. Saporin, a highly potent protein, which is able to inactivate ribosomes and nonpenetrable into the cell membrane, has demonstrated better antitumor efficacy than CC, confirming the formulation as a good option for cationic protein delivery.

Another approach for pep/pro use in polymeric nanocarriers is to improve the system stability [12,121,122] carrying proteins or chemicals. Zhang et al. [113] developed TRAIL and GA coloaded BSA-PEI NPs with CMCS-FA-based outer shell encapsulating the NPs. They tested this NP complex in MCF-7 (FR+) and A549 (FR-) cells analyzing the synergistic mechanism of action of TRAIL interacting with cell membrane receptors, leading to a caspase enzyme cascade, and GA being released intracellular via proton sponge effect reaching the nucleus, both inducing cell death and apoptosis. The CS-derived enabled targeted release of NPs because of their pH-responsive behavior, considering that tumor tissues are more acidic than healthy tissues. Moreover, Liu et al. [123] explored the well-known self-assembly property of CMC and BSA, focusing on the effective radionuclide ^{131}I and the CT drug camptothecin codelivery, to achieve combined chemoradioisotope synergistic therapy of cancer. Surprisingly, the CMC−BSA complex presented a pH-dependent drug release profile and high drug loading capacity, and following LLC cells, the combined therapy was significantly superior to single therapy.

Protein delivery by PDS matrix is also being evaluated as a model vaccine delivery platform, using the protein antigen for presentation and the polysaccharide polymer, providing low cytotoxicity, good antigen-loading capacity, and targeted and sustained release. Its mechanism can improve cell or humoral immunity, through induced T cell proliferation and cytokine secretion by a pH-dependent release [124] and MHC I/II response, promoting intracellular processing of the antigen based on polymer bioreduction [125].

Furthermore, the P-PDS combination can make up theragnostic (tumor targeting and magnetic resonance imaging) applications in brain tumor 9L-glioma cells when complexed with heparin-coated magnetic NPs. In detail, the protein can perform as a helper for tissue targeting as B-galactosidase [126] and even as a cationic model to prove the binding capacity of the heparin-functionalized complex, as protamine [127].

Some authors studied the liberation kinetics of pep/pro encapsulated or complexed in nanocarriers [128] based in carrageenan/CS and BSA [129], starch and BMP-4 [130], mannan and bFGF [131], glucomannan/CS and bFGF [132], fucoidan/CS and BSA [133], pullulan and BoHc/A [134], heparin and bFGF [135]. Despite the promising protein delivery results, in vitro or in vivo antitumor evaluations were not conducted; therefore, they need more deep and straightforward studies to be considered.

4. Clinical trials of PDS

Although several PDSs have been studied to develop an effective anticancer treatment, only a small amount of studies reached clinical trials (Table 19.5).

Overall, the reasons that can explain this issue are mostly given to the impurity of some developed products that can cause systemic toxicity. Therefore, future studies using PDS should include systemic delivery evaluations [7]. In addition, codeliveries and smart designed PDS seem to be the most promising approaches in this field of research.

Table 19.5: Clinical trials with PDS.

Polysaccharide	Product	Formulation	Cancer type	Development stage/phase	References
Dextran	DE-310	Exatecan mesylate, carboxymethyl dextran	Advanced solid tumors	I	[136]
	Delimotecan (MEN 4901-T/0128)	Camptothecin (T-2513), carboxymethyl dextran	Solid tumors	I	[137,138]
Chitosan	Milican	Holmium-166, CS	Small hepatocellular carcinoma	II	[139]
Hyaluronic acid	ONCOFID-R-B	PTX and HA	Bladder cancer	I, II	[140]
Cyclodextrin	CALAA-01	siRNA (RRM2), cisplatin, AD-PEG, hTf	Solid tumors	I	[141]
	CRLX101/ IT-101	β-Cyclodextrin and PEG copolymer—camptothecin	Ovarian/tubal/ peritoneal cancer	I, II	[142]
			Rectal cancer	I, II	[143]
			Advanced solid tumors	I	[144]
			Lung cancer	I, II	[145]

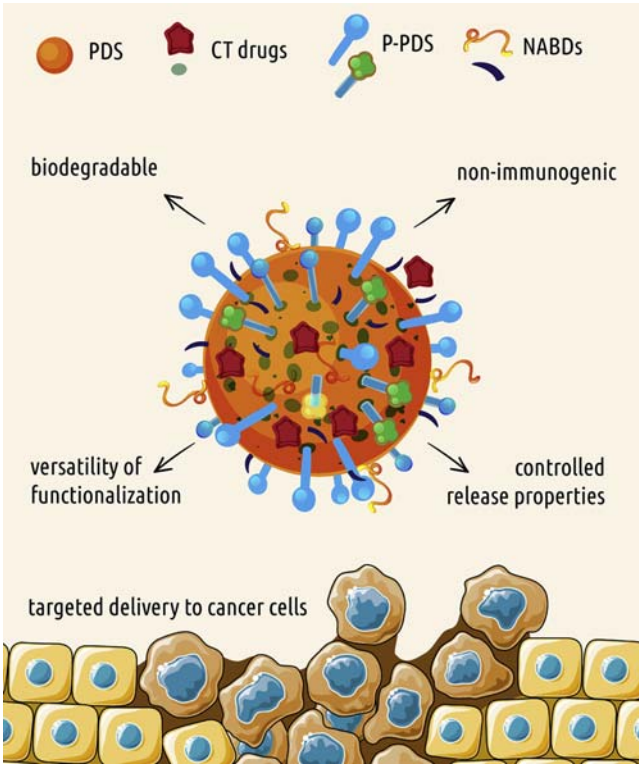

Figure 19.2
A schematic representation of polysaccharides-based drug delivery systems and their biofunctionalization.

5. Conclusion

There are several strategies being developed to improve therapeutics biological stability, blood half-life, and specificity to target cancer cells, with attention given to incorporation and targeting with polymers. Polysaccharide polymers represent a promising approach to promote targeted delivery and overcome these molecular deficiencies because of low toxicity, natural abundance, and biodegradability. In addition, PDSs exhibit the ability to mimic the natural extracellular matrix, self-assembling properties, and also easy ionic manipulation, which allows various possibilities of drug targeting and release (Fig. 19.2). However, further studies are necessary to improve production methods, considering synthesis issues such as stability, product variability, and impurity. Additionally, more immunogenic evaluations are needed to prove that these bioproducts are safe for systematic use.

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APÊNDICE B

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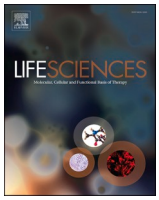

Review article

Recent developments in drug delivery strategies for targeting DNA damage response in glioblastoma

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ABSTRACT

Glioblastoma is the most frequent and malignant brain tumor. The median survival for this disease is approximately 15 months, and despite all the available treatment strategies employed, it remains an incurable disease. Preclinical and clinical research have shown that the resistance process related to DNA damage repair pathways, glioma stem cells, blood-brain barrier selectivity, and dose-limiting toxicity of systemic treatment leads to poor clinical outcomes. In this context, the advent of drug delivery systems associated with localized treatment seems to be a promising and versatile alternative to overcome the failure of the current treatment approaches. In order to bypass therapeutic tumor resistance mechanisms, more effective combinatorial therapies should be identified, such as the use of cytotoxic drugs combined with the inhibition of DNA damage response (DDR)-related targets. Additionally, critical reasoning about the delivery approach and administration route in brain tumors treatment innovation is essential. The outcomes of future experimental studies regarding the association of delivery systems, alternative treatment routes, and DDR targets are expected to lead to the development of refined therapeutic interventions. Novel therapeutic approaches could improve the life's quality of glioblastoma patients and increase their survival rate.

1. Introduction

Glioblastoma (GBM) is the most malignant and frequent primary central nervous system (CNS) tumor, representing 30% of all CNS tumors and 80% of CNS primary malignant tumors [1]. Despite all recent progress in treatment strategies, there are no current curative therapeutic options for GBM, and its median survival is approximately 15 months from the time of diagnosis [2]. GBM is histologically defined as astrocytoma and molecularly classified according to the isocitrate dehydrogenase (IDH) status and O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation [3,4]. GBM exhibits a diffuse nature, and its complete etiology and pathophysiology are not yet known. Although GBM can occur at any age, it should be noted that the incidence increases with age, with the average diagnosis age being around 65 years [5].

The standard therapy of newly diagnosed GBM consists of maximal safe resection, followed by radiotherapy (RT) plus concomitant and adjuvant temozolomide (TMZ)-based chemotherapy (CT). However, there are several challenges. The highly infiltrative nature of GBM makes complete surgical resection nearly impossible, and the recurrence is inevitable [6,7]. RT and CT directly or indirectly induce cell death through DNA damage [8,9], and several biochemistry pathways influence the therapy success. Also, the genetic background significantly affects the treatment outcome. The cellular response comprises a complex signaling cascade named the DNA damage response (DDR), which is responsible for recognizing, signaling, and correcting DNA damage (Fig. 1).

Different kinds of lesion formed in the DNA require specific DNA repair pathways which allow damage resolution and might contribute to radio and chemoresistance [10]. In this regard, a plethora of treatment approaches aiming at novel molecular targets have been developed, which could be used as therapeutic alternatives. Nevertheless, most of them fail during clinical trials, suggesting that a single targeting strategy does not improve therapeutic outcomes [5,11]. The failure related to

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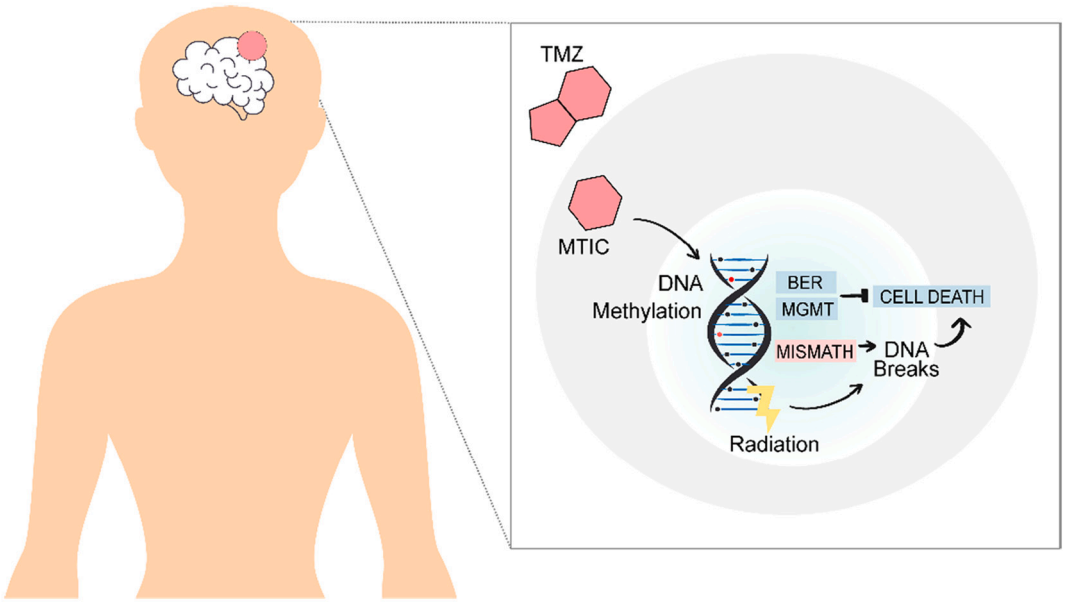

Fig. 1. Direct and indirect DNA damage induced by chemo- and radiotherapy. The mechanism of action of chemotherapy based on temozolomide administration as well as radiotherapy is associated with the modulation of DDR pathways, which can promote cell survival through DNA repair or lead cells to cell death.
Abbreviations: Base excision Repair (BER), O-6-methylguanine-DNA methyltransferase (MGMT), 5-(3-methyl triazen-1-yl)imidazole-4-carbozamide (MTIC). Temozolomide (TMZ).

these approaches might be associated with the compensatory DDR mechanisms, high systemic toxicity, lack of drugs' stability, and insufficiency of *in vitro* and *in vivo* studies demonstrating the efficacy of novel drugs [12].

To bypass GBM resistance mechanisms and decrease treatment side effects, well-designed drug delivery systems (DDS) associated with alternative exposure pathways have become crucial for GBM therapy. Hence, the purpose of this review is to explore how DDR mechanisms contribute to GBM treatment resistance and to present how drug delivery strategies could be used to fine-tune the therapy targeting DNA repair and revert the unhappy out in GBM treatment.

2. The current clinical treatment protocol of glioblastoma (GBM)

GBM is known for its diffusely infiltrative profile that invades multiple lobes and both hemispheres of the brain. Hence, establishing the extent of resection and defining how to balance the benefits and risks of surgery prove to be extremely complicated [13]. Even though high resection rates are usually predictive of survival, it is necessary to thoroughly evaluate the improvement in survival with postoperative neurological deficits as neurological morbidity [14]. Despite being beneficial, surgery has limited efficacy, thus indicating that a combined treatment modality is required.

After surgery, GBM treatment involves a partial-brain fractionated RT of 30 fractions of 2-Gy over six weeks with concomitant daily TMZ (75 mg/m^2 P.O.) 1 h prior. This multimodal treatment is followed by six to twelve cycles of adjuvant TMZ at 150 mg/m^2/day (days 1–5 of a 28-day cycle). The dose can be increased if it is well tolerated by the patient. Wang et al. [15] showed that oral CT plus RT contributed significantly to the improvement in the overall survival (OS) and progression-free survival (PFS) in patients with newly diagnosed GBM when compared to RT alone [16,17]. Still, establishing the ideal therapy in the management of GBM is challenging due to the complexity of this disease and response variability among the general population.

There are several other drugs considered for recurrent gliomas (to review treatment and resistance mechanisms of recurrent GBM, read Campos et al. [18], Diaz et al. [19] and Nam and de Groot [4]). A second alkylating agent, namely lomustine, is frequently used and applied in association with a monoclonal antibody, bevacizumab, in some cases [20]. Recently, Herrlinger et al. [21] demonstrated the effectiveness of the association between lomustine and TMZ *versus* TMZ in a phase III study (ClinicalTrials.gov number NCT01149109). The average OS rate was increased from 31.4 to 48.1 months [21], suggesting a significant improvement in treatment outcome.

RT is a treatment approach that uses ionizing radiation (IR) (*e.g.*, X-ray) to eliminate tumors or prevent malignant cells from growing and metastasizing [22]. Although the cellular death mechanism associated with RT exposure is not completely understood, cellular death is known to be caused by two main reasons, namely cellular stress and DNA damage [9,22]. The DNA damage is caused by the direct interaction of X-ray with several small and macromolecules, inducing DNA single- and double-strand breaks (SSB and DSB respectively), DNA crosslinks, and DNA-protein crosslinks, and by an indirect mechanism caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS). The cellular water radiolysis induced by IR can produce superoxide anion ($O2^{-}$•), hydroxyl radicals (OH•), and hydrogen peroxide (H2O2), which cause organelles and macromolecules damage, including oxidation of DNA bases, SSB, abasic sites, and DSB [23].

Despite the several different types of damage triggered by IR, the most harmful effect of radiation is a clustered DNA damage, defined as two or more lesions formed by the passage of one radiation track, which could be bistrand or in tandem to the DNA strand localization [24]. DSB clusters can lead to chromosome aberrations and cell death, and non-DSB clusters can induce mutations and chromosomal abnormalities that increase the genomic instability of cancer cells [25]. However, tumor cells can adapt their responses to radiation by increasing cellular defenses to neutralize cellular stress activating hypoxia in order to decrease the oxygen supply and limit the production of ROS [26,27]. Furthermore, tumor cells can augment their antioxidant defenses to neutralize oxidative molecules before promoting any cell damage [23].

CT is generally used in combination with RT to treat gliomas, and the most frequently used drug is TMZ, which is rapidly absorbed intact and can cross the blood-brain barrier (BBB). After being absorbed, TMZ undergoes a spontaneous hydrolysis process in pH $>$ 7 and is converted to its active metabolite 5-(3-methyl triazen-1-yl) imidazole-4-

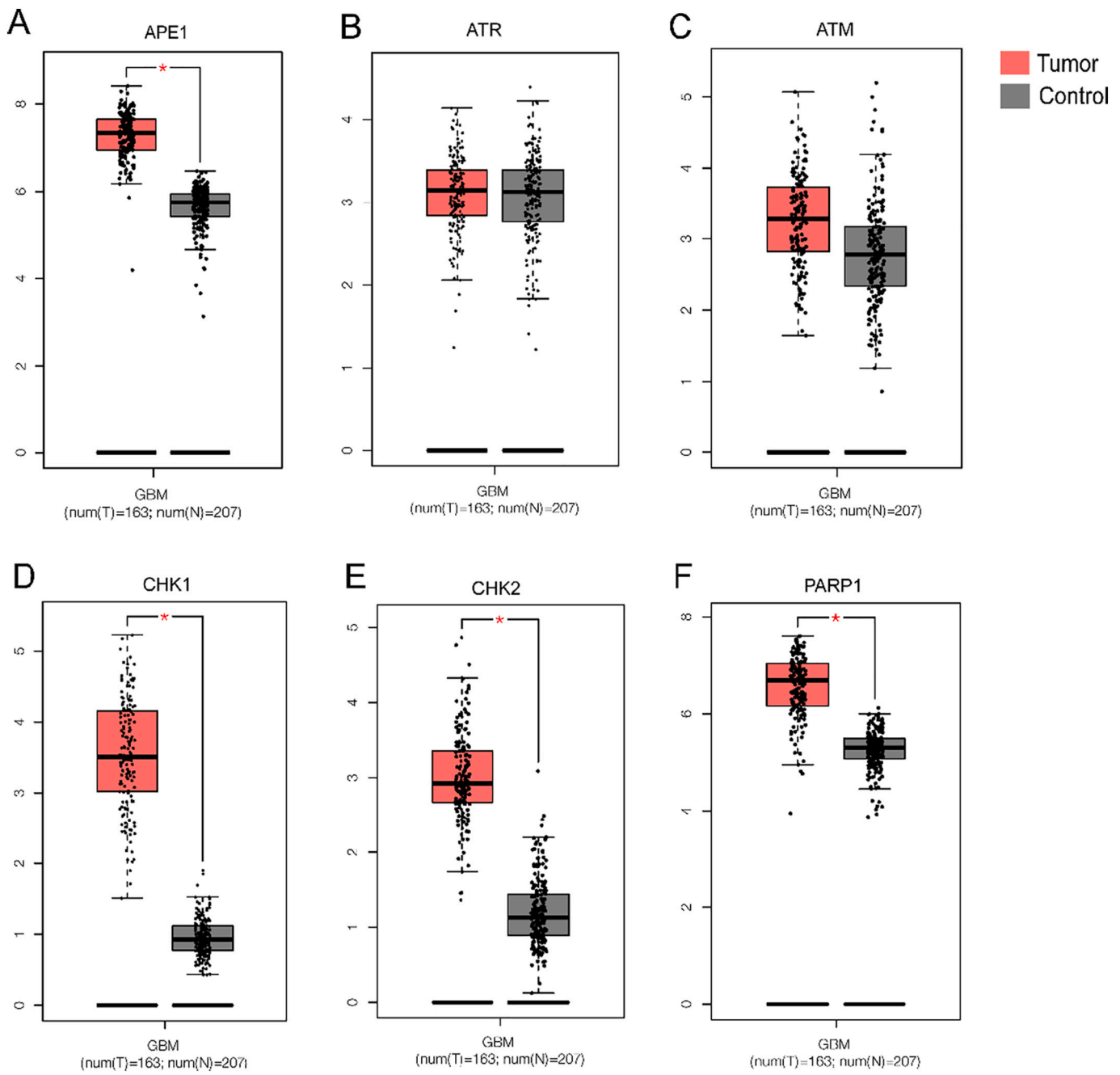

Fig. 2. Expression of DNA repair genes. The expression of the main repair genes involved in the resistance process was evaluated by comparing 163 patients with GBM to 207 controls derived from the GEPIA dataset (http://gepia.cancer-pku.cn/index.html) [34]. The analysis was performed by ANOVA, the $|Log_2FC|$ Cutoff was 0.01 (q-value 0.01) with the match between TCGA and GTEx data.
Abbreviations: DNA-(apurinic or apyrimidinic site) endonuclease (APE1), Ataxia telangiectasia and Rad3 related (ATR), Ataxia telangiectasia mutated (ATM), Checkpoint kinase 1 (CHK1), Checkpoint kinase 2 (CHK2), poly(ADP-ribose) polymerase 1 (PARP-1), Glioblastoma (GBM).

carbozamide (MTIC) [28]. MTIC is further hydrolyzed to methyldiazonium cation and 5 aminoimidazole-4-carboxamide (AIC) [29].

TMZ belongs to the class of alkylating agents, and its mechanism of action consists of the transference of its electrophilic alkyl group to the most nucleophilic atom within the DNA [29]. The methyldiazonium cation preferentially methylates DNA at the N3 position of adenine and N7 position of guanine (90%) but also methylates O^6-guanine (5–10%). Despite being the least frequent lesion, O^6-methylguanine (O^6-MG) is the most cytotoxic and mutagenic lesion leading to the insertion of thymine during the subsequent DNA replication phase [29]. TMZ also induces methylation of macromolecules, such as proteins and lipids. Unfortunately, even though the TMZ-induced methylation of macromolecules has been of great biological importance, it is poorly understood and little is known about its therapeutic contribution [30].

Certainly, the inclusion of TMZ in the newly diagnosed glioma treatment regimen represented an increase in the patient's survival. However, there are several critical consequences associated with the systemic administration of TMZ, such as gastrointestinal or hematological effects [31]. In addition, its clinical use is limited by the need for higher systemic doses for achieving therapeutic effects in the brain [12].

3. Therapeutic resistance process in GBM

Improvements in the GBM treatment have contributed to

ameliorating patients' quality of life and survival outcomes. Nevertheless, the improvements do not translate the considerable technological advances that have occurred in the biomedical areas in the past years. Several resistance mechanisms are related to the failure of current treatments against GBM, including the DNA repair mechanisms, the BBB and blood-brain tumor barrier (BBTB), and the glioma stem cells [10,12], which can be addressed to improve therapy outcomes.

3.1. Lesions induced by chemotherapy (CT) and radiotherapy (RT) can be restored by DNA repair mechanisms

The role of DDR in carcinogenesis and tumor resistance is closely dependent on the timing of evaluation and DNA damage type. The DDR-related proteins have been associated with the two following important aspects: at the beginning of gliomagenesis, DDR breaks the expansion of malignant cells. However, when the cancer cells and tumor niche are installed, DDR contributes to enduring the genomic instability and correcting damages caused by external agents such as CT drugs or radiation [8]. Both CT and RT aim to generate direct DNA damage triggering cell death. A complex protein network is activated in response to different DNA lesions to mediate cellular changes (*e.g.*, cell-cycle arrest) and to directly repair the lesion. Cells activate different repair mechanisms depending on the cellular context and the type of substrate or lesion to be corrected. The repair mechanisms are pro-survival and are related to the resistance process and tumor recurrence [32,33].

In order to evaluate the expression of the members of DNA repair systems, the gene expression of therapeutic targets in human samples was investigated (Fig. 2). The specific role of each of these genes will be discussed in sequence, however, it can be observed in Fig. 2 that the DDR markers are upregulated in tumor samples compared to control samples, contributing to the resistance process in the GBM cells.

There are three main DNA repair pathways that process TMZ alkylation lesions: direct repair by MGMT, base excision repair (BER), and mismatch repair (MMR) [32,33]. O^6-MG lesions are directly repaired by the one-step enzyme MGMT. The expression of *MGMT* is correlated with the resistance to TMZ, mainly because MGMT removes the methyl group from O^6-MG, restoring the integrity of guanine bases in the DNA. The benefits of alkylating agents are largely restricted to patients whose tumors show methylation of *MGMT* promoter [35,36]. If not repaired by MGMT, the thymine misincorporation that occurred during the replication of O^6-MG activates the MMR pathway. This process enters in a futile cycle which replaces the misincorporated thymine with another thymine, leading to energy-consuming cycles, replication fork arrest, and DNA breaks. The conversion of misincorporation errors to a DSB activates DSB repair pathways, and, if the repair fails, apoptosis is triggered [8].

Most of the TMZ-induced lesions, such as N3-methyladenine and N7-methylguanine, are primarily repaired by the BER pathway. Consequently, a functional BER pathway contributes to TMZ resistance and is associated with a worse prognosis in GBM [29]. Additionally to alkylation lesions, the oxidative lesions in DNA bases are usually repaired by BER. In this pathway, a glycosylase initiates the repair process by breaking the glycosidic bond and forming an abasic site. Endonucleases are responsible for cleaving the phosphodiester bond, and finally, the gap is further repaired by DNA polymerases, DNA ligases, and XRCC1 ([23,37]; to review this repair pathway in detail, read [38]).

APE1 is a multifunctional enzyme involved in different activities depending on the protein domain [29]. The main function of APE1 in BER is to create a nick in the phosphodiester backbone of the AP site that has been established when the DNA glycosylase removed the damaged base. When the repair process is concluded, the damage is restored, which contributes to the survival of tumor cells [39]. *APE1* expression is shown in Fig. 2A as being overexpressed in tumor samples. Hudson et al. [40] investigated pre-treatment and posttreatment GBM to identify molecular changes following treatment and recurrence of disease and also demonstrated that specimens had molecular changes that correlated with known resistance mechanisms, including increased expression of APE1. Moreover, APE1 contributes to chemoresistance, facilitating the BRCA1-mediated Homologous recombination (HR) repair in response to DSB [41]. Thus, inhibiting APE1 is an interesting strategy to induce toxicity and decrease the resistance of GBM to TMZ [42].

As previously mentioned, DSBs are highly toxic radiation-induced DNA lesions, and their repair can trigger genomic rearrangements and mutation or apoptosis. The response to DSB starts with the MRN complex (MRE11, RAD53, and NBS1 proteins) sensing the lesion. This complex activates the damage signaling mediated by two proteins related to cell cycle checkpoint mechanisms, namely Rad3-related protein (ATR) and ataxia telangiectasia mutated (ATM), whose action will culminate in DNA damage repair. These proteins phosphorylate downstream targets to coordinate several processes. ATM activates Chk1 and Chk2 to control cell cycle, allowing cell cycle arrest, and 53BP1 and H2AX to model chromatin [43]. ATM phosphorylates p53, inducing G1-arrest by p53-p21 pathway and preventing cells with damaged DNA from entering S-phase [5,44]. As shown in Fig. 2, ATR and ATM are not differently expressed in the evaluated samples, however, Chk1 and Chk2 are highly expressed in GBM patients' samples (Fig. 2B–E).

The repair of DSB can be mediated by HR, an error-free pathway that is dependent on DNA ends resection and template availability, or by Non-homologous end joining (NHEJ) which repairs the damage without the necessity of a template and is hence error-prone [8,45]. Defining which pathway the cells will activate depends on several regulation steps, including DSB extension and availability of DNA repair proteins ([5]; to review these pathways, consult Kakarougkas and Jeggo [43] and Ranjha et al. [46]).

The repair of SSB involves the participation of a protein named poly (ADP-ribose) polymerase 1 (PARP-1). PARP1 bound to DNA strand breaks and assists the repair signaling of this type of lesion by producing a poly (ADP-ribose) chain from the substrate NAD+ [47]. Furthermore, its expression is higher in GBM than non-tumoral tissue (Fig. 2F). In accordance, Galia et al. [48] demonstrated that *PARP-1* is expressed in 27 GBM samples, and Murnyák et al. [47] showed that *PARP-1* upregulation is a characteristic of high-grade astrocytomas and that high *PARP-1* levels are negatively associated with patient survival. Since PARP1 is overexpressed in GBM and its function corresponds to an anti-apoptotic factor that generates cell death resistance, it has been studied as a promising therapeutic target [49].

Contrary to TMZ-related lesions, RT induces damage in clusters whose repair involves a more complex mechanism than individual damage sites. The efficiency of DNA repair proteins can be inhibited by the difficult access to the DNA damage site, thus increasing the repair process time. These unrepaired clusters may generate additional DSB, which increases the genomic instability [25]. DSB in clusters can also induce a shift between DSB repair pathways, which increases genomic instability and cell death even more [50]. In consequence, the cells' ability to repair DNA lesions is compromised by increasing the complexity of the damage, which indicates the importance of RT-induced lesions in therapy and presents possibilities of radioresistance modulation.

3.2. Selective permeability of blood-brain barrier (BBB) and blood-brain tumor barrier (BBTB) can cause treatment resistance

The brain is a complex and delicate organ that involves a vast mechanism of defense formed by a huge vascular network of over 100 billion capillaries tightly joined to the endothelial cells, pericytes, astrocytes, and microglia of the CNS [51,52]. As a result, it causes several challenges to therapeutic approaches, including chemotherapeutic interventions, which are often unable to cross the BBB, rendering the therapy ineffective in many cases of brain cancer [53].

The transport across the BBB may occur by two different pathways: (i) the paracellular transport where substances pass between the

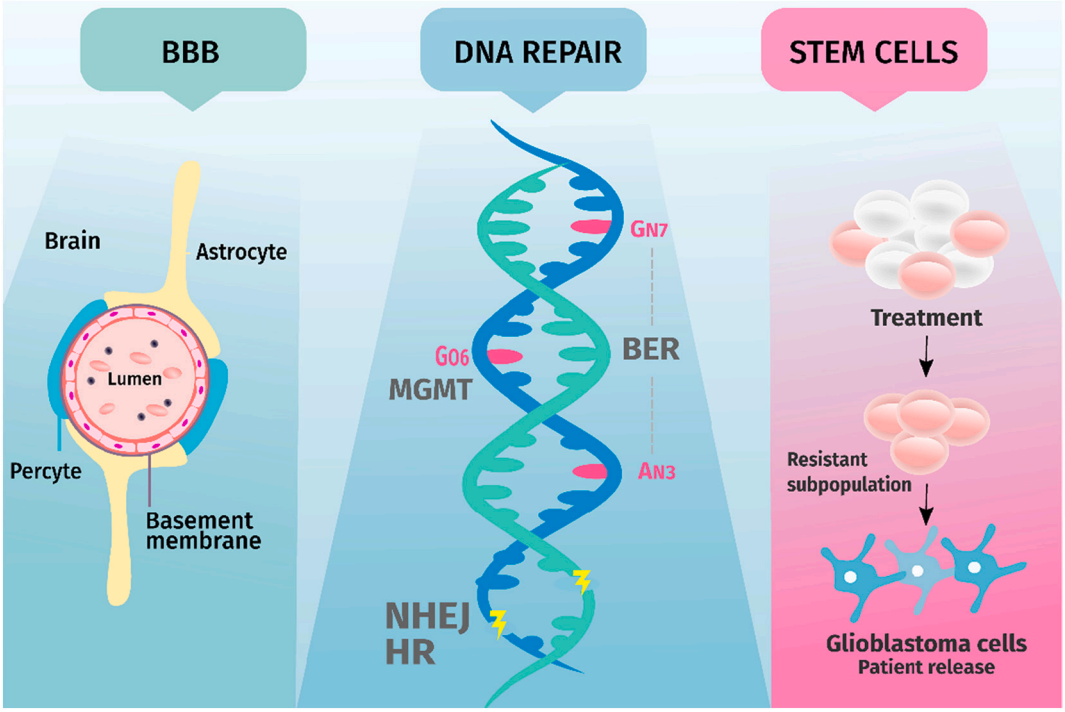

Fig. 3. Cellular mechanisms contributing to GBM resistance. Several resistance mechanisms are related to the failure of current treatments against GBM including Blood-brain barrier, DNA repair mechanisms, and the glioma stem cells. The restricted permeability of the blood-brain interface decreases drug levels in the brain, while the DNA repair pathways act on DNA methylations caused by TMZ and the breaks caused by radiation inducing tumor cell survival, and finally, the resistance of tumor stem cells to treatment and later differentiation in GBM cells contribute to GBM recurrence.
Abbreviations: Blood-brain barrier (BBB); Base excision Repair (BER), O-6-methylguanine-DNA methyltransferase (MGMT); Non-homologous End Join (NHEJ); Homologous Recombination (HR).

endothelial cells and (ii) the transcellular transport where substances pass across the luminal side of the endothelial cells. Similarly, some substances may require other transport mechanisms such as the carrier and receptor-mediated transport. Molecular size and weight, surface charge and lipophilicity of molecules, and integrity of the BBB are common characteristics that regulate these pathways [54]. Besides, the intravascularly administered drug is known to be distributed asymmetrically in smaller amounts to the brain but in larger amounts to other tissues [55,56].

The specialized brain endothelium cells exert barrier properties and are essential to protect the brain from potentially neurotoxic compounds. The normal BBB is vital not only to protect the brain but to also supply it with nutrients and oxygen. The functioning and organization of BBB can be altered in pathological conditions like high-grade gliomas [57]. Thus, in gliomas, their rapid growth and migration are maintained by a structure that resembles the BBB [58]. In this structure, tumor vasculature is in most cases different from the normal vasculature, with branching pattern and cellular and molecular components considerably different from a normal vasculature. The tumor cells damage the BBB and as an effort to grow even more, they create new vascular networks and form a BBTB, which is distinct from the BBB, allowing greater permeability in bulk tumor areas and the opposite in the peripheral ones [59]. Moreover, the capillaries from this vascular system are distended and formed by leaky walls, presenting a sluggish flow and a high interstitial pressure due to the internal accumulation of fluid, which makes this set of factors responsible for variability in drug delivery [60].

Although BBB plays a crucial role in maintaining the local homeostasis in healthy brains by hindering the entrance of substances from the blood, it has been a significant obstacle for brain drug delivery [61,62].

3.3. Glioma stem cells as key drivers of tumor resistance

Glioma stem cells (GSCs) are a small subpopulation of cells within tumors with capabilities of unlimited self-renew and are different to all cell populations present in original tumors [63]. After the first line of chemoradiotherapy, a restricted cell population of stem cells activates DDR, inducing a resistant profile. This cell population might be responsible for the inevitable recurrence of GBM ([64,65,175]).

One explanation for resistance mediated by GSCs is the high levels of DNA replication stress caused by radiation exposure that activates DDR. GSCs constitutively exhibit stress replication caused by replication/transcription collisions and consequent upregulation of DDR, which triggers radioresistance [66]. Previous studies found an association between radioresistance and CD133 status, where the results demonstrated that CD133+ cell populations increase the basal response to DSB, exhibiting active phosphorylation of proteins related to cell cycle checkpoints, such as Rad17, Chk1, and Chk2. Besides, the activation of ATM after radiation exposure is exacerbated in CD133+ cell populations [8,67,68]. The properties of GSCs allow these cell populations to be exposed to CT and later differentiated into highly proliferative tumor cells that might be more resistant than non-stem tumor cells. Consequently, as the local concentration of TMZ cannot eliminate the differentiated cells, tumor recurrence might occur [69].

Fig. 3 summarizes the three main GBM resistance mechanisms. The restricted permeability of the blood-brain interface decreases drug levels in the brain, while the DNA repair pathways act on DNA methylations caused by TMZ and the breaks caused by radiation inducing tumor cell survival. Finally, the resistance of tumor stem cells to treatment and later differentiation in GBM cells contribute to GBM recurrence.

Table 1
Clinical studies (clinical trials database - NIH) carried out with inhibitors of DNA damage response-related targets.

Author or clinical trial ID	Year	Study type	Target	Repair pathway	Drug	Outcome
Quinn et al.	2009a	Phase I	MGMT	Direct repair	O^6-BG	Schedule dose definition. Tolerance limited by myelosuppression
Quinn et al.	2009b	Phase I	MGMT	Direct repair	O^6-BG	1 of 34 patients responded to the treatment. Hematological effects in 48% of the patients.
Adair et al. (NCT00669669)	2014	Phase I/II	MGMT	Direct repair	O^6-BG	increase tolerated cycles of 1.7 to 4.4 PFS = 9 months OS = 20 months
NCT01587144	2012	Phase II	APEI	BER	Lucanthone	Trial in progress
Grupta et al.	2016	GBM PDX lines grown as orthotopic xenografts	PARP1	BER	Veliparib	Increase the TMZ efficiency in MGMT-hypermethylated lines
NCT02152982	2014	Phase II/III	PARP1	BER	Veliparib	Trial in progress
NCT01514201	2012	Phase I/II	PARP1	BER	Veliparib	OS 3 years = 5.3%; PFS 3 years = 2,9%
Abida et al. (NCT02588105)	2018	Phase I	ATM PARP1	DDR DDR	AZD0156 Olaparib	Tolerance limited by hematological toxicity
NCT03423628	2018	Phase I	ATR	DDR	AZD1390	Trial in progress

Abbreviations: ataxia-telangiectasia mutated (ATM); Poly [ADP-ribose] polymerase 1 (PARP1); DNA-(apurinic or apyrimidinic site) endonuclease (APE1); O^6-benzylguanine (O^6-BG); O^6-methylguanine-DNA methyltransferase (MGMT); DNA damage response (DDR); Base Excision Repair (BER).

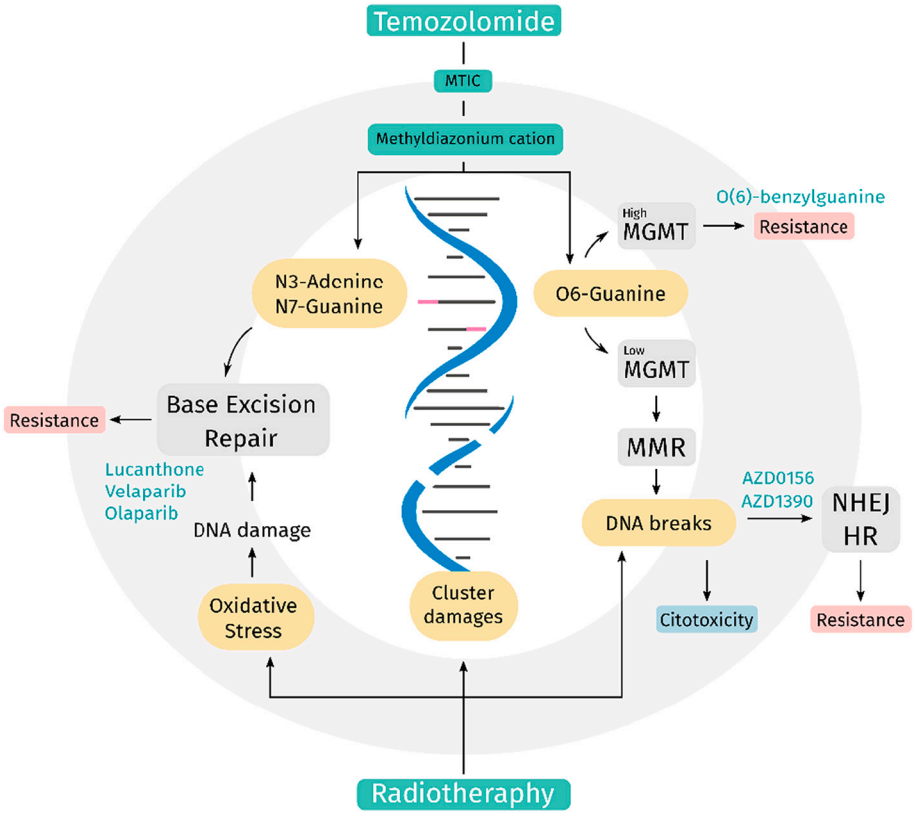

Fig. 4. Repair pathways activated in each damage caused by classical therapies. TMZ is a prodrug that is spontaneously converted to its active metabolite MTIC in a spontaneous hydrolysis process in pH > 7. MTIC is further hydrolyzed to methyldiazonium cation and 5 aminoimidazole-4-carboxamide (AIC). The cation is the active compound responsible for the delivery of methyl groups to DNA, mostly at guanine residues. Approximately 90% of lesions are N3-methyladenine and N7-methylguanine, however, just 5–10% are O^6-MG. Radiotherapy can cause direct damages as DNA breaks or indirect by reactive species generation. However, the most harmful effect of radiation is a clustered DNA damage, defined as two or more lesions formed by the passage of one radiation track. Each of these damages is repaired by specific pathways, which increase the resistance. Several inhibitor molecules have been tested in clinical trials as adjuvants drugs (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Abbreviations: O^6-methylguanine-DNA methyltransferase (MGMT); Mismatch Repair (MMR); Non-homologous End Join (NHEJ); Homologous Recombination (HR); 5-(3-methyl triazen-1-yl)imidazole-4-carbozamide (MTIC).

4. Therapies for glioblastoma targeting DNA damage response

One of the disadvantages of traditional cancer therapy is its absence of specificity. This is easily observed by the fact that the current chemotherapeutic protocols do not only affect the cancerous cells but also damage healthy cells and tissues surrounding the tumor or, in some cases, compromise the entire system, making the risk-benefit ratio even more doubtful [70,71]. Inhibitors of DDR could be a strategy to overcome the resistance, which may provide a therapeutic advantage to reduce tumor recurrence [72]. In this sense, many efforts have been expended to improve the effectiveness of RT and CT. Clinical results of drugs that are emerging as options for modulating the response to DNA damage in GBM (Table 1 and Fig. 4) will be discussed below [73,74].

O^6-benzylguanine (O^6-BG), a synthetic derivative of guanine and competitor of O^6-MG, acts as an inhibitor of MGMT. There are 18 clinical trials registered with O^6-BG. Quinn et al. [75] published studies of phase I and phase II that evaluate the treatment of TMZ plus O^6-BG. The phase I trial established the best schedule of treatment and found that myelosuppression was the limiting effect. The phase II trial demonstrated that only one of 34 patients with GBM responded to the addition of O^6-BG in the treatment schedule and the main signal of toxicity was hematological side effects [76]. This compound, when combined with TMZ or BCNU, was able to bypass tumor resistance when tested in clinical trials (phase I and II) [75–79,176]. Adair et al. [80] reported an interesting strategy to increase the tolerance and efficacy of the combination of O^6-BG with other alkylating agents (NCT00669669). Patients received

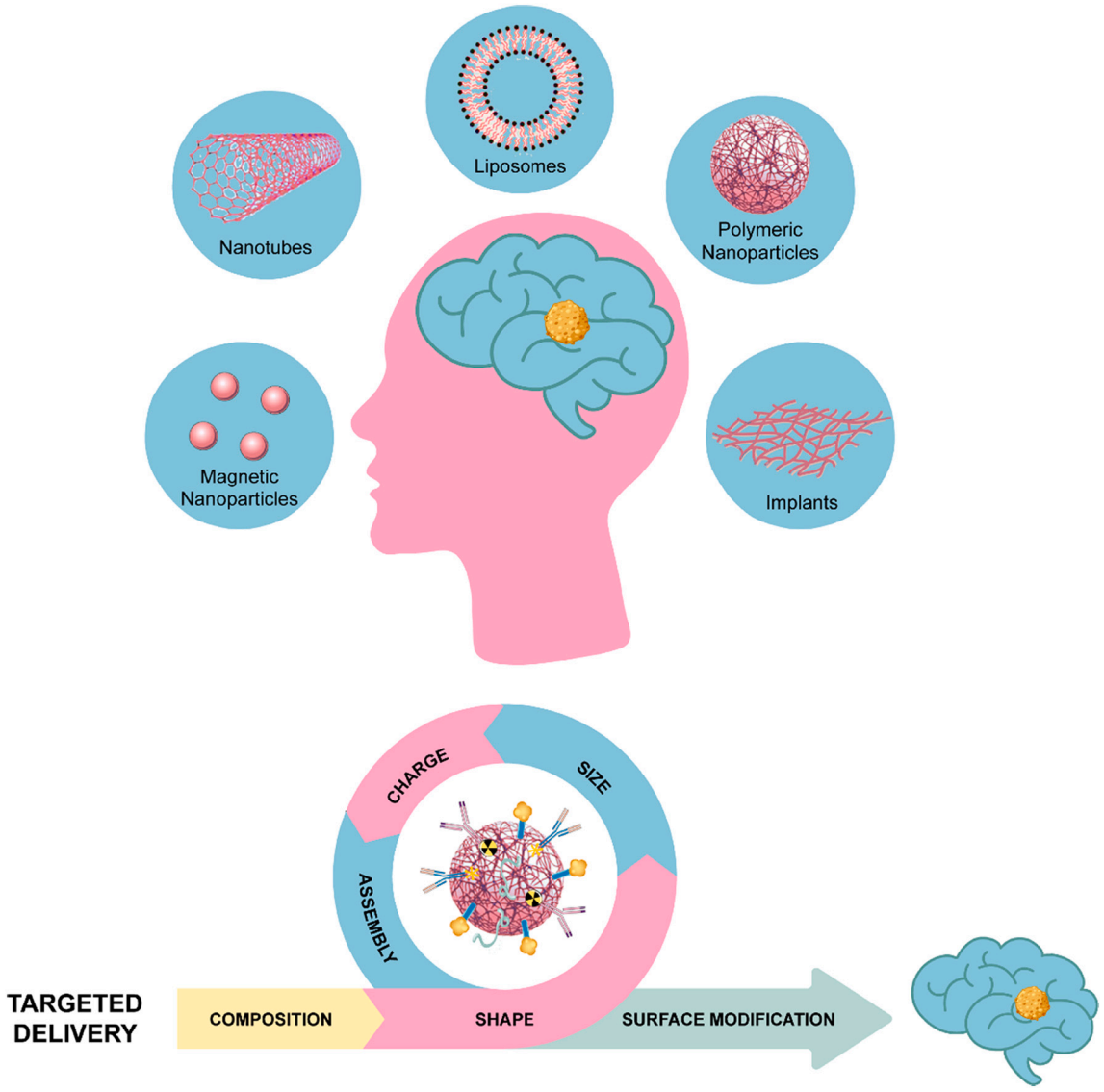

Fig. 5. Drug delivery strategies to target GBM. DDS including magnetic nanoparticles, nanotubes, liposomes, polymeric nanoparticles, and implants can be developed to deliver CT drugs and gene-based therapies to the brain. These strategies can be modified among their physical and chemical properties to generate nanosystems able to recognize and target cancerous cells.

MGMT mutant *P*140*K* gene-modified autologous hematopoietic $CD34^{+}$ cells which provide chemoprotection against hematopoietic toxicity. The gene therapy increases the mean number of tolerated cycles from 1.7 to 4.4, and the main outcomes were PFS from a diagnosis of nine months and median OS of 20 months. This strategy implies that an increase in the TMZ dose leads to better tolerance of patients to TMZ and survival outcomes. Nevertheless, the study was carried out with only a few patients, and a larger scale study is certainly needed to determine the real impact of this therapy. However, the strategy indicates that adverse effects can be reduced and patients' tolerance to TMZ can be increased.

Lucanthone is a thioxanthone-based DNA intercalator and inhibitor of DNA repair proteins like topoisomerases and APE1 [81]. A phase II study (NCT01587144) evaluates the safety and efficacy of lucanthone as an adjunct with TMZ and RT, however, the results were not available at the time of the manuscript's submission.

Five registered clinical trials can be found with the terms GBM and PARP1. Gupta et al. [82] demonstrated that veliparib increases TMZ efficiency in *MGMT*-hypermethylated GBM cell lines evaluated in orthotopic xenografts, and this was established as an eligibility criterion for the Clinical Trial NCT02152982, a phase II/III study that investigates the combination of TMZ and veliparib compared to only TMZ (there were no results reported at the time of the manuscript's submission). However, a phase I/II study (NCT01514201) that evaluated veliparib, RT, and TMZ in the treatment of younger patients with newly diagnosed diffuse pontine gliomas showed that hematological and gastrointestinal adverse effects can limit veliparib tolerance and that the OS and PFS do not demonstrate a major impact of adding PARP1 inhibitor to the treatment (OS 3 years = 5.3%; PFS 3 years = 2.9%). These unsatisfactory observations could be due to the employment of a systemic treatment approach (oral administration).

As previously discussed, radioresistance is responsible for the treatment failure and relapse in GBM [44], and ATM/Chk2 certainly represents one of the factors that contribute to radioresistance owing to its role as a DSB sensor [83]. Previous studies indicated that ATM is a prognosis factor related to longer survival in GBM [8,84–87]. Romano et al. [44] evaluated ATM protein expression in 21 GBM patients, and a high p-ATM score (++/+++) strongly correlated to shorter survival ($p = 0.022$). In accordance, Squatrito et al. [83] showed in pre-clinical models that the loss of a single copy or both copies of ATM

significantly accelerates glioma formation in mice. Based on these reports, researchers have been testing the clinical application of ATM inhibitors. Due to ATM's prosurvival role in DDR, its inhibition induces DNA damage accumulation, which improves the RT response. AZS1390 and AZD0456 are oral drugs available in GBM tests that have been included in clinical evaluations [88]. Even though ATM has been described as a promising target, there is no clinical data to support the application of ATM inhibitors. In the clinical trials database, there is one study with AZD0156 (NCT02588105) that aims to assess the safety of ATR inhibition in combination with other anticancer drugs in patients with advanced solid tumors. A summary published by the authors' reports that hematologic toxicity decreases the tolerance of AZD0156 with PARP inhibitors [88,89]. Moreover, a phase I study is currently evaluating the safety of AZD1390 in GBM and other brain tumors (NCT03423628) but is still at the recruitment step.

The clinical data shows that although there are many targets considered as promising in the DDR context (Fig. 4), the results are not highly promising in the GBM therapy. It is important to understand that in the context of DNA repair, the inhibition of an overexpressed target associated with the resistance process, for example, is only suitable if it happens at the tumor tissue. Otherwise, it can trigger side effects in normal cells, changing the normal DDR of healthy cells, and this can explain the hematological toxicity caused by the combination of olaparib and AZT0156 for instance [89]. Therefore, the pursuit for alternative therapeutic methods is a necessity for GBM therapy and could be achieved by using DDS that can deliver drugs into the brain and maintain the drugs stable.

5. Overview of drug delivery systems (DDS) for cancer therapy

In a way to overcome the lack of success of the systemic administration of drugs, the advent of DDS seems to be a promising and versatile perspective once this field of research has a range of materials that can be used to increase the cellular uptake of several chemotherapeutic agents [70]. Since this approach allows the mixture of diverse materials by several techniques, it facilitates the combination of different molecules (even the poorly soluble ones), increases their protection from earlier degradation, modifies their targets, modulates membrane interactions, and decreases side effects [90].

Those formulations can be administered by using different routes, which facilitates the delivery to several tissues, especially in brain tumors such as GBM, since this organ is constantly surrounded and shielded by the BBB, not to mention the BBTB that provides extra protection for the tumor microenvironment. Even in cases where those strategies might not be available, the use of localized treatment approaches to bypass some DDS drawbacks is still possible [91,92].

5.1. Use of nanotechnological structures as delivery systems to decrease toxicity and increase efficiency

The conception of complex nanocarriers (Fig. 5) allows the internalization and protection of drugs improving aspects related to the therapy outcome, including the efficiency of drug delivery in a specific target, the physicochemical stability, the surpassing of biological barriers and pharmacological properties, and the administration of different structures, such as proteins and RNAs; besides, multiple agents conjugate in a single nanosystem [93]. Since their surfaces can be modified with targeting molecules, nanocarriers acquire functions that not only deliver molecules to a specific location but also hold a more ample functionality since they may avoid bioactive compounds' early degradation and the consequent loss of effectiveness [94]. Also, the drug targeting concentrates the molecules in the aimed location, avoiding several effects and sometimes allowing the use of lower concentrations and spacing treatment schemes [95], not to mention that these nanocarriers may be able to accumulate inside tumors due to their reduced lymphatic drainage, which is one of the main characteristics of the enhanced permeability and retention (EPR) effect which will be discussed below.

With regard to their main advantages, nanotechnological structures can be designed by several approaches using lipidic and/or polymeric materials, which will generate structures such as liposomes, micelles, exosomes, polymeric and inorganic nanoparticles, and polymer conjugates. The selection of the most suitable material depends on the physicochemical characteristics and interactions between the system and the molecule to be integrated, the route of administration, and the target [96,97].

Liposomes are the most common form of lipid nanosystems and are based on phospholipidic vesicles that allow the loading of hydrophilic agents inside them and hydrophobic molecules at their lipid bilayer [97]. Their composition assembles to the cell membrane, increasing their biocompatibility compared to other nanomaterials, and their structure acts as a shell that protects the drug from early degradation. Even so, disadvantages such as poor solubility and oxidation of their phospholipids could appear. Nevertheless, these disadvantages may be solved by physicochemical and structural adjustments [98,99].

Micelles are colloidal nanostructures with the amphiphilic feature, formed by an external shell with sufficient polarity grade to dissolute in aqueous solutions and an internal hydrophobic core. They are a good approach to increasing the bioavailability of low-solubility drugs [100,101]. Contrary to liposomes and micelles, the exosomes are extracellular vesicles secreted by almost all cell types, and these multiple origins summed to their bilipid layer allow them to interact with different tissues and carry different molecules [102–104].

Polymeric nanoparticles are biodegradable and biocompatible solid colloidal structures that enable therapeutic agents with different characteristics to be entrapped, encapsulated, or absorbed onto the polymeric matrix. They encompass numerous types of natural or synthetic polymers, such as chitosan, albumin, poly(lactide-co-glycolide) (PLGA), poly(acrylic acid) (PAA), polylactide (PLA), and polyvinyl alcohol (PVA), all of them being capable to result in nanocapsules or nanospheres, depending on the process of formation. For differentiation purposes, when compared to natural polymers, the synthetic ones are feasible for better controlled or sustained drug release systems, whereas the difference between nanocapsules and nanospheres is based on the drug allocation (confined to the first one and dispersed all over the second one) [105–107]. Finally, polymers can also be covalently bonded to proteins, drugs, and other molecules as a way of avoiding a possible lack of interaction that may be present by polymeric nanoparticles. Once formed, these polymer conjugates can extend drug circulation time in the blood and improve their bioavailability and local burst release, which reduces adverse effects [108–110].

Inorganic nanoparticles are a wide group that consists of silica, magnetic, silver, and gold structures, which have a relatively easy development process in common, showing a surface engineering and physicochemical properties that provide them with special biocompatibility features [111,112]. Among these different types of nanoparticles, the gold ones have shown interesting results owing to their surface plasmon resonance which turns light into heat and kills local cells by the hypothermia caused [113,114]. Similarly, silver nanoparticles have remarkable conductivity properties that allow them to internalize into cells by endocytosis and release their content to the cytoplasm [115].

5.2. Administration routes to overcome blood-brain barrier (BBB) selectivity

The GBM resistance process is partially imposed by the BBB, which acts as a filter for several molecules, and is not a task to be easily overcome. Other consequences of GBM occurrence may constitute obstacles for brain drug delivery, such as the aggressive infiltration of the tumor, which releases cancer cells into neighboring areas, leading to a recurrent glioma, and the low level of drug diffusion that may cause unexpected local toxicity, reinforcing the requirement of DDS with

Table 2
Strategies and routes for the delivery of nano carriers on the brain.[a]

Strategies	Route	Advantages	Limitations	Reference
Intravascular delivery	Cross[a]	Selective drug delivery High-dose chemotherapy	Lack of treatment to the contralateral hemisphere	[60]
Intracerebral delivery	Bypass[a]	Targeting	Slow diffusion within the brain	[60,116]
Intrathecal and intraventricular infusion	Bypass	High concentrations in cerebrospinal fluid	Invasive Limited concentrations	[51,173]
Intranasal deliver	Bypass	Non-invasive	Local irritation Low efficiency	[51,116]
Intracarotid infusion	Bypass	Free drug diffusion	Passage of pathogens	[61]
Interstitial delivery	Bypass	Sustained and/or controlled release	Invasive Limited distribution through extracellular space	[51]
Transmucosal drug delivery	Bypass	High concentrations delivery	Cost	[61]
Implantable DDS	Bypass	DDS depot directly placed into the extracellular space	Cost	[118]
CED	Bypass	Minimally invasive	Cost	[119]
Direct intratumoral drug administration	Bypass	Fewer side effects	Not feasible depending on the tumor location	[118]
Targeted delivery	Cross	Selective drug delivery High-dose chemotherapy	BBB	[120]
BBB disruption	Bypass	Momentary and localized effects	Non-selective for specific drug/purpose	[118,120]

Abbreviations: blood-brain barrier (BBB); convection-enhanced delivery (CED).
[a] Strategies that cross the BBB are considered to be those that are systematically distributed and can cross the barrier, reaching the brain tissue. On the other hand, strategies that bypass the BBB are locally distributed, not needing to cross the BBB.

reasonable diffusion levels [91,92].

To date, several strategies have been explored to overcome BBB and therefore enhance local drug delivery [116]. These approaches can be divided into physiological, pharmacological, and invasive models [117], encompassing conventional strategies, such as the barrier disruption and transport systems modifications, and alternative methods involving DDS into the brain that can cross or bypass the BBB completely, such as nanoparticle carriers, focused ultrasound, and intrathecal, intraventricular, intranasal and interstitial routes (Table 2).

Once these DDS find a gateway for drug delivery to the brain, they can take advantage of the permeable tumor site vasculature, poor local lymphatic drainage, and compression of lymphatic vessels to reach tumoral environment and accumulate *in situ*, thus reducing systemic cytotoxic effects [121]. This peculiarity, referred to as the EPR effect, can be manipulated by modifications in the nanoparticles' structures, such as their size, shape, physicochemical properties, and porosity [93].

Although the EPR effect diverges between both patients and tumor characteristics, considering its relevance has become the center of several cancer treatments [90], especially when this effect can be used as an enhancer in the treatment of tumors placed beyond physiological barriers that make the therapeutic target almost unreachable, such as brain tumors.

5.2.1. Localized treatment of glioblastoma (GBM) can overcome chemotherapy drawbacks

Localized treatment involves the direct administration of drugs, such as gene delivery, chemotherapeutics, and immunotherapeutics, to the tumor site. This approach has been considerably studied in view of decreasing the adverse effects related to systemic CT. Among the benefits of local administration, the increased amount of drug at the tumor site and the decreased side effects on healthy cells significantly improve the efficacy of treatment. Thus, combining localized treatment with DDS nanocarriers can significantly increase drug stability by targeting the drug release directly into the tumor without the need to bypass biological barriers. Moreover, when these systems are directly delivered to the tumor site, formulations can be generated with diminished drug load. Owing to these advantages, the local treatment modality has been widely studied in the literature [118,122,123] and will only be briefly discussed here. The concept of localized treatment is known to be an easy solution for bypassing BBB, and the DDS used locally can be delivered in different ways as follows:

(i) local implant: depot of DDS directly placed into the extracellular space;
(ii) convection-enhanced delivery (CED): using a pressure head to drive a drug or DDS through the extracellular space. The CED is a method that delivers therapeutics directly through the interstitial spaces of the CNS by an infusion catheter. Results demonstrated prolonged survival of mice treated with CED etoposide compared to control mice [119];
(iii) direct intratumoral drug administration;
(iv) target delivery: cellular receptors-mediated delivery;
(v) BBB disruption: opening the tight junctions in the BBB for a short period and in a specific site.

In GBM, the localized approach is being used mainly to avoid the recurrence by targeting vascular endothelial growth factor (VEGF) overexpressing cells with monoclonal antibodies and the drug bevacizumab since the recurrent tumor is highly vascular [18,120]. Until now, the results indicated that the localized application significantly improves the efficacy of these drugs [124].

Regarding the implantable DDS, several attempts have been made to produce functional brain implants [125–130]. However, the only implant that has been approved by the Food and Drug Administration (FDA) is the Gliadel®. This wafer aims the sustained release of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU or carmustine), but its use has only produced modest improvement in patients' survival [131,132]. The implantable therapy approach, combined with controlled-release polymer-based DDS, could allow the use of drugs that were previously not utilized for GBM treatment due to their systemic toxicity, poor availability, and BBB bypassing. Thus, it opens a possibility to target new cellular pathways to treat GBM.

6. Drug delivery strategies applied in the DNA damage context for glioblastoma (GBM)

Despite the treatment management used against GBM, this disease is still an important challenge for the current medicine although several drugs and DDS have been tested. In several cases, the *in vitro* results are very promising but without success in *in vivo* GBM models.

Many reasons can explain this discordant result, including the low selectivity of the treatment and the incapacity of crossing the BBB. For instance, the application of Topoisomerases II poisons against GBM reflects one of these problems. These drugs impair the action of Topoisomerase II, an enzyme that removes the supercoiled DNA events by creating DSB, passing a separate DNA duplex through the breaks generated and rejoining the DNA ends [133]. While the preclinical data are promising, the low levels of intratumoral drug concentrations limit

Table 3
Drug delivery strategies applied in the DNA damage context for GBM tested *in vitro* and/or *in vivo*.

Delivery system	Conventional treatment	DDR target	Delivery approach	Outcome	Reference
Targeting direct repair					
Cationic liposome (LipoTrust™ EX Oligo)	–	MGMT siRNA	Intratumoral injection	The liposome efficiently delivered MGMT-siRNA *in vivo* and enhanced TMZ cytotoxicity.	[137]
Cationic liposome (LipoTrust™ EX Oligo)	–	MGMT siRNA	Intratumoral injection and CED infusions	The DDS did not achieve enough distribution.	[138]
Electrospun PLGA nanofiber	TMZ and BCNU	O^6-BG	Surgically implanted onto the surface of the brain parenchyma	Controlled and sustained release; The treatment efficiency was improved *in vivo*.	[139]
Apoferritin nanocage	TMZ	N3-propargyl imidazotetrazine analog (N3P)	*In vitro*	Greater cells uptake; Increased O^6-MG formation.	[140]
Targeting BER					
Superparamagnetic iron oxide nanoparticles coated with chitosan, PEG, and PEI	RT	APE1 siRNA	Intravenous injection	Mice treated with the combination (nanoparticle + RT) exhibited double extension in survival.	[141]
Oxidized graphene nanoribbons coated with DSPE-PEG	–	Lucanthone	*In vitro*	Enhanced cytotoxicity against GBM cells.	[142]
A fluorescent virus-like particle with a modified surface (cell-penetrating peptide and apolipoprotein E peptide)	TMZ	c-MET siRNA and RNAi	Intravenous injection	It significantly bypassed TMZ-resistance promoting cell death in time and dose-dependent manners, but no improvement was found in animal survival.	[143]
Targeting RT-related DDR					
MDH-DSPE-PEG-2000- cholesterol liposome	RT	DNA repair inhibitor Dbait	Intravenous injection	The treatment was able to effectively sensitize GBM cells to RT inhibiting tumor growth and augmenting the survival of mice.	[144]
PLA-PEG nanoparticle	Fractionated RT	Inhibitors for DNA-PK, ATM, ATR, and Chk1 proteins	Delivered locally *via* CED	The use of the ATR inhibitor impaired HR with insignificant influence on NHEJ and increased animals' survival.	[145]

Abbreviations: convection-enhanced delivery (CED); DNA strand break bait (Dbait); 1,2-distearoyl-sn-glycero- 3-phosphoethanolamine-N–amino (DSPE); O′1,O′1-(3-(dimethylamino) pro-pane-1, 2-diyl) 16-bis (2-(2-methyl-5-nitro1-1H-imidazol-1-yl)ethyl) di (hexadecanedioate) (MDH); polyethylene glycol (PEG); polyethyleneimine (PEI); polylactic acid (PLA); poly(lactic-co-glycolic acid) (PLGA); radiotherapy (RT); temozolomide (TMZ).

the efficacy of clinical applications [134]. In order to overcome this issue, a few delivery strategies were developed. Bruce et al. [135] demonstrated the safety of CED in the treatment of recurrent malignant gliomas treated with the topoisomerase poison Topotecan. Topotecan has an interesting antitumor activity with minimal drug-associated toxicity [135]. In this sense, clinical trials for GBM treatment with doxorubicin have been conducted using delivery strategies, including the Laser Interstitial Thermal Therapy (LITT) to modulate the BBB (NCT01851733) and a nanoparticle delivery targeting cells using bispecific antibodies (NCT02766699) [136].

Table 3 shows studies that developed DDS for targeting DNA repair in a GBM context.

6.1. Drug delivery strategies targeting direct repair

Owing to the importance of MGMT for the effectiveness of GBM CT, since this protein reverses the DNA damage effect of TMZ, several strategies have been developed to improve the efficacy of alkylating agents by inhibiting MGMT activity and diminishing CT resistance (reviewed by [146,147]). Among these strategies is the LipoTrust™ EX Oligo liposome DDS that delivers siRNA downregulating MGMT [137], which suggests that even TMZ-resistant cells could be sensitized to TMZ in both *in vitro* and *in vivo* tumor models after transduction. Similarly, Tsujiuchi et al. [138] used the same DDS for MGMT siRNA delivery. However, even by using CED in the application, the liposomes did not achieve enough distribution in the brain of rats and pigs.

As previously discussed, several studies have revealed that O^6-BG can improve the therapeutic efficacy of alkylating drugs by modulating MGMT activity [148,149]. However, the systemic administration of O^6-BG includes the inability to cross membranes and toxic side effects that can be overcome by an *in situ* approach. Therefore, Liu et al. [139] developed an electrospun poly(lactic-co-glycolic acid) (PLGA) nanofiber loaded with O^6-BG, TMZ, and BCNU. This system presented a controlled and sustained release of O^6-BG for two weeks followed by TMZ and BCNU for >14 weeks, and the *in vivo* results performed in F98 tumor-bearing rats suggested that the treatment efficiency was improved compared to a combined treatment of O^6-BG intraperitoneally, Gliadel® wafer implantation, or oral TMZ. Finally, the authors concluded that the O^6-BG-loaded nanofibers could be potentially used in therapy owing to their release properties that enhance the treatment and decrease systemic toxic effects [139]. This study shows the importance of targeting DNA repair while treating cancer cells with DNA-damaging agents.

An interesting study was designed by Bouzinab et al. [140] where TMZ was loaded into a nanocage made of apoferritin, which can be internalized by the transferrin receptor-1 and can facilitate cell uptake. Following GBM cell exposure to these nanosystems, an increased O^6-MG formation and consequent DNA damage burden were observed. Moreover, the N3-propargyl imidazotetrazine analog (N3P) was used with the apoferritin-nanosystem to overcome TMZ-resistant cells, which suggests that this approach could be further evaluated *in vivo* to confirm its enhanced therapy efficacy.

6.2. Drug delivery strategies targeting BER

A promising approach was established by Kievit et al. [141] in which nanoparticles comprising superparamagnetic iron oxide cores coated with chitosan, PEG, and polyethyleneimine (PEI), transporting anti-APE1 siRNA, were produced and injected intravenously through the tail vein of genetically modified mouse models, and finally, after 24 h, the animals were exposed to RT. The results showed that a reduction of 40% in APE1 activity was achieved only in the tumor tissue and that mice treated with the combination (nanoparticle + RT) exhibited double extension in survival compared to RT alone, which indicates that APE1 inhibition could improve RT outcomes. Another study targeting APE1

was developed by Chowdhury et al. [142] in which nanoribbons of oxidized graphene coated with amphiphilic polymer 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N -amino (DSPE)-PEG were prepared and loaded with lucanthone, an endonuclease inhibitor of APE1. Certain cancer-specificities were observed since this system enhanced cytotoxicity against GBM cells, but there was no toxicity when exposed to other cells such as breast cancer and rat glial progenitor cells [142].

As discussed before, PARP1 is another interesting target for GBM therapy [68] since this protein is considered as a cell-survival factor that acts during the repair of SSB, maintaining the genomic integrity. Several PARP1 inhibition attempts have been performed [68] by using siRNA or commonly known drugs like olaparib. Nevertheless, the results *in vitro* present great potential, while the outcomes *in vivo* do not seem to improve the treatment. It was shown that the inhibition of PARP1 with siRNA or the drug 3-aminobenzamide combined with silencing of MGMT followed by TMZ exposure significantly increased GBM cell death, which suggests that targeting BER and MGMT could be a promising strategy to solve TMZ-resistance [150].

Recently, Pang et al. [143] developed an elegant strategy to improve GBM therapy that aims to target DNA repair synergistically with TMZ exposure and enhances cell uptake by adding a surface modification in the nanosystem. Fluorescent virus-like particle/RNAi nano complexes modified with cell-penetrating peptide and apolipoprotein E peptide (dP@VLP/RNAi) were produced, targeting the tyrosine-protein kinase Met (c-MET), and evaluated *in vivo*. It is classically known that c-MET is a growth factor receptor but is also associated with the maintenance of genomic stability and DDR signaling (reviewed by [151]). c-MET siRNA and the entire complex, dP@VLP/c-MET RNAi, significantly revert TMZ-resistance, promoting cell death in time and dose-dependent manners. Importantly, the stability of the siRNA in the complex was greater than the naked siRNA. The complex was able to cross the BBB and, together with oral TMZ, significantly improved the animals' median survival from 25 days in the oral TMZ group to 42 days in the combination, indicating that the downregulation of c-MET can decrease the repair efficiency and revert CT resistance.

6.3. Drug delivery strategies targeting the radiotherapy-related DNA damage response (DDR)

A promising approach for maximizing GBM therapy is the combination of DNA repair inhibitors and RT. Recently, Liu et al. [144] produced a radiosensitizer-prodrug liposome for the delivery of the DNA repair inhibitor Dbait (DNA strand break bait), which mimics DSB by trapping DNA repair proteins, thus inhibiting the repair of DNA damage associated with RT. Due to the synergistic effects of this combination, the treatment was able to effectively sensitize GBM cells to RT inhibiting tumor growth and augment the survival time of mice [144]. Following the same line, King et al. [145] developed polylactic acid (PLA)-polyethylene glycol (PEG) nanoparticles loaded with DNA repair protein inhibitors, including DNA-PK, ATM, ATR, and Chk1, to be delivered *via* CED, aiming to radiosensitize the gliomas. The authors showed that nanoparticles containing VE822 (ATR inhibitor) could impair HR with insignificant influence on NHEJ, which increases the survival of *in vivo* models when in combination with fractionated RT. The study demonstrated the applicability of combinations of standard therapy and inhibitors for the local treatment of gliomas with the possibility of using this approach in different types of cancers [145].

7. Nanoparticles in cancer research

The most used strategies to drug deliver in the brain have been the pharmacological, neurosurgical-based approaches and destabilization of BBB [119,152]. Regardless of the delivery method, all drugs trigger tumor resistance and tolerance mechanisms that might limit the doses. Nevertheless, understanding the molecular basis of GBM can assist in novel combinatorial therapies such as the use of cytotoxic drugs and DDR-related proteins or synthetic lethality strategies. All approaches have advantages and disadvantages, and hence, understanding the characteristics of each tumor is important in this choice [152]. Many treatment strategies are based on the development of nanoparticles. The following topic will discuss relevant aspects in this context and the importance of evaluating the interaction between biological systems and nanocarriers.

Table 4
NanoEL effect observed in nanoparticles evaluations.

NP	Features	Outcome	Ref
TiO_2	Size: 23.5 and 680 nm Concentration: 5–1.250 μM	NP migrated into the inter-endothelial adherens junction niche, bounded directly to VE-cadherin and disrupted cell-cell interactions causing cell leakiness. This disruption resulted in the loss of interaction between VE-cad with β-catenin and with p120, triggering actin-rearrangement.	[158]
Silica	Size: 48 nm Charge: −18 mV Density: 1.45 g/cm^3 Concentration: 2.0 × 10^{11} NP/ml	NP disrupted the VE-cad-VE-cad interaction at the cell-cell junction of the endothelial cells. The overall gap formation process initiated by interactions of heavy NP. Underflow conditions the chance of NanoEL occurring increases.	[160]
Au	Size: 10 to 30 nm Charge: −16 mV Concentration: 25 μM	NP-induced micrometers sized gaps between endothelial cells within 30 min of exposure. The NanoEL occurred *via* disruption of VE-cad-VE-cad triggering actin remodeling.	[159]
PEI	Size: 25 kD	NP had the potential to activate the immune response *in vivo*. The ROS generation and inflammation activation contributed to immune system dysfunction and cancer metastasis.	[174]
Iron oxide	Size: 16 and 33 nm *application of an external magnetic field	The external magnetic field temporarily disrupted endothelial adherens junctions through internalized iron oxide NP, activating the paracellular transport pathway and facilitating local extravasation of circulating substances.	[157]
Au	Size from 37.66 to 68.43 nm Charge: −40, −20, +15 and +40 mV Concentration: 10 μM	The negative charge on Au NP induced more NanoEL. This NP could be repelled by the negatively charged glycocalyx in a bouncing manner toward the cell-cell junctions.	[161]
Titanium dioxide, silica, Au	Size: around 18 to 23 nm Charge: −20 mV Concentration: 10–40 μg/ml	Intravenously injected NP accelerated both extravasation and intravasation of breast cancer cells *in vivo*, inducing metastasis.	[166]

Abbreviations: nanoparticles (NP); titanium dioxide (TiO2); vascular endothelial (VE); gold (Au); Polyetherimide (PEI).

7.1. Nanoparticles: the cell leakiness drawback

DDS offers several prospects in treating and diagnosing tumors thanks to their many promising and attractive approaches [153–156]. Given the fact that some nanoparticles can provoke endothelial leakiness (NanoEL) [157–161], DDS aiming cancer therapy [162–164] can also accidentally induce NanoEL of the tumor vasculature, thus decreasing the impediment for intravasation entrance of persisting tumor cells into the circulation.

The term NanoEL is related to the cell-cell interaction disruption

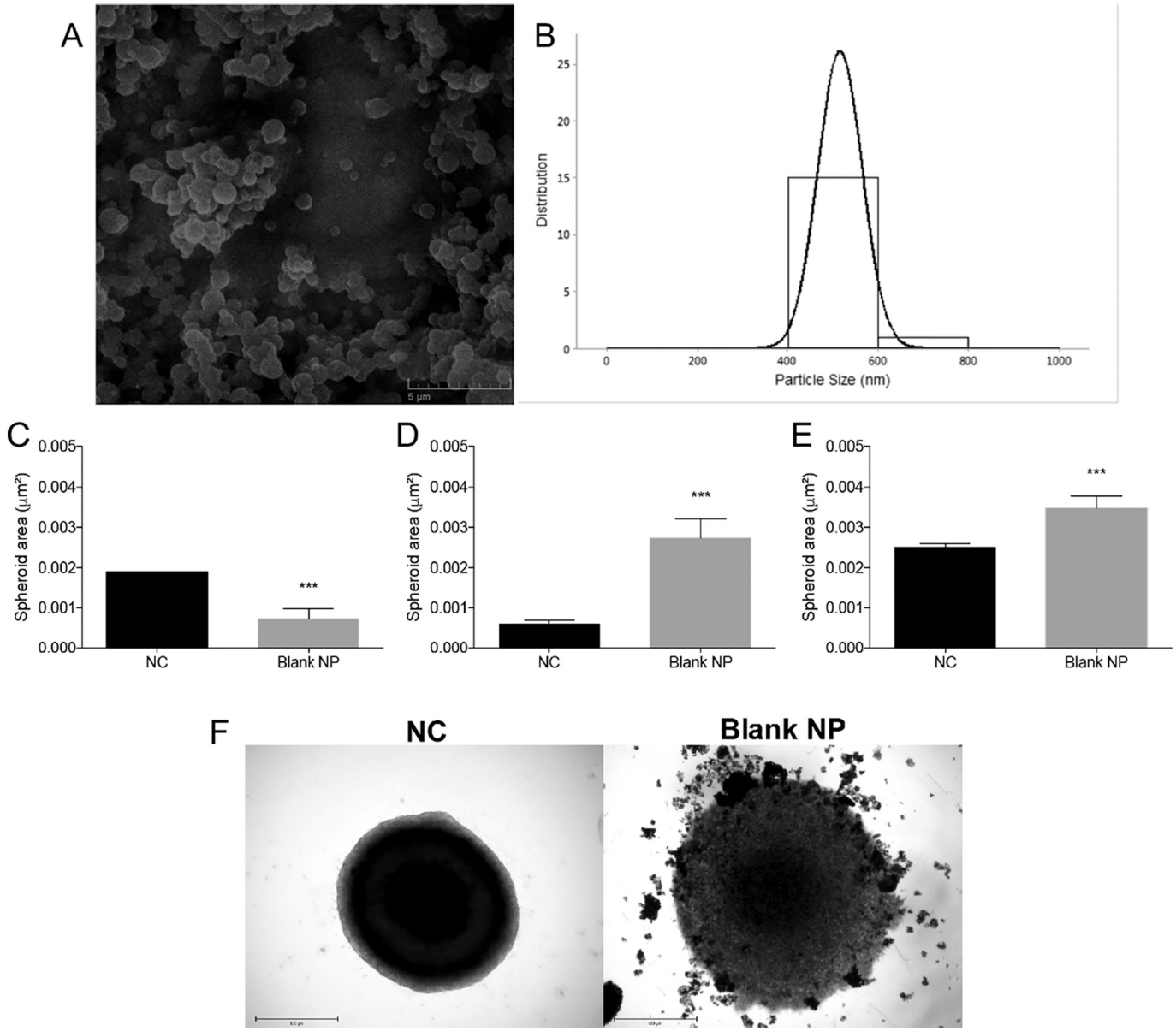

Fig. 6. Characterization of stearic acid nanoparticles. (A) SEM analysis of the nanoparticles. (B) Particle size distribution. (C) Spheroid size. (D) Expansion spheroid area (halo of live cells), (E) total spheroid size (spheroid area plus halo area), and (F) images of spheroids after 5 days of exposure. Results are expressed as mean spheroid area ± SD. Statistical analysis was achieved using one-way ANOVA. Data was considered significant at ***$p < 0.005$. NC: negative control.

caused by nanocarriers when it binds to adherent junction proteins, including vascular endothelial-cadherin (VE-cadherin) [158]. After the nanoparticle binds, there is an establishment of a force that disrupts the VE-cadherin interface [160]. The subsequent micrometer-sized gaps formed between the neighboring cells are caused by the intracellular signaling and the endothelial cell tension [158]. These cell junctions' disruptions were discovered to be linked with nanoparticles' size [159], the charge of the surface [161], and intrinsic mass density [160]. Given the importance that this drawback of nanocarriers could imply in nanotechnological approaches for cancer therapy, there is rising evidence of nanoproducts that can trigger endothelial gaps (Table 4) [158,159,165]. Exploring nanoparticles' effects on metastasis can increase the side effects knowledge of nanocarriers and stimulate more examinations on how to reduce the side effects and improve the proposed antitumor effect.

An impressive study developed by Peng et al. [166] systematically analyzed the effects of NanoEF induced by TiO_2 nanoparticles through the investigation of the multiphasic process of metastasis and its various cellular steps. The authors reported that these nanoparticles can promote the adhesion and migration of breast cancer cells to vascular endothelial cells and the permeability induced by the nanoparticle triggered extravasation and intravasation of breast cancer cells *in vivo* by interacting with the capillary endothelium and accelerating the extravasation of circulating cancer cells.

Our research group has prepared and characterized stearic acid nanoparticles (Fig. 6). These nanoparticles were produced by the emulsion method according to Hafeez and Kazmi [167] with minor modifications and characterized according to their size and zeta potential. These carriers presented a mean size of 519.5 ± 52 nm, and the zeta potential was found to be −31.6 ± 0.7 mV (Fig. 6A–B). Its efficacy *in vitro* was evaluated by using 3D cell culture of the U87 cell line. It was observed that after treatment with the nanoparticles, the total spheroid size (Fig. 6E) - spheroid area (Supplementary Fig. 1D) plus the halo formed by the expansion of live cells (Fig. 6C) - was bigger than the control spheroids (negative control). Moreover, the cells were slowly released from the spheroid and the spheroid exhibited a loose

morphology (Fig. 6F), suggesting a decreased tightness of cell contact which could provoke cell extravasation.

As suggested by our data, during the DDS development for cancer treatment, it is necessary to evaluate the interaction between biological systems and nanocarriers. Since nanoproducts can be related to new metastatic sites, an in-depth investigation of NanoEL during the DDS efficacy evaluation must be conducted, mainly in nanocarriers that can accumulate in human tissues and degrade slowly. Therefore, more studies are needed to better understand and regulate the NanoEL effect [168].

8. Conclusions

To date, the most acceptable therapy for newly diagnosed GBM is the combination of surgical resection, chemoradiotherapy with TMZ as adjuvant therapy. GBM remains incurable, and therefore, several new targets have been proposed to increase the patient's responsiveness to the treatment. Some of these targets include DDR proteins with the strategy of decreasing the DNA damage reparation capacity and inducing cell death. However, the low tolerability of these therapies impairs the efficacy and the improvement of survival outcomes [177]. In order to overcome these negative features, extensive attention has been drawn to the field of DDS and several new technologies have been developed to combat GBM [119,152].

The pharmacological approaches based on liposomes, polymeric nanoparticles, and wafers have been used in the context of DNA damage response inhibition [137–139,144,145,169]. These approaches allow the incorporation of several effective antitumor agents in the GBM treatment protocol, whose application was impaired by low BBB permeability such as doxorubicin [152]. Overcoming BBB selectivity with DDS unlocks a range of possibilities with known drugs whose tolerance and toxicity are already known. Nevertheless, the main challenge of using nanoparticles and liposomes is the short half-life in a systemic application [170,171].

In light of this, neurosurgical-based approaches allow the delivery at specific regions, which increases the bioavailability and efficiency of drugs [152]. The CED approach allows a continuous drug delivery *via* a catheter using positive pressure to increase the circulation throughout the brain tissue [119]. The invasiveness of this technology and the infection risk are some of the disadvantages. However, the local administration enables high drug concentrations, decreasing resistance mechanisms and avoiding systemic toxicity [119,135,152]. Furthermore, the delivery systems could assist in drug repositioning for GBM, which is a promising option as it aims to expand the possibilities of drugs whose pharmacokinetics and toxicity are already known [128,172].

Lastly, as described by this review, recent progress in DDS to the brain demonstrates the potential of new delivery strategies to permit novel and commonly used CT drugs and gene-based therapies to target brain cancer cells. These strategies can be modified among their physical and chemical properties to generate nanosystems that can recognize and target cancerous cells. It is expected that the ideas resulting from future experimental studies with the association of delivery systems and DDR targets will lead to the development of improved therapeutic interventions, which could bring hope to the incurable scenario of GBM patients.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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APÊNDICE C

Multifunctional nanocomposites for theranostics

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1.Introduction

Multifunctional nanosystems that associate diagnostic and therapeutic modalities are a novel trend in nanotechnology. Progresses in nanotechnology expressively influenced therapy and detection of illnesses (1-4). It is known that, prior to treatment, clinical diagnosis is a critical step that significantly affects therapy outcome and overall survival. Recently, clinicians are targeting to accomplish diagnosis and therapy simultaneously, thus, multifunctional nanosystems including nanocomposites offer a platform for theranostics to concurrently achieve diagnosis and treatment (5), making possible the monitoring of drug localisation and image the biological outcome of a therapeutical approach (Figure 1).

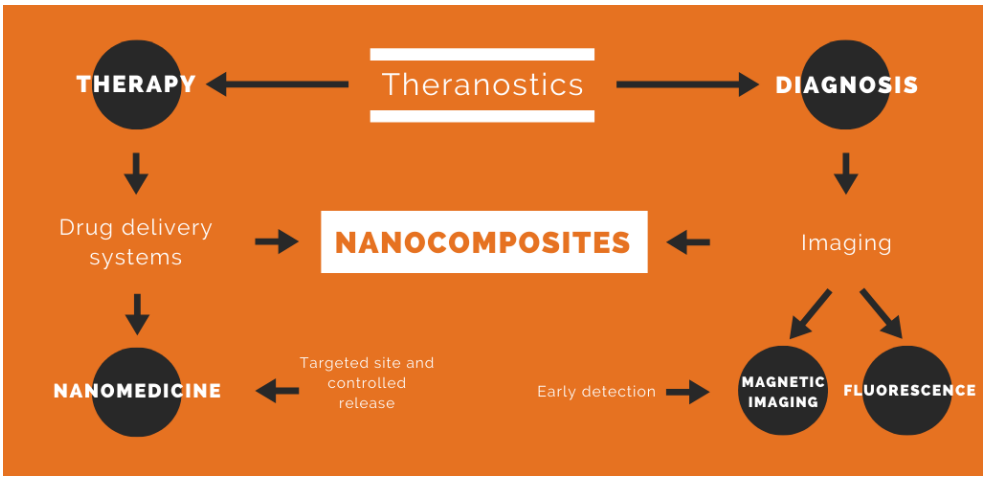

Figure 1. Nanocomposites as multifunctional drug delivery systems for theranostics approach.

The theranostic strategies can be produced by using several different approaches including solid lipid nanoparticles, micelles, dendrimers, silica nanoparticles, magnetic nanoparticles, quantum dots and polymeric carries that are able to achieve improved synergistic effects and diminished side effects when compared to drugs without a drug delivery system (6-8). The main aim of theranostics is to enable the diagnosis and treatment at the earliest stage of diseases. In this chapter, various nanocomposites designed for imaging as well as for the controlled release of drugs are described.

2.Nanocomposites

A nanocomposite is a two-phase structure, where, at least one constituent must present a nano-sized dimension up to 100 nm (9). A key property of a nanocomposite is a large surface area which results in higher interaction between its nano components with the matrix (10). The nanocomposite approach can avoid nanoparticle agglomeration by using a matrix where the nanoparticle can be dispersed (11), moreover, the nanocomposite biodegradability increases after producing a composite with nano-sized systems (12) (Figure 2).

Figure 2. Nanocomposites drug delivery systems.

Similarly, a nanocomposite drug delivery system hypothetically can accomplish important requirements aiming to provide effective therapy since these systems:

I. Improve drug pharmacodynamics and pharmacokinetics profiles.
II. Can eradicate unhealthy cells without affecting healthy cells.

III. Prolong and control the release of compounds.
IV. Enhance cellular uptake of delivered drugs.
V. Can reduce side effects by targeting therapy and by decreasing the dose of drugs (13, 14).

Owing to the fact that the nanocomposites field is a novel and rapidly expanding area, it is consistently generating new materials with different properties. Nanocomposite science produces a flexible platform for manufacturing new nanomaterials, which have diverse properties and functionalities. There are three different morphologic types of nanocomposites that are obtained including phase-separated systems, exfoliated systems and intercalated systems given the different preparation methodologies and nature of the components (15).

The types of framework systems for nanocomposites can be one, two and three-dimensional combination of organic and inorganic materials. According to the matrix of the systems, the nanocomposites can be classified as ceramic-matrix, metal-matrix or polymer-matrix nanocomposites. Specifically for biomedical applications, the bionanocomposites are usually hybrid systems derived from synthetic or natural biodegradable polymers and organic/inorganic fillers (16). Eminent areas of interest in nanocomposites for biomedical sciences are the production of nanofibers by the electrospinning method, which can be used as tissue scaffolds (17), the use of metallic nanoparticles incorporated into polymeric matrices, which present antimicrobial activity and can be used as a contrast agent for magnetic resonance imaging (18), and finally, the hydroxyapatite-based systems for bone repair and implantation (19).

Nanocomposites exhibit numerous advantages to manufacture drug delivery systems given their capacity to preserve the system’s stability and improve its physical and mechanical properties; nevertheless, since these systems are heterogeneous, the application of the nanocomposite is subjected by the composition of the system. Therefore, the production of multifunctional composites that are able to bypass drawbacks related to specific characteristics of individual components may enhance the quality of drug delivery systems and improve the treatment outcome of several challenging diseases.

3.The dual approach of theranostics

Advanced theranostic nanosystems retaining multifunctional properties have the capacity to diagnose and deliver therapeutics to the unhealthy tissue with the assistance of targeting ligands and biomarkers (20, 21) (Table 1). The entrapment, conjugation or encapsulation of imaging agents and drugs in nanosystems can result in combined loading and, it can eventually accomplish the purpose of theranostics at the cellular level (22, 23). The therapeutic agents used in theranostics comprise small chemical molecules, proteins, peptides and genetic material.

Aiming to deliver the combination of imaging agents and drugs concurrently, drug delivery systems including nanocomposites have been designed and developed to achieve a controlled and sustainable release of the therapeutic agents and to treat and image the affected cells in specific diseases. For precise circumstances such as pH, hypoxia and temperature, specific markers have been carried out to accurately regulate the drugs' pharmacokinetics (24).

Nanocomposites produced for cancer theranostics are intelligent platforms, which usually comprehend a synergic treatment with chemotherapy and stimuli-responsive nanocarriers for the photothermal therapy (PTT) and/or photodynamic therapy (PDT) (Figure 3). These combinations are capable of significantly enhancing the therapeutic efficiency (25). PTT is a local therapy modality, which is minimally invasive and non-toxic. This method is based on activating a photosensitiser agent by electromagnetic radiation, including near-infrared (NIR), microwaves and visible light aiming to transform the energy into heat. The heat generates hyperthermia, which triggers several cellular phenomena, such as cell membrane lysis and protein denaturation resulting in cell apoptosis (26). Differently of PTT, PDT induces an antitumour response by triggering the generation of radical oxygen species (ROS), thus, the presence of oxygen is mandatory (27). Even though PTT presents suitable efficacy, it is not an easy task to perform optimised NIR radiation on each individual tumour and, while heating the tumour tissue to approximately 50°C or higher, this method can damage the adjacent tissue, thus causing unwanted side effects (28). Thus, a targeted therapy seems to be an important option to overcome possible side effects towards healthy cells.

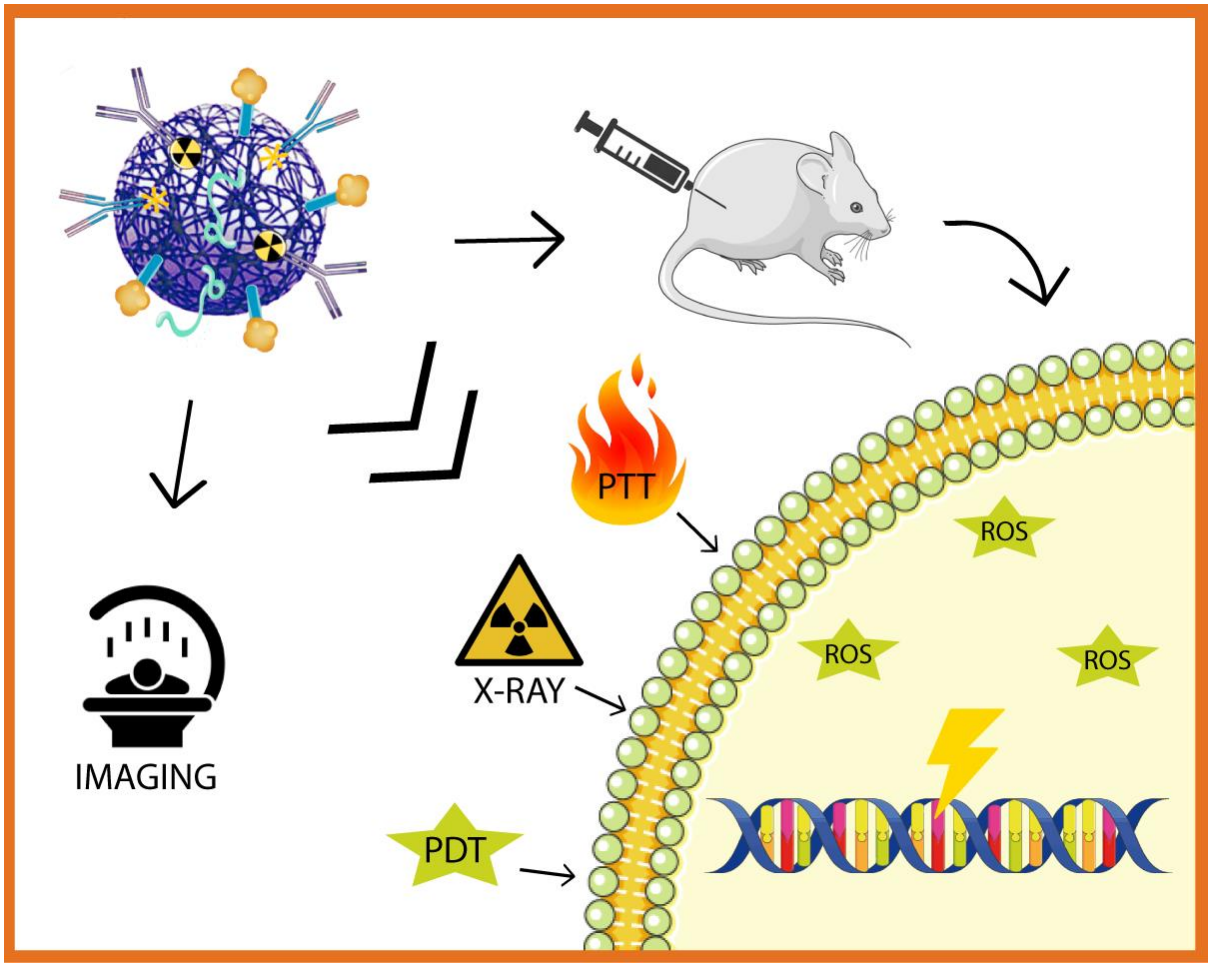

Figure 3. Nanocomposites produced for cancer theranostics offer different treatment combinations including PTT, PDT, chemotherapy and radiotherapy combined with diagnosis by using different imaging methods.

Imaging agents in drug delivery systems can be used to observer nanosystems accumulation and affinity in tissues and to detect interactions between the microenvironment and nanocarriers throughout a non-invasive way. The theranostic probes are a type of nano-agents that can deliver an improved therapeutic response through imaging guidance and positioning of the treatment (29).

Table 1. Properties of multifunctional theranostic systems and combination therapy options.

Combination treatment	**Imaging probe type**	**Characteristics**
Chemotherapy Radiotherapy	Small molecule dyes	Easy metabolise High quantum yield

Photothermal (PTT) Photodynamic (PDT)		
Radiotherapy Photothermal (PTT) Photodynamic (PDT) Gene therapy Immunotherapy	Quantum dots Carbon dots Metal nanoclusters Upconverting nanoparticles	Anti-photo bleaching Long cycle time
Radiotherapy Photothermal (PTT) Photodynamic (PDT) Gene therapy Immunotherapy	Fluorescent nanoparticles	Targeting and safety

The traditional diagnostic methods are magnetic resonance imaging (MRI), X-ray, positron emission tomography (PET), photoacoustic imaging, and computed tomography (CT), however, fluorescence imaging offers benefits when compared to those, including low toxicity, radiation and invasiveness and real-time rapid response. Based on these advantages, several fluorescent probes haven been described predominantly for tumour diagnosis. One interesting example is the development of targeted probes, which help to visualise the normal tissue boundary with the tumour aiming surgical assistance; moreover, the use of NIR fluorescent probes seems to be a promising approach for tumour vascular imaging. Amid the developed theranostic probes, glutathione-responsive prodrugs, proteinase-responsive prodrugs, hypoxia-activated prodrugs, H_2O_2-activated prodrugs, photon-activated prodrugs, quantum dots, carbon dots, metal nanoclusters and upconversion nanoparticles have been extensively studied. These probes present different features, but normally they exhibit suitable performance for diagnosis in biomedical research (Reviewed in (29)).

3.1.Nanocomposites for theranostics

In this section, recent developments in multifunctional nanocomposites theranostic platforms for the therapy of cancer (Table 2) and other disorders including brain disorders such as cerebral ischemia, cardiovascular diseases, osteoporosis, infections and bone regeneration will be summarized, including magnetic

nanoparticles, drug-polymer conjugates, micelles, polymeric/magnetic nanoparticles and polymeric/silica nanoparticles.

Table 2. Recent developments of multifunctional nanocomposites for cancer theranostics.

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
Bimetallic zeolitic imidazolate framework nanocomposites (Mn-ZIF-8)	Chemotherapy: 5-Fu	T_1-weighted MRI (Mn-based NPs)	Glioma (U87-MG tumour-bearing mice)	After injecting the system into the mice, a high accumulation of Mn2+ in tumours was observed. Targeted delivery significantly improved the therapeutic efficacy of Mn-ZIF-8/5-Fu, resulting in 80% survival rate over 40 days of treatment.	(30)
PLGA-based hybrid nanocomposites (LDM-PLGA/PPF/VEGF shRNA)	Chemotherapy: DOX and VEGF shRNA (targeted therapy)	T_2-weighted MRI and fluorescence imaging (quantum dots, superparamagnetic Fe_3O_4)	Breast cancer (EMT-6 tumour-bearing mice)	The co-delivery of DOX and VEGF shRNA into tumour cells effectively suppressed VEGF expression, exhibiting synergistic antitumour effects both *in vitro* and *in vivo*.	(31)
Nickel ferrite/carbon nanocomposite ($NiFe_2O_4$/C)	SDT	T_2-weighted MRI ($NiFe_2O_4$/C)	Melanoma (B16/F10 tumour-bearing mice)	The results established the applicability of the nanocomposite as a theranostic agent for concurrent SDT and MRI.	(32)
Nano-sized graphene oxide (GO)-PEG-folate nanocomposite	PDT PTT	Multi-colour fluorescence imaging (GO)	Melanoma (B16/F10 tumour-bearing mice)	The average half-life span of the mice treated with the nanocomposite GO-PEG-folate and PDT was ~1.8 times longer than the mice treated with DOX. The study accomplished an effective system of using GO-PEG-folate nanocomposite as a theranostic nanomedicine for simultaneous *in vivo* fluorescent imaging and combined PDT and PTT for antitumour treatment.	(33)
Zinc(II) phthalocyanine mono-α-substituted with 4-sulfonatophenoxyl (PcS) nanovesicle structure nanocomposite (NanoPcS)	PDT (zinc(II))	Fluorescence imaging	Hepatocellular carcinoma and adenocarcinoma (HepG2 and HeLa tumour-bearing mice)	The *in vivo* specific binding between albumin and PcS, of injected NanoPcS, was confirmed using a transgenic mouse system. Fluorescence imaging and antitumour tests suggested that NanoPcS has superior tumour-targeting ability and the potential for PDT.	(34)
Iron platinum-dimercaptosuccinnic acid/PEGylated	FePt (Fe catalyse H_2O_2 decomposition into ROS and	T_2-weighted MRI (Fe) and CT imaging	Breast cancer (4T1 tumour-bearing mice)	The results indicated that FePt-based NPs displayed suitable biocompatibility and favourable MRI/CT imaging ability *in vivo* and *in vitro*. The	(35)

graphene oxide-folic acid (FePt-DMSA/GO-PEG-FA) composite nanoassemblies (FePt/GO CNs)	induce cell apoptosis)			decomposition of FePt decreased the T_2-weighted MRI signal and increase the ROS signal. This enabled real-time and *in situ* monitoring of Fe release in tumour cells.	
Iron oxide (Fe_3O_4) NPs (G23-DOX/alg-Fe_3O_4) nanocomposite	Chemotherapy: DOX	T_2-weighted MRI	Glioma (U87-MG-luciferase tumour-bearing mice)	The nanocomposite was able to cross the BBB by targeting gangliosides. In the mice treated with DOX, algFe_3O_4 NPs and G23-alg-Fe_3O_4 NPs, the tumour sizes showed no obvious variation until 7 days post-injection, however, the tumours shrank significantly in the mice treated with the system.	(36)
Polypyrrole@MIL-53 nanocomposite	Chemotherapy: DOX PTT	T_2-weighted MRI (MIL-53)	Breast cancer (4T1 tumour-bearing mice)	The nanocomposite displayed *in vitro* and *in vivo* synergism of chemotherapy and PTT, MRI-guided.	(37)
PFH/DOX@PLGA/Fe_3O_4-FA nanocomposite	Chemotherapy: DOX HIFU	T_2-weighted MRI (superparamagnetic iron oxide NPs)	Hepatocellular carcinoma (Bel-7402 tumour-bearing mice)	The system suppressed tumour growth based on the enhanced and synergistic chemotherapy and HIFU ablation, providing an efficient theranostic nanoplatform for cancer treatment.	(38)
PEGylated hollow gold NPs (mPEG@HGNPs)	Radiotherapy PTT	CT imaging	Breast cancer (4T1 tumour-bearing mice)	mPEG@HGNPs exhibited a favourable tumour targeting effect and good CT contrast enhancement in breast tumour models.	(39)
Carbon nanoparticles (CPs), silver nanoparticles (AgNPs) and MnO_2 (CPs@MnO_2-AgNP) nanocomposite	PTT	Fluorescence imaging	Hepatocellular carcinoma (SMMC-7721 cell line)	This redox-responsive nanocomposite was constructed as a multifunctional nano-sensor for glutathione and improved the MnO_2 nanomaterial-based applications in GSH sensing.	(40)
Transferrin receptor antibody (TfR Ab) and DOX–loaded Fe3O4@ZnO nanocomposites	Chemotherapy: DOX Radiotherapy	MRI	Hepatocellular carcinoma (SMMC-7721 tumour-bearing mice)	After the treatment, a non-invasive visualisation monitoring exhibited a suppression in tumour growth by the targeted chemo radiotherapy.	(41)
Doped titanium dioxide NPs (TiO_2(Gd) NPs)	Radiotherapy with nanosensitiser (TPP)	Fluorescence imaging (IR806)	Breast cancer (MCF-7 tumour-bearing mice)	TPP in combination with a single X-ray radiation exposure achieved complete tumour ablation without side effects during treatment. Moreover, the mitochondria-targeted nanosensitiser could	(42)

				significantly reduce treatment doses and greatly amplify antitumour efficiency.	
Double-mesoporous core-shell nanosystems based on Pt NPs functionalised with lanthanide complexes (mPt@mSiO_2-GdDTPA)	PTT (Pt NPs)	MRI (Gd)	Adenocarcinoma (HeLa tumour-bearing mice)	The nanosystems displayed a higher r_1 value than the medical contrast agent magnevist and were successfully applied to *in vivo* MRI.	(43)
Gd-doped silicon NPs, zeolitic imidazolate framework-8 (ZIF-8), HOOC-PDMAEMA-SH, and FA-PEG into one single nanoplatform (FZIF-8/DOX-PD-FA) nanocomposite	Chemotherapy: DOX PTT: Ce6	MRI (Si-Gd NPs) and fluorescence imaging (Ce6)	Breast cancer (MCF-7 tumour-bearing mice)	The pH-responsive ability of HOOC-PDMAEMA-SH was able to prevent drug leakage. Moreover, The tumour volume of the FZIF-8/DOX-PD-FA + PTT-treated mice significantly decreased when compared to the other treatment groups.	(44)
Mesoporous silica-coated gold cube-in-cubes core/shell (RGD-CCmMC/DOX)	Chemotherapy: DOX, RGD (targeted therapy) PDT (singlet oxygen) PTT (gold cube-in-cube core)	MRI and fluorescence imaging	Breast cancer (4T1 tumour-bearing mice)	The nanocomposite was found to be biocompatible and effectively obliterates the tumours *in vivo*.	(45)
Au-BSA core/shell NPs (Au-BSA-DOX-FA) nanocomposites	Chemotherapy: DOX	CT imaging (Au)	Gastric cancer (MGC-803 tumour-bearing mice)	*In vivo* antitumour experiments demonstrated that Au-BSA-DOX-FA nanocomposites have selective antitumour activity effects and no adverse effects on normal tissues and organs. Additionally, the system exhibited selective targeting activity, X-ray attenuation activity and pH-sensitive drug release activity.	(46)
Mn-porphyrin&Fe_3O_4@SiO_2@PAA-c(RGDyK) nanocomposite	Chemotherapy: DOX	T_1- and T_2-weighted MRI and fluorescent imaging	Lung cancer (A549 tumour-bearing mice)	The nanocomposites exhibited highly sensitive MRI contrast function by synergistically enhancing positive and negative MRI signals. The nanocomposites showed great potential for	(47)

				integrating imaging diagnosis and drug controlled release providing real-time imaging with greatly enhanced diagnostic accuracy during targeted therapy.	
Mesoporous organosilica NPs (HMONs) loaded with ICG and PFP	Chemotherapy: PTX PTT: ICG	Ultrasound (PFP) and photoacoustic imaging	Breast cancer (MDA-MB-231-tumour-bearing mice)	The platform was found to have suitable properties for both ultrasound and photoacoustic imaging. In addition, both *in vitro* and *in vivo* results show that the NPs provide potent synergistic chemo-PT therapy.	(48)
AuNPs-PEI nanocomposites	PTT	Photoacoustic imaging	Colon carcinoma (CT26 tumour-bearing mice)	The nanocomposites containing PEI outperformed the other tumours as measured by tumour growth rate.	(49)
HMONs with PDA interlayer (DI@HMONs-PMOF)	Chemotherapy: DOX PTT: ICG	MRI and photoacoustic imaging	Breast cancer (4T1 tumour-bearing mice)	The results suggested that the existence of ICG can cooperatively enhance the MRI. In addition, the significantly improved synergistic therapeutic efficacy was confirmed both *in vitro* and *in vivo*.	(50)
Bio-Metal-Organic Framework (Fe_3O_4@Bio-MOF) coated FA-chitosan conjugate	Chemotherapy: Curcumin and 5-Fu	T_2-weighted MRI	Breast cancer (MDA-MB-231 tumour-bearing mice)	The selective uptake of 5-Fu-loaded Fe_3O_4@Bio-MOF by folate receptor-positive cells was confirmed. The nanocarrier exhibited no significant toxicity, while drug-loaded nanocarrier showed selective and higher toxicity against the cancerous cells than normal cells.	(51)
Hybrid Au/Ag doped carbon quantum dot nanocomposite	PTT	Not tested *in vivo*	Adenocarcinoma (HeLa tumour-bearing mice)	*PTT* heating experiments were promising.	(52)
A single-light-triggered ICG-loaded PEGylation AgNPs core/polyaniline shell (Ag@PANI) nanocomposites (ICG-Ag@PANI)	PTT: ICG	Photoacoustic and NIRF imaging	Adenocarcinoma (HeLa tumour-bearing mice)	The dual-modal imaging confirms the accumulation and distribution of ICG-Ag@PANI in the tumour region via EPR effect.	(53)
Neodymium vanadate ($NdVO_4$)/Au heterojunction nanocrystals (NCs)	PTT PDT	PTT and photoacoustic imaging	Adenocarcinoma (HeLa tumour-bearing mice)	$NdVO_4$/Au were internalised efficiently via endocytosis and cause apparent phototoxicity on HeLa cells. *In vivo* experiments showed that the system could act as a high-efficiency NIR light-triggered anticancer agent with suitable tumour inhibition effect.	(54)

PEGylated Fe@Bi_2S_3 nanocomposites	Radiotherapy PTT	*T2*-weighted MRI (Fe core) and CT (Bi_2S_3)	Breast cancer (4T1 tumour-bearing mice)	The imaging effect provided by the system was better than commercial products. When the nanocomposites were used for synergistic therapy there was a significantly reduction in tumour size and the nanocomposites exhibited no obvious toxicity to cells and mice at a therapeutic dose.	(55)
HA coated Fe_3O_4@polydopamine NPs	Chemotherapy: DOX PTT	MRI	Adenocarcinoma (HeLa tumour-bearing mice)	The nanocomposite presented a suitable anti-tumour effect by photothermal-chemo combination therapy. H&E and Ki67 staining tests showed obvious necrosis and weak cell proliferation at the region of the tumour.	(56)
Magnetic HMONs loaded with Ce6 and DOX, assembled with alginate/chitosan PEM and adsorbed with P-gp shRNA (M-MSN(Dox/Ce6)/PEM/P-gp shRNA) nanocomposite	Chemotherapy: DOX PDT: Ce6	MRI and CT imaging	Breast cancer (EMT-6 tumour-bearing mice)	The system presented great potential as a multifunctional delivery platform, which is promising for imaging-guided cancer combination therapy with high efficacy.	(57)
Au and ferroferric oxide NPs coating polypyrrole particles (PPy@Fe_3O_4/Au) nanocomposite	PTT	MRI and CT imaging	Adenocarcinoma (HeLa tumour-bearing mice)	The PPy@Fe_3O_4/Au nanocomposites exhibited suitable CT imaging and MRI performance, which provide more comprehensive and accurate diagnostic information. Moreover, PPy@Fe_3O_4/Au nanocomposites could efficiently kill cancer cells by hyperthermia and even completely ablate tumours.	(58)
SPIONs with PCLA-PEG-PCLA (NC-SPIOs-IR820-PTX) nanocomposite	Chemotherapy: PTX PDT: IR820 – photosensitiser)	MRI and NIR fluorescence imaging	Breast cancer (4T1 tumour-bearing mice)	The synergistic therapeutic effects of NC-SPIOs-IR820-PTX was able to induce tumour targeting and enhance the co-therapy results, resulting in significant tumour inhibition effects. Thus, NC-SPIOs-IR820-PTX theranostics could be applied for magnetic field guided tumour targeting as well as multimodal imaging, and imaging-guided combined therapy.	(59)
GO and AuNPs core with polyaniline shell	Chemotherapy: DOX	SERS imaging	Breast cancer (4T1 tumour-bearing mice)	The GO-Au@PANI system presented a high-performance as a chemo-photothermal therapeutic nanoagent. The theranostic applications of GO-	(60)

(GO-Au@PANI) nanocomposite	PTT: PANI			Au@PANI provide it with great potential for personalized and precise cancer medicine.	
FA and PEG modified octopod platinum-copper alloy nanoframes (OPCNs-PEG-FA) nanocomposite	Radiotherapy PTT: OPCNs	Infrared thermal *imaging and* photoacoustic *imaging*	Hepatocellular carcinoma (HepG2 tumour-bearing mice)	The multifunctional nanotheranostics of OPCNs-PEG-FA achieved simultaneous imaging and synergistic dual-modal radiotherapy/PTT tumour ablation. The nanotheranostic agent not only improved synergistic tumour suppression, but also exhibited no obvious systemic toxicity *in vivo.*	(61)
HA-coated FeOOH@polypyrrole (FeOOH@PPy) nanorods (HA-FeOOH@PPy NRs) nanocomposite	PTT	Photoacoustic *imaging*	Breast cancer (MDA-MB-231 tumour-bearing mice)	The photothermal anticancer activity results of the designed nanocomposite evidenced its promising potential in cancer treatment. The tumour-bearing mice completely recovered after 17 days of PTT treatment without obvious side effects.	(62)
Surface-superparamagnetic iron-oxide functionalised tantalum carbide (Ta_4C_3) MXene (Ta_4C_3-IONP-SPs composite MXenes)	PTT	T_2-weighted MRI and CT	Breast cancer (4T1 tumour-bearing mice)	The high photothermal-conversion efficiency of Ta_4C_3-IONP-SPs composite nanosheets achieved complete tumour eradication without reoccurrence, demonstrating the highly efficient breast-tumour hyperthermia performance.	(63)
MOF@POP-PEG (HUC-PEG) nanocomposite	PTT PDT	CT and photothermal imaging	Cervical cancer (U14 tumour-bearing mice)	The HUC-PEG exhibited suitable physiological stability and favourable biocompatibility. For the tumours treated with PTT, HUC-PEG had a highly efficiency in tumour inhibition.	(64)
Zeolitic imidazolate framework (ZIF-8) core-shell with MnO_2 on the surface of porphyrinic ZrMOF NPs (ZrMOF@MnO_2) nanocomposite	PDT	T_1-weighted MRI	Glioma (U87-MG-tumour-bearing mice)	ZrMOF@MnO_2 hybrid NPs have enhanced PDT efficiency owing to the intracellular balance of GSH and MnO_2.	(65)
Fe_3O_4-black TiO_2 (Fe-Ti NCs) nanocomposite	PTT PDT	MRI	Breast cancer (MCF-7 tumour-bearing mice)	Fe–Ti NCs had superior photothermal properties compared to those of individual NPs. Moreover, their therapeutic applications were confirmed.	(66)
Polydopamine stabilized graphene quantum dots (GQD)-	Immunotherapy PTT	MRI and fluorescence imaging	Breast cancer (EMT6 tumour-bearing mice)	CpG ODN was delivered to the targeted endosomal Toll-like receptor 9 to stimulate the secretion of pro-inflammatory cytokines and the maturation of	(67)

GCpD/CpG oligodeoxynucleotide (CpG ODN) NPs PC@GCpD(Gd)) nanocomposite	PDT			dendritic cells, thereby resulting in the activation and infiltration of T-lymphocytes. almost completely suppress the tumours under laser irradiation.	
GSH-platinum (IV) (Pt(IV)) prodrug-loaded phase-transitional NPs (Pt(IV) NP-cRGD) nanocomposite	Chemotherapy: Pt(IV)	Ultrasound imaging	Ovarian cancer (SKOV3 tumour-bearing mice)	Pt(IV) NP-cRGD combined with ultrasound imaging exhibited excellent echogenic signals, suitable therapeutic efficacy and limited side effect, suggesting precise theranostics against ovarian cancer.	(68)
Bismuth-based NPs coated with SiO2 and functionalised with S-nitrosothiol (Bi-SNO NPs)	Radiotherapy PTT	CT imaging and NIR thermal imaging	Cervical cancer (U14 tumour-bearing mice)	Synergistic tumour inhibition was found and no obvious toxicity of Bi-SNO NPs was observed in the treated mice within 14 days. Thus, the Bi-SNO was found to be an effective nano-agent for cancer theranostics with well-controlled morphology and uniform size.	(69)
Fe_3O_4 with ^{99m}Tc and IR-1061 (FIP-^{99m}Tc) nanocomposite	PTT: IR-1061	NIR fluorescence imaging, photoacoustic imaging (IR-1061) and CT imaging (^{99m}Tc)	Breast cancer (4T1-Luc tumour-bearing mice)	FIP-^{99m}Tc confirmed the fast accumulation and clear delineation of metastatic lymph nodes and it could effectively prevent further lung metastasis after resection of the primary tumour.	(70)
GO/bismuth selenide/PVP NP (GO/Bi2Se3/PVP) nanocomposite	PTT	CT imaging and photoacoustic imaging	Adenocarcinoma (HeLa tumour-bearing mice)	After intratumoural or intravenous injection of the nanocomposites, irreversible photothermal ablation of tumours was achieved.	(71)
Gadolinium porphyrin and Zinc porphyrin (GdTPP/ZnTPP) nanocomposites	PDT	MRI and fluorescence imaging	Adenocarcinoma (HeLa tumour-bearing mice)	The system achieved combined functions for visualised cancer theranostics.	(72)
Electrospun hyaluronic acid-ceramide (HACE) and Soluplus (SP) nanocomposite	Chemotherapy: resveratrol	NIR fluorescence imaging	Breast cancer (MDA-MB-231 tumour-bearing mice)	The HACE/SP NC can be a promising theranostic nanosystem for CD44 receptor-expressed cancers.	(73)
Maghemite (γ-Fe2O3) nanoflower-like multicore NPs and a	Magnetic hyperthermia	MRI and photoacoustic imaging	Prostate cancer (PC3 tumour-bearing mice)	Complete tumour regression was obtained for the PTT-treated animals.	(74)

spiky copper sulfide shell (IONF@CuS) (Iron Oxide Nanoflowers @ CuS Hybrids) nanocomposite	PTT PDT			The integration of the dual heating capability (magnetic hyperthermia + PTT) with the PDT offered a unique asset to tackle tumours by multiple cytotoxic strategies in order to improve the therapeutic outcome in a broader spectrum of clinical conditions.	
Lu-based upconversion nanophosphor (UCNP) and Bi-based nanomaterial loaded with iron phthalocyanine and coated with folate-conjugated amphilic PEG (UCNP@NBOF-FePc-PFA)	Radiotherapy PTT PDT	CT imaging and luminescence	Breast cancer (4T1.2 tumour-bearing mice)	A highly effective tumour ablation effect was verified, thus, the nanomaterial offered a novel method for the construction of a new theranostic platform.	(75)
β-cyclodextrin-$(76)_{21}$ [β-CD-(PLA-PDMAEMA-PEtOxMA)$_{21}$] unimolecular micelles loaded with AuNPs and DOX nanocomposite	Chemotherapy: DOX	CT imaging	Hepatocellular carcinoma (HepG2 tumour-bearing mice)	The system achieved high CT imaging and antitumour efficacy under *in vitro* and *in vivo* acid tumour condition.	(76)
Gadolinium oxide (Gd_2O_3) NPs with PPy, modifying with HA and loaded aluminum phthalocyanine (AlPc) (Gd_2O_3@PPy/AlPc-HA) nanocomposite	PTT PDT	MRI, fluorescence and photoacoustic imaging	Breast cancer (4T1 tumour-bearing mice)	HA and AlPc were adsorbed on PPy for HA-mediated tumour targeting and PDT respectively. It was observed enhanced tumour uptake effect after intravenous injection and the anti-tumour efficiency was achieved under the combined therapy, which was significantly better than any other mono-therapy.	(77)
Tyrosine (Tyr)-HA-PEI, radiolabelled with $^{131/125}$ I and loaded with a p53 mutant restoring regent, Prima-1	Chemotherapy: Prima-1 (targeted therapy) Radiotherapy	SPECT and CT imaging	Thyroid cancer (8305C tumour-bearing mice)	The system displayed a suitable tumour imaging and a long radiation treatment cycle. The ^{131} I-labeled NPs demonstrated anti-tumour effects *in vitro* and *in vivo*, due to radiosensitisation of Prima-1 by reactivation of the p53 mutants.	(78)

(Prima-1@PEI–HA–Tyrs–131I) nanocomposite					
T-UCNPs@Ce6@mSiO$_2$ nanocomposite	PDT	MRI	Breast cancer (MDA-MB-435 tumour-bearing mice)	The designed nanocomposite could improve the uptake of HER2-positive cells and tumours by modifying the site-specific peptide, and the *in vivo* experiments showed suitable MRI and PDT via intravenous injection.	(79)

5-Fu: 5-Fluorouracil; AgNPs: silver nanoparticles; Au: gold; BBB: blood-brain barrier; BSA: bovine serum albumin; Ce6: chlorine e6; CN: composite nanoassemblies; CP: carbon nanoparticles; CT: computed tomography; DOX: doxorubicin; EPR: enhanced permeability and retention; FA: folic acid; Fe: iron; Gd: Lanthanide; GO: graphene oxide; GSH: glutathione; H_2O_2: oxygen peroxide; HA: hyaluronic acid; HIFU: high-intensity focused ultrasound; HMONs: mesoporous organosilica NPs; HOOC-PDMAEMA: succinic-poly(2-(diethylamino)ethyl methacrylate); ICG: indocyanine green; Mn: Manganese; MOFs: metal-organic frameworks; MRI: magnetic resonance imaging; NPs: nanoparticles; NIR: near-infrared; NIRF: near-infrared fluorescence; PAA: poly(acrylic acid); PANI: polyaniline; PCLA: poly(ε-caprolactone-co-lactide); PcS: zinc(II) phthalocyanine mono-α-substituted with 4-sulfonatophenoxyl; PDA: polydopamine; PDT: photodynamic therapy; PEG: poly(ethylene glycol); PEI: polyethyleneimines; PEM: polyelectrolyte multilayers; PFH: perfluorohexane; PFP: perfluoropentane; PLGA: poly(lactic-co-glycolic acid); POPs: porous organic polymers; PPy: polypyrrole; PPF: PEI-PEG-FA; Pt: platinum; PTT: photothermal therapy; PTX: paclitaxel; PVP: polyvinylpyrrolidone; RGD: Arg-Gly-Asp peptide; ROS: reactive oxygen species; SDT: sonodynamic therapy; SERS: surface-enhanced Raman scattering; shRNA: short hairpin RNA; SPIONs: superparamagnetic iron oxide NPs; TfR Ab: transferrin receptor antibody; TPP: 4-carboxybutyl triphenylphosphonium bromide; VEGF: vascular endothelial growth factor.

3.1.1.Multifunctional nanocomposites for cancer therapy

Cancer is one of the most lethal diseases in the world, and the number of new cases rises each year (80). In 2018, it was estimated over 18 million new cases, resulting in more than 9.5 million deaths worldwide (81). The most common cancer types are lung, breast, and colorectal cancer, which comprehend for more than thirty percent of all cancer cases (81).

The conventional approaches for cancer handling comprise surgery, chemotherapy, immunotherapy and radiotherapy. There is a rising number of preclinical and clinical studies showing satisfactory outcomes for patients with different cancer types and stages (82-85) where overall cancer morbidity and mortality are decreasing. Even though there are novel improvements in diagnostic and treatment approaches, the patients' overall survival rate has not significantly improved over the past decades (86, 87). Therefore, there is a necessity for the development of novel approaches to specifically detect early-stage cancers and to target the therapies established on specific markers of different types of tumours, which could lead to personalised treatment.

Currently, innovative treatments including targeted therapies are being extensively studied (88), due to the fact that chemotherapy targets rapidly dividing cancerous cells and normal cells (89) resulting in severe side effects (90). Novel strategies for cancer therapy are extremely necessary to not only reduce side effects but also to improve patient diagnosis and prognosis. Recent advances in nanocomposites materials focused on passive and active targeting approaches for improving drug concentrations at the tumour site whereas preventing the undesirable toxicity to healthy tissue (91). Nanotechnology combined with molecular biology appears to be an important path for new therapy strategies guaranteeing personalised oncological care linked to low-cost and non-invasive treatments (91).

The targeted delivery of nanocomposites can overcome complications related to the conventional chemotherapy, including rapid clearance, insolubility of compounds, lack of selectivity and several side effects associated to the toxicity to healthy cells (92). A possible direction to resolve these problems is the use of theranostics

formulations. Several theranostic systems have been studied for tumour therapy and imaging (93) and particular attention has been found in the use of nanocomposites, as a consequence of their capacity to cross biological barriers and accumulate in tumour cells.

Nanocomposites theranostics can significantly advance therapeutic and diagnostic effectiveness (20, 92). Thus, these multifaceted drug delivery systems could reach challenging regions of the body such as the brain. Along with that, some biomolecules or biofunctionalised nanocarriers could penetrate the brain-blood barrier (BBB) and the brain tumour barrier (BTB), contributing to a better outcome for the brain cancers' treatment, including the glioblastomas.

Recently, a bimetallic zeolitic imidazolate framework (Mn-ZIF-8) was synthesised and it presented high surface area and good dispensability, which could be used for possible high drug loading. This drug delivery system was produced as a pH-responsive nanocomposite for the delivery of 5-Fluorouracil (5-Fu) targeting gliomas. The results demonstrated a significantly improved in the therapeutic efficacy of 5-Fu and it was able to prolong the survival of a glioblastoma mice model. Moreover, given the pH responsiveness property, it enhanced the accumulation of Mn^{2+} at the tumour site, resulting in a suitable T_1-weighted MRI signal (30). Another nanocomposite based on ZIF-8 containing MnO_2 (ZrMOF@MnO_2) used photodynamic therapy (PDT) to treat gliomas and it provided T_1-weighted MRI signal improving the PDT efficiency owing to the intracellular balance of glutathione (GSH) and MnO_2 (65). By using iron oxide (Fe_3O_4) nanoparticles, Liu et al. developed a nanocomposite loaded with doxorubicin (DOX) and alginate tagged with a peptide (G23; sequence: HLNILSTLWKYRC) on the surface, which is permeable to the BBB. This system nanocarrier is able to cross the BBB and enter the brain to treat gliomas while providing enhanced T_2-weighted images. The tumours of mice treated with G23-Dox/alg-Fe_3O_4 reduced significantly when compared to DOX only. Interestingly, the nanoparticles and alginate have their used authorised by the United States Food and Drug Administration (U.S. FDA), thus, this nanocomposite would be safe for human use since it does not show side effects (36).

It was observed a recent increase in the number of systems targeting breast cancer (31, 35, 37, 39, 42, 44, 45, 48, 50, 51, 55, 57, 59, 60, 62, 63, 66, 67, 70, 73, 75, 77, 79). These nanocomposites exhibited promising antitumour results and they were able to significantly achieve an efficient theranostic effect. Shen and collaborators proposed a strategy of encapsulating quantum dots, superparamagnetic Fe_3O_4 nanocrystals, and DOX into a biodegradable poly(d,l-lactic-*co*-glycolic acid) (PLGA) polymeric nanocomposite (31). This system was modified with polyethylene glycol (PEG) and folic acid (FA) and loaded with a vascular endothelial growth factor (VEGF)-small hairpin RNA (shRNA). The nanocomposite enhanced T_2-weighted MRI signal and the co-delivery of VEGF shRNA and DOX effectively suppressed VEGF expression resulting in a synergic antitumour effect (31).

An interesting study produced a hyaluronic acid-ceramide-based nanocomposite with Soluplus by using electrospraying method for the release of resveratrol (73). This drug is a known anticancer agent that can induce oxidative stress resulting in cell death, however, it has poor aqueous solubility and it is unstable in solution, thus, the authors improved the drug solubility by trapping it into a micellar composite structure. The results confirmed a selective tumour targetability with an increased uptake for the CD44+ cells in a breast cancer mouse model using a NIRF imaging, therefore, this system could be a promising theranostic option for CD44-receptor-positive tumours (73).

An impressive research was developed by Liu et al. (75), in which, the authors proposed a unique strategy combining PTT, PDT and radiotherapy to treat breast cancer. The developed nanocomposite contained a Lu-based upconversion nanophosphor (UCNP) and an iron phthalocyanine-loaded Bi-based nanomaterial (UCNP@NBOF-FePc-PFA). The system provided a tri-modal tumour treatment and the *in vivo* results demonstrated a greatly efficient tumour ablation effect, moreover, this nanocomposite exhibited upconversion luminescence capacity, X-ray attenuation, PTT effect, and X-ray and NIR that triggered ROS generation (75).

Other elegant nanocomposites approaches for cancer theranostic targeted hepatocellular carcinomas by using different tactics (34, 38). It is known that the overexpression of albumin receptors and albumin-binding protein SPARC (secreted

protein, acidic and rich in cysteine) on tumour cells increases albumin accumulation and degradation. Consequently, albumin is considered an interesting candidate as a cancer diagnosis biomarker and possibly a target for specific drug delivery. In view of that, a research group designed a theranostic nanocomposite system to target these cells that overexpress albumin (34). The design strategy of this system comprises a nanostructured self-assembly nanocomposite based on the zinc(II) phthalocyanine mono-α-substituted with 4-sulfonatophenoxyl (PcS) molecule, displaying an albumin-dependent disassembly (NanoPcS), resulting in an effective theranostic agent for tumour-targeted fluorescence imaging and time-modulated PDT (34). Finally, Tang et al. conjugated folate onto the surface of a nanocomposite for targeting hepatocellular carcinoma cells by receptor-ligand interaction, which facilitates the uptake of these systems into the tumour cells (38). Perfluorohexane (PFH) was used to generate PFH/DOX@PLGA/Fe_3O_4-FA nanocomposite, which significantly improved the high-intensity focused ultrasound (HIFU) ablation efficacy and when combined with DOX, it caused a higher percentage of tumour necrosis when compared to the other treated groups; moreover, it achieved the contrast-enhanced ultrasound imaging (38). Thus, this system successfully suppressed tumour growth providing an efficient theranostic nanocomposite for cancer treatment.

3.1.2.Multifunctional Nanocomposites for other diseases

Multipurpose nanodevices are a fascinating prospect towards more personalised and efficient medicine. As a future perspective, it is challenging since each disease and individual have unique characteristics that have to be taken into account to meet its needs when building a nanotheranostics system (94). Nevertheless, its application reduces prognosis mistakes and therapeutically failure due to empirical treatment, since it can provide an early and more accurate diagnosis, targeted release, and real-time imaging. Commonly, the nanotheranostics agent is a nanocomposite system, especially for its multiple functionalisation opportunities, targeting possibilities, polymer coating/stabilisation ability, and carrier function and not to mention its varied bioimaging approaches (either through fluorescence or MRI/electronic transmission). Most of the nanocomposites have been assessed for anticancer therapy, however, other applications are arising reported in the literature.

Herein we outline utmost research on different diseases treatment with nanocomposite theranostics (Figure 4).

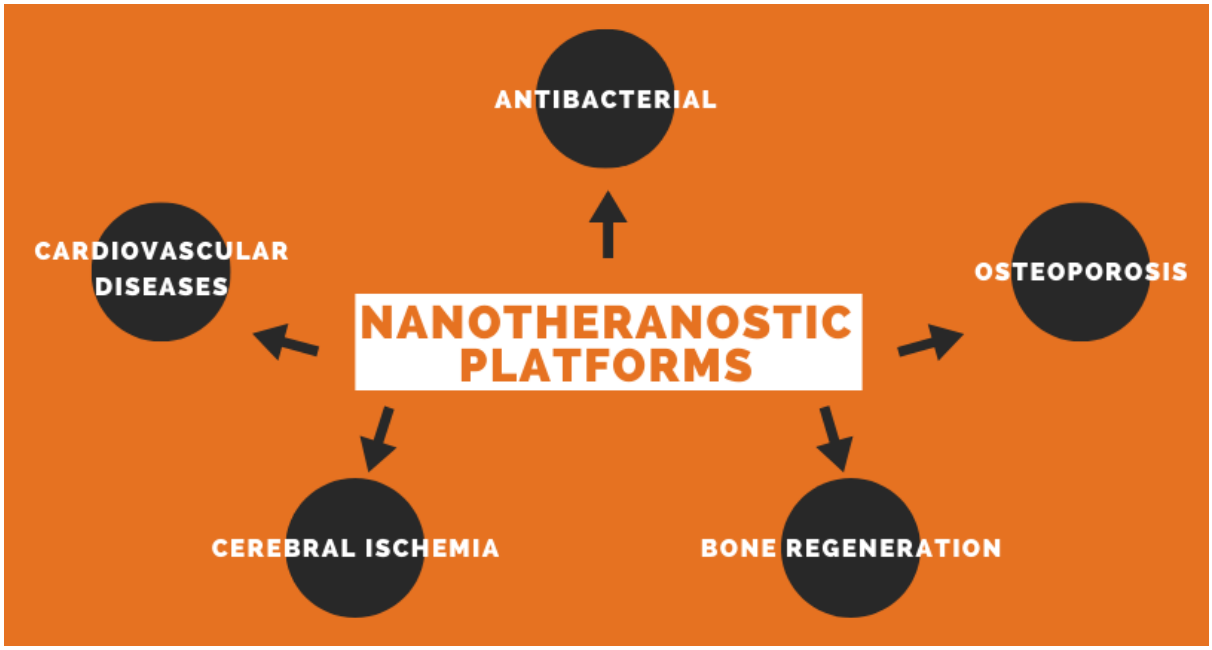

Figure 4. Scheme illustrating currently approached diseases with nanotheranostics composites based on published research.

Recently, Kumar and collaborators (95) highlighted the potential of nanotheranostics to address current issues in the neurodegenerative diseases field, such as Alzheimer's and Parkinson's diseases. Up-to-date studies call attention to nanocomposites, metal nanoparticles, biosensors, quantum dots, and biomarkers that can be further explored on a combined strategy such as nanotheranostics. A limitation for the treatment of neurodegenerative diseases is the individuality of each patient, in line with other diseases. Every patient has a different genome coding and its neural functioning characteristics, thus, it is difficult to assess all of them on a standard-treatment basis. Another issue found is proper targeting of proposed treatments, due to absorption by the systemic circulation and the challenge of the BBB. Also relevant, monitoring of the treatment is currently inefficient and the therapeutic alternatives are expensive. All of these characteristics urge the attention to the development of a complex nanocomposite combined with a theranostic approach that could potentially solve most of the issues related to neurodegenerative diseases treatment. In 2018, Chen and collaborators (96) highlighted that the current approach to treat Alzheimer's disease by inhibiting

amyloid-β aggregation might be uncertain, since most of the treatments lead to failure, and this mechanism has been weakly correlated with cognition loss in the literature. Thus, researchers have proposed a nanocomposite focused on diminishing tau hyperphosporilation and oxidative stress, to reduce cognition decrease *in vivo.* The proposed nanocomposite had methylene blue as carried drug, which inhibits tau aggregation, in combination with and iron oxide nanocrystals (IONCs, for bioimaging) onto the surface of mesoporous silica nanoparticles (MSNs) for synergistic effect. The nanoplatforms had also self-assembled ultra-small ceria nanocrystals (CeNCs), which are tau hyperphosphorylation inhibitors, and coated with Amino-T807 (selective tau binder). The Amino-T807 was grafted via the macrocyclic chelator NOTA, which could be further conjugated to $_{68}$Ga for PET imaging. With this approach, the authors achieved a simultaneous inhibition of tau hyperphosphorylation and aggregation, accompanied by the inhibition of neuronal apoptosis and consequent therapeutically success. However, the study did not assess BBB barrier crossing, which might be a topic for further improvement and analyses on this promising nanocomposite (96).

Beyond Alzheimer's disease, brain stroke (or infarction) is also an important disease that leads to many deaths every year. Almalki and collaborators (97) recently highlighted the importance of nanotheranostics to address common issues regarding stroke treatment, like specificity, targeting and bioimaging, and enumerated in their review some outstanding studies with nanoparticles and nanocomposites for this purpose. For example, a study using core-shell nanopolymerosomes with magnetic nanoparticles and targeting siRNA revealed a theranostic potential to targeted deliver, provide imaging contrast and affect the neural stem cells (97).

In a similar situation, osteoporosis is a disease in which its current hormonal treatment lacks specificity and targeting. Serious side effects occur in patients undertaking hormonal therapy to treat osteoporosis, due to the accumulation of the hormone in non-selective sites (such as breast, heart, and uterus). Therefore, the nanotheranostics approach is a good choice to mitigate this problem. Chen et al. (98) have reported an upconversion nanocomposite of 17β-estradiol (E2)-laden, mesoporous silica surface-modified with EDTA, for targeted osteoporosis hormonal treatment. The upconversion core (nanoscale crystals doped with rare-earth ions)

coated with mesoporous silica provided imaging capacity to the complex, together with a slow-release profile (drug release within 50 hours). Moreover, EDTA functionalisation provided bone-targeting ability. The proposed nanocomposite proved to be efficient on bone targeting and local delivery of the hormone (good cellular uptake rates and osteogenic activity), decreasing side effects and increasing the efficiency of the therapy (98).

Tissue engineering and bone regeneration can also be assessed by nanotheranostics composites, in order to monitor the treatment *in situ* and increase its efficiency. Li and collaborators (99) indicated the application of nanotheranostics to bone regeneration through a composite nanogel. The authors developed a simple poly(citrate-siloxane) elastomer to provide desired mechanical properties, osteogenic capacity, and photoluminescent property (bioimaging) to conjugate with BGNs (silica-based bioactive glasses). The research group used sol-gel derived BGNs nanoparticles monodispersed in the elastomer environment to potentiate biomineralisation and osteoblastic cells response. It could be seen that the biomineralisation activity, osteogenic differentiation ability, low inflammatory response, and osteoblasts biocompatibility was achieved within the proposed nanocomposite. Besides, bioimaging capacity was demonstrated with its detection for more than 2 months *in vivo* (99).

In a recent review, Agrawal and collaborators (100) shed light on nanomaterials-based theranostics to approach vascular diseases, such as atherosclerosis. Mostly using nanoparticles, research is going towards nanocomposites applications due to its multiple mechanisms possibilities and addressing of current disadvantages and limitations listed by them, to note, nanomaterial-related toxicity, limited targeted ability, and difficulty to assess the blood distribution (100).

Considering all previously mentioned studies, it is ultimate that the second most addressed field for nanotheranostics composites is bacterial infections. In a recent review, Mosselhy and collaborators (101) claimed attention to the application of nanotheranostics to assess drug-resistant *Staphylococcus aureus* (MRSA) infection and biofilm formation. They highlighted different nanosystems like nanofibers and nanoparticles as potential platforms for treatment, diagnosis, and real-time

accompanying of the pathogen infection. According to the review, different mechanisms can be approached by nanotheranostics, such as PTT and PDT (similar as for cancer treatment). Moreover, it can provide rapid and accurate bacterial identification, together with treatment monitoring. In agreement, some recent studies have approached this technology to fight different bacteria with varied nanocomposite systems (101).

In 2017, Huang et al. (102) developed a polypeptidic ruthenium/selenium nanotheranostics composite for antibacterial activity. The targeting function was achieved by the peptide UBI29-41, which also helped the stabilisation of the selenium nanoparticle core. The selenium nanoparticle core, beyond its antibacterial activity, is also efficient in wound healing function. Selective imaging function was assessed by coating with fluorescent ruthenium complexes, also possessing antibacterial activity. The signalisation of the system with ruthenium and the peptide provided specificity to the nanocomposite, being able to distinguish between cancer-induced infection, bacterial infection, and inflammation sites. Moreover, the selenium and ruthenium combination proved to have a synergistic inhibitory effect, especially against gram-positive bacteria. Mechanisms involved in antibacterial activity were adsorption via electrostatic interactions with the phosphoric acid on bacteria cell wall, disruption of cell membrane followed by ROS generation inside the bacteria, and DNA destruction. Also, biocompatibility assays were performed (cytotoxicity and haemolysis) and its toxicity was considered negligible, however, there is still a place for optimisation of biocompatibility in further assessed nanoplatforms in this fashion (102). It is worth mentioning that Enshaei and collaborators (103) used a different nanotheranostics approach to treat bacterial infections. They used the electro-responsive polymer (poly(3,4-ehtylendioxythiphene)) loaded with chloramphenicol, forming nanoparticles with activity against *Escherichia coli* and *Streptococcus sanguinis.* This smart nanotheranostic electro-responsive platform provided antibiotic controlled release by cyclic voltammetry and diagnostic capability through electrochemical detection of $\boldsymbol{\beta}$-nicotinamide (microbial metabolism product) (103).

More recently, Liu and collaborators (104) proposed a self-assembled nanogel of thiolated silver nanoclusters impregnated in chitosan for both gram-positive and gram-negative infections, *in vitro*. Ultra-small silver nanoclusters are known for their

antibacterial activity, however, their potential of toxicity and aggregation capacity complicates their usage. Thus, the group used chitosan to enhance biocompatibility, antibacterial activity, diminish aggregation by fine dispersion and promote the slow release of silver ions. Moreover, these nanoclusters are also photo-luminescent at UV-vis range and had their photoluminescence increased by 1.8 fold with the chitosan conjugation. The nanocomposite demonstrated to have almost the same activity after 6-month storage at 4ºC, indicating stability. It was tested against *Staphylococcus aureus, Escherichia coli, Bacillus subtillis,* and *Pseudomona aeruginosa*, bacteria representative of the main pathogenic ones, and functioned as both bacteriostatic and bactericidal with all of them, having a proposed mechanism of action by ROS production (slight increase). The complex had reduced cytotoxicity in comparison to non-conjugated nanoclusters, but it still needs improvements for biocompatibility in further animal testing (104).

Taking into account the utmost research on nanocomposites for theranostics, it can be stated that this technology has potential to be widely used for many diseases. The nanocomposite complexity can range from multi composed platforms of more than four components, with complicated synthesis and individual roles; or it can comprise a conjugation of up to three components in affordable and easy synthesis, with multi skilled components. Even promising, the technology is still at its basics steps towards development out of the cancer field, and it is of great contribution for the science of the future.

4.Perspectives

One of the most attractive options for theranostic applications is the nanocomposites that associate biofunctionalised nanoparticles and the suitable photosensitiser. Nevertheless, the limited available data related to unknown effects that these approaches could trigger on biological systems turn the applicability of multifunctional nanocomposites challenging. For complex drug delivery systems as nanocomposites to be recognised as safe for human treatment, more studies including the use of other *in vivo* models, besides immunocompromised mice models, are indispensable.

In a cancer therapy point of view, to achieve an efficient antitumour effect without harming the healthy adjacent tissue is still a demanding goal of novel therapies' approaches. Thus, multifunctional nanocomposites could bypass several treatment drawbacks including systemic toxicity, drug degradation and bloodstream circulation time. The data assessed in this chapter exhibited promising theranostic systems; however, it was also observed the lack of standardisation of evaluation methods between research laboratories. Importantly, the complex mixtures could be a risk if the nanocomposite release dynamic is not assessed. For commercially applicable nanocomposites, there is a necessity for prediction of the metabolisation of secondary products aiming to develop a risk assessment framework for the delivery systems.

With that said, the perspective of theranostics' applications is optimistic. With key attention on personalised medicine, theranostics offers an evolution from conventional therapy to a precise medicine approach. The theranostics systems are able to achieve the target tissue avoiding host defences and delivering treatment drugs and diagnostic agents to treat and diagnose at a cellular and molecular levels. It is also important to state that theranostic systems could move directly in clinical trials for established markers and drugs.

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69. Zhang F, Liu S, Zhang N, Kuang Y, Li W, Gai S, et al. X-ray-triggered NO-released Bi-SNO nanoparticles: all-in-one nano-radiosensitizer with photothermal/gas therapy for enhanced radiotherapy. Nanoscale. 2020;12(37):19293-307.
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71. Zhang Y, Zhang H, Wang Y, Wu H, Zeng B, Zhang Y, et al. Hydrophilic graphene oxide/bismuth selenide nanocomposites for CT imaging, photoacoustic imaging, and photothermal therapy. J Mater Chem B. 2017;5(9):1846-55.
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96. Chen Q, Du Y, Zhang K, Liang Z, Li J, Yu H, et al. Tau-Targeted Multifunctional Nanocomposite for Combinational Therapy of Alzheimer's Disease. ACS Nano. 2018;12(2):1321-38.
97. Almalki WH, Alghamdi S, Alzahrani A, Zhang W. Emerging paradigms in treating cerebral infarction with nanotheranostics: opportunities and clinical challenges. Drug Discov Today. 2020.
98. Chen X, Zhu X, Hu Y, Yuan W, Qiu X, Jiang T, et al. EDTA-Modified 17β-Estradiol-Laden Upconversion Nanocomposite for Bone-Targeted Hormone Replacement Therapy for Osteoporosis. Theranostics. 2020;10(7):3281-92.
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Biomineralization Activity, Real-Time Bioimaging Tracking, and Decreased Inflammatory Response. ACS Appl Mater Interfaces. 2018;10(21):17722-31.
100. Agrawal S, Nooti SK, Singh H, Rai V. Nanomaterial-Mediated Theranostics for Vascular Diseases. Journal of Nanotheranostics. 2021;2(1):1-15.
101. Mosselhy DA, Assad M, Sironen T, Elbahri M. Nanotheranostics: A Possible Solution for Drug-Resistant Staphylococcus aureus and their Biofilms? Nanomaterials (Basel). 2021;11(1).
102. Huang N, Chen X, Zhu X, Xu M, Liu J. Ruthenium complexes/polypeptide self-assembled nanoparticles for identification of bacterial infection and targeted antibacterial research. Biomaterials. 2017;141:296-313.
103. Enshaei H, Puiggalí-Jou A, del Valle LJ, Turon P, Saperas N, Alemán C. Nanotheranostic Interface Based on Antibiotic-Loaded Conducting Polymer Nanoparticles for Real-Time Monitoring of Bacterial Growth Inhibition. Advanced Healthcare Materials.n/a(n/a):2001636.
104. Liu J, Liu L, Li S, Kang Q, Zhang R, Zhu Z. Self-assembled nanogels of luminescent thiolated silver nanoclusters and chitosan as bactericidal agent and bacterial sensor. Materials Science and Engineering: C. 2021;118:111520.

CURRÍCULO LATTES

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Última atualização do currículo em 06/05/2022

Possui graduação em Toxicologia Analítica pela Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA (2016), mestrado em Biociências pela UFCSPA, com período sanduíche no Athlone Institute of Technology - Irlanda (2019). Atualmente é doutoranda em Genética Médica na Universidade de Newcastle - Austrália e no Programa de Pós-Graduação em Biociências na UFCSPA. Tem experiência da área de reparo de DNA, genotoxicidade, mutagenicidade, ensaios in vitro e in vivo, câncer, polímeros para aplicações biomédicas e nanotecnologia. **(Texto informado pelo autor)**

Identificação

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Endereço

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Formação acadêmica/titulação

2019 Doutorado em andamento em BIOCIÊNCIAS (Conceito CAPES 4).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.
Orientador: Dinara Jaqueline Moura.

2019 Doutorado em andamento em Medical Genetics.
The University of Newcastle Australia, NEWCASTLE, Austrália.
Orientador: Kelly Avery-Kiejda.
Bolsista do(a): university of newcastle, UON, Austrália.

2017 - 2019 Mestrado em BIOCIÊNCIAS (Conceito CAPES 4).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.
com **período sanduíche** em Athlone Institute of Technology (Orientador: Michael Nugent).
Título: Desenvolvimento e avaliação do potencial terapêutico de sistemas de entrega de drogas antitumorais para o tratamento de gliomas,Ano de Obtenção: 2019.
Orientador: Dinara Jaqueline Moura.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.

2014 - 2016 Graduação em Toxicologia Analítica.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2010 - 2012 Ensino Médio (2º grau).
Colégio Marista Nossa Senhora do Rosário, MARISTA/Rosário, Brasil.

Formação Complementar

2022 - 2022 I2N?s Navigator for Researchers. (Carga horária: 16h).
The University of Newcastle Australia, NEWCASTLE, Austrália.

2020 - 2020 Writing Amazing Abstracts. (Carga horária: 4h).
The University of Newcastle Australia, NEWCASTLE, Austrália.

2020 - 2020 Secrets and strategies in peer review. (Carga horária: 4h).
The University of Newcastle Australia, NEWCASTLE, Austrália.

2019 - 2019

Hunter Informatics Training. (Carga horária: 72h).
The University of Newcastle Australia, NEWCASTLE, Austrália.

2018 - 2018 Communications Module. (Carga horária: 45h).
Athlone Institute of Technology, AIT, Irlanda.

2017 - 2017 Minicurso de Quantificação de Imagens. (Carga horária: 3h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2016 - 2016 Extensão universitária em I Encontro do Programa de Pós-Graduação em Biociências. (Carga horária: 12h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2016 - 2016 Minicurso em Bioinformática. (Carga horária: 3h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2015 - 2015 Extensão universitária em V Curso de Inverno - Resposta a danos no DNA: Impl. (Carga horária: 50h).
Universidade de São Paulo, USP, Brasil.

2015 - 2015 I Curso de Toxicidade Genética: Causas, Consequências e Ensaio Pré-Clínico. (Carga horária: 50h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

Atuação Profissional

Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

Vínculo institucional
2019 - Atual Vínculo: Doutoranda, Enquadramento Funcional: PhD candidate, Carga horária: 8

Vínculo institucional
2017 - Atual Vínculo: Bolsista, Enquadramento Funcional: Mestranda, Carga horária: 40, Regime: Dedicação exclusiva.

Vínculo institucional
2017 - 2017 Vínculo: Bolsista, Enquadramento Funcional: Bolsista de Apoio Técnico, Carga horária: 40, Regime: Dedicação exclusiva.

Outras informações Bolsista de apoio técnico no Laboratório de Genética Toxicológica.

Vínculo institucional
2016 - 2017 Vínculo: Bolsista, Enquadramento Funcional: Iniciação Científica, Carga horária: 20

Vínculo institucional
2016 - 2016 Vínculo: Estagiário, Enquadramento Funcional: Estagiário, Carga horária: 30

Outras informações Estagiário em Genética Toxicológica.

Vínculo institucional
2016 - 2016 Vínculo: Voluntário, Enquadramento Funcional: Monitora, Carga horária: 6

Outras informações Monitora da Disciplina de Bioquímica e Genética Toxicológica

Vínculo institucional
2015 - 2016 Vínculo: Membro de Comissão Interna, Enquadramento Funcional: Membro Efetivo da Comissão Graduação, Carga horária: 1

Outras informações Membro da Comissão de Graduação do Curso de Toxicologia Analítica, como representante discente.

Vínculo institucional
2015 - 2016 Vínculo: Bolsista, Enquadramento Funcional: Iniciação Científica, Carga horária: 20

Outras informações Atuação como IC no projeto de pesquisa "Desenvolvimento de extrato padronizado de Plantago major L. em ácidos triterpênicos e determinação do potencial cicatrizante, anti-inflamatório e da sua segurança toxicológica.

Vínculo institucional
2015 - 2016 Vínculo: Coordenadora Geral de CA, Enquadramento Funcional: Coordenadora Geral do Centro Acadêmico Orfila

Vínculo institucional
2014 - 2015 Vínculo: Bolsista, Enquadramento Funcional: Iniciação Científica, Carga horária: 20, Regime: Dedicação exclusiva.

Outras informações Atuação como IC no projeto de pesquisa "Desenvolvimento de extrato padronizado de Plantago major L. em ácidos triterpênicos e determinação do potencial cicatrizante, anti-inflamatório e da sua segurança toxicológica.

Universidade Federal do Rio Grande do Sul, UFRGS, Brasil.

Vínculo institucional
2016 - 2016 Vínculo: Estagiário, Enquadramento Funcional: Estagiário na área de Toxicologia Analítica, Carga horária: 30

Athlone Institute of Technology, AIT, Irlanda.

Vínculo institucional
2018 - 2019 Vínculo: Pesquisador visitante, Enquadramento Funcional: Pesquisador

Vínculo institucional
2018 - 2018 Vínculo: Lab instructor, Enquadramento Funcional: Lab instructor, Carga horária: 20

The University of Newcastle Australia, NEWCASTLE, Austrália.

Vínculo institucional

2019 - Atual Vínculo: Bolsista, Enquadramento Funcional: PhD candidate, Carga horária: 40, Regime: Dedicação exclusiva.

Projetos de pesquisa

2017 - Atual NIMA-related Kinase 1 e seu papel na resistência tumoral
Descrição: Considerando estes estudos que indicam que Nek1 está relacionada ao processo tumoral, e que a resistência à quimioterapia ainda representa um importante obstáculo clínico e científico, sendo importante buscar novas opções de alvos terapêuticos para propor alternativas metodológicas em tumores que ainda possuem alta taxa de mortalidade, como os gliomas, este projeto visa caracterizar a proteína Nek1 em gliomas, buscando avaliar seu papel no prognóstico e tratamento, assim como na proposição de novas estratégias terapêuticas..
Situação: Em andamento; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (1) / Mestrado acadêmico: (1) / Doutorado: (1) .

Integrantes: Luiza Steffens Reinhardt - Integrante / MORÁS, ANA MOIRA - Integrante / SAFFI, JENIFER - Integrante / MOURA, DINARA JAQUELINE - Coordenador / Elizandra Braganhol - Integrante / Felipe Lopes Schenider - Integrante.

2014 - Atual Estudo de novos parceiros moleculares na resposta a danos no DNA: Avaliação da proteína Nek1 como parceira molecular das proteínas da via de Fanconi
Descrição: Este projeto tem como objetivo determinar uma possível interação entre NEK1 com proteínas da via de Fanconi, mais especificamente as proteínas FANCA, FANCC e FANCD2, na resposta celular a agentes indutores de pontes intercadeias, utilizando linhagens mutantes ou silenciadas nas proteínas Nek1, FANCA, FANCC e/ou FANCD2 expostas a indutores de pontes intercadeia..
Situação: Em andamento; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (1) / Mestrado acadêmico: (1) .

Integrantes: Luiza Steffens Reinhardt - Integrante / Dinara Jaqueline Moura - Coordenador / Helen Taís da Rosa Silva - Integrante / Jenifer Saffi - Integrante / Guido Lenz - Integrante / João Antonio Pegas Henriques - Integrante.

2013 - Atual Desenvolvimento de um extrato padronizado de Plantago major L. em ácidos triterpênicos e determinação do potencial cicatrizante, anti-inflamatório e da sua segurança toxicológica
Descrição: Este projeto tem como objetivo elaborar um extrato padronizado de P. major, em ácidos triterpênicos, e determinar o potencial cicatrizante e anti-inflamatório deste extrato em modelos in vitro e in vivo. Também serão conduzidos os ensaios biológicos in vitro com células T-helper (TH-1), para avaliação da atividade anti-inflamatória, com células de queratinócitos (HaCaT), para avaliação da atividade cicatrizante. Paralelamente serão realizados ensaios in vitro para determinação do potencial mutagênico. Os ensaios toxicológicos in vivo complementarão os ensaios biológicos e incluirão as avaliações farmacológicas (anti-inflamatória e cicatrizante) e os ensaios de toxicidade aguda, toxicidade de doses repetidas e genotoxicidade utilizando ratos Wistar. Adicionalmente, este projeto prevê a identificação e a caracterização morfoanatômica desta espécie, visando à elaboração de uma descrição detalhada de suas características macroscópicas e microscópicas..
Situação: Em andamento; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (2) / Mestrado acadêmico: (2) .

Integrantes: Luiza Steffens Reinhardt - Integrante / Dinara Jaqueline Moura - Coordenador / Jenifer Saffi - Integrante / Jeferson henn - Integrante / Nathalia Sperotto - Integrante / Valéria Peres - Integrante / Rodrigo Veríssimo - Integrante / Eliane Dallegrave - Integrante.
Financiador(es): Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul - Auxílio financeiro.

Revisor de periódico

2020 - Atual Periódico: International Journal of Neurological Disorders
2019 - Atual Periódico: Nanomaterial Chemistry and Technology
2021 - Atual Periódico: International Journal of Pharmaceutics
2022 - Atual Periódico: Scientific Reports

Áreas de atuação

1. Grande área: Ciências Biológicas / Área: Genética / Subárea: Genética Toxicológica.
2. Grande área: Ciências Biológicas / Área: Genética / Subárea: REPARO DE DNA.
3. Grande área: Ciências Biológicas / Área: Genética / Subárea: Bioquímica.
4. Grande área: Ciências Biológicas / Área: Biologia Geral / Subárea: Biologia Celular.
5. Grande área: Ciências Biológicas / Área: Biologia Geral / Subárea: Toxicologia.
6. Grande área: Ciências Biológicas / Área: Biotecnologia.

Idiomas

Português	Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.
Inglês	Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.

Prêmios e títulos

2021	Highly Commended Oral Presentation, HCRA.
2021	Beautiful Science, School of Biomedical Sciences and Pharmacy, University of Newcastle.
2020	Melhor Dissertação da UFCSPA - 2019, UFCSPA.
2020	Best Poster ? People?s Choice award, Hunter Cancer Research Symposium.
2019	Melhor resumo Mutagen-Brasil, Mutagen-Brasil.
2019	Tercer Premio en la comunicación oral, XI Congreso de Mutagénesis, Carcinogénesis y Teratogénesis Ambiental,.
2017	Menção Honrosa, UFCSPA.
2016	Menção Honrosa, Federação de Sociedades de Biologia Experimental - FeSBE.
2015	Destaque na Categoria Trabalhos Relacionados à Pesquisa, UFCSPA.

Produções

Produção bibliográfica

Artigos completos publicados em periódicos

Ordenar por

Ordem Cronológica

1. **STEFFENS REINHARDT, LUIZA**; MOIRA MORÁS, ANA ; GUSTAVO HENN, JEFERSON ; RICARDO ARANTES, PABLO ; BERNARDES FERRO, MATHEUS ; BRAGANHOL, ELIZANDRA ; OLIVEIRA DE SOUZA, PRISCILA ; DE OLIVEIRA MERIB, JOSIAS ; RAMOS BORGES, GABRIELA ; SILVEIRA DALANHOL, CAROLINA ; COX HOLANDA DE BARROS DIAS, MABILLY ; NUGENT, MICHAEL ; JAQUELINE MOURA, DINARA . Nek1-inhibitor and temozolomide-loaded microfibers as a co-therapy strategy for glioblastoma treatment. INTERNATIONAL JOURNAL OF PHARMACEUTICS JCR, v. 1, p. 121584-1, 2022.
2. HAMMERSCHMIDT, TATIANE G. ; DONIDA, BRUNA ; FAVERZANI, JÉSSICA L. ; MOURA, ALANA P. ; DOS REIS, BIANCA G. ; MACHADO, ANDRYELE Z. ; KESSLER, REJANE G. ; SEBASTIÃO, FERNANDA M. ; **REINHARDT, LUIZA S.** ; MOURA, DINARA J. ; VARGAS, CARMEN R. . Cytokine profile and cholesterol levels in patients with Niemann-Pick type C disease presenting neurological symptoms: in vivo effect of miglustat and in vitro effect of N-acetylcysteine and coenzyme Q10. EXPERIMENTAL CELL RESEARCH JCR, v. 416, p. 113175, 2022.
3. **REINHARDT, LUIZA STEFFENS**; HENN, JEFERSON GUSTAVO ; MORÁS, ANA MOIRA ; DE MOURA SPEROTTO, NATHALIA DENISE ; FERRO, MATHEUS BERNARDES ; CAO, ZHI ; ROEHE, ADRIANA VIAL ; PETRY, ADRIANA UBIRAJARA SILVA ; NUGENT, MICHAEL ; MOURA, DINARA JAQUELINE . Plantago australis Hydroethanolic Extract-Loaded Formulations: Promising Dressings for Wound Healing. BRAZILIAN JOURNAL OF PHARMACOGNOSY JCR, v. 1, p. 1, 2021.

   Citações: WEB OF SCIENCE 1
4. FAVERZANI, JÉSSICA LAMBERTY ; STEINMETZ, ALINE ; DEON, MARION ; MARCHETTI, DESIRÈE PADILHA ; GUERREIRO, GILIAN ; SITTA, ANGELA ; DE MOURA COELHO, DANIELLA ; LOPES, FRANCIELE FATIMA ; NASCIMENTO, LEOPOLDO VINICIUS MARTINS ; **STEFFENS, LUIZA** ; HENN, JEFERSON GUSTAVO ; FERRO, MATHEUS BERNARDES ; BRITO, VERÔNICA BIDINOTTO ; WAJNER, MOACIR ; MOURA, DINARA JAQUELINE ; VARGAS, CARMEN REGLA . L-carnitine protects DNA oxidative damage induced by phenylalanine and its keto acid derivatives in neural cells: a possible pathomechanism and adjuvant therapy for brain injury in phenylketonuria. METABOLIC BRAIN DISEASE JCR, v. 36, p. 1957-1968, 2021.
5. ZHANG, XIAJIE ; GROEN, KIRA ; MORTEN, BRIANNA C. ; **STEFFENS REINHARDT, LUIZA** ; CAMPBELL, HAMISH G. ; BRAITHWAITE, ANTONY W. ; BOURDON, JEAN'CHRISTOPHE ; AVERY'KIEJDA, KELLY A. . Effect of p53 and its N-terminally truncated isoform, -40p53, on breast cancer migration and invasion. Molecular Oncology JCR, v. 1, p. 1, 2021.
6. SEBA, VIVIANE ; DE LIMA, GABRIEL GOETTEN ; PEREIRA, BRUNO L. ; SILVA, GABRIEL ; **REINHARDT, LUIZA STEFFENS** ; ARANTES, PABLO RICARDO ; CHEE, BOR SHIN ; DOS SANTOS, MARIANA BASTOS ; FRANÇA, SUZELEI C. ; REGASINI, LUIS OCTAVIO ; FACHIN, ANA LÚCIA ; CAO, ZHI ; NUGENT, MICHAEL J. D. ; MARINS, MOZART . Development, Characterization and Cell Viability Inhibition of PVA Spheres Loaded with Doxorubicin and 4--Amino-1-Naphthyl-Chalcone (D14) for Osteosarcoma. Polymers JCR, v. 13, p. 2611, 2021.

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7. MORÁS, A.M. ; HENN, J.G. ; **STEFFENS REINHARDT, L.** ; LENZ, G. ; MOURA, D.J. . Recent developments in drug delivery strategies for targeting DNA damage response in glioblastoma. LIFE SCIENCES JCR, v. 287, p. 120128, 2021.

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8. DONIDA, BRUNA ; RAABE, MARCO ; TAUFFNER, BÁRBARA ; FARIAS, MARCELO A. ; MACHADO, ANDRYELE Z. ; TIMM, FERNANDA ; KESSLER, REJANE G. ; HAMMERSCHMIDT, TATIANE G. ; **REINHARDT, LUIZA S.** ; BRITO, VERÔNICA B. ; PORTUGAL, RODRIGO V. ; BERNARDI, ANDRESSA ; FROZZA, RUDIMAR ; MOURA, DINARA J. ; GIUGLIANI, ROBERTO ; POLETTO, FERNANDA ; VARGAS, CARMEN R. . Nanoparticles containing β-cyclodextrin potentially useful for the treatment of Niemann-Pick C. JOURNAL OF INHERITED METABOLIC DISEASE JCR, v. 1, p. jimd.12210-1, 2020.

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10. ☆ **STEFFENS REINHARDT, LUIZA**; ZHANG, XIAJIE ; WAWRUSZAK, ANNA ; GROEN, KIRA ; DE IULIIS, GEOFFRY N. ; AVERY-KIEJDA, KELLY A. . Good Cop, Bad Cop: Defining the Roles of -40p53 in Cancer and Aging. Cancers JCR, v. 12, p. 1659, 2020.

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11. HAUSCHILD, TATIANE CRISTINA ; GUERREIRO, GILIAN ; MESCKA, CAROLINE PAULA ; COELHO, DANIELLA MOURA ; **STEFFENS, LUIZA** ; MOURA, DINARA JAQUELINE ; MANFREDINI, VANUSA ; VARGAS, CARMEN REGLA . DNA damage induced by alloisoleucine and other metabolites in maple syrup urine disease and protective effect of l-carnitine. TOXICOLOGY IN VITRO JCR, v. 57, p. 194-202, 2019.

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12. LUFT, JORDANA GRIEBLER ; **STEFFENS, LUIZA** ; MORÁS, ANA MOIRA ; DA ROSA, MATEUS STRUCKER ; LEIPNITZ, GUILHIAN ; REGNER, GABRIELA GREGORY ; PFLÜGER, PRICILA FERNANDES ; GONÇALVES, DÉBORA ; MOURA, DINARA JAQUELINE ; PEREIRA, PATRÍCIA . Rosmarinic acid improves oxidative stress parameters and mitochondrial respiratory chain activity following 4-aminopyridine and picrotoxin-induced seizure in mice. NAUNYN-SCHMIEDEBERGS ARCHIVES OF PHARMACOLOGY JCR, v. 1, p. 1-12, 2019.

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13. **STEFFENS REINHARDT, LUIZA**; CHEE, BOR SHIN ; CAO, ZHI ; JAQUELINE MOURA, DINARA ; NUGENT, MICHAEL . Freeze-thaw electrospun PVA-dacarbazine nanoparticles: preparation, characterization and anticancer evaluation. International Journal of Polymeric Materials and Polymeric Biomaterials JCR, v. 1, p. 1-12, 2019.

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14. Zancan, M. ; MALYSZ, T. ; MOURA, D. J. ; MORAS, A. M. ; **STEFFENS, LUIZA** ; RASIA-FILHO, A. A. . Gap junctions and expression of Cx36, Cx43 and Cx45 in the posterodorsal medial amygdala of adult rats. HISTOLOGY AND HISTOPATHOLOGY JCR, v. 4, p. 1-10, 2019.

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15. ZANCAN, MARIANA ; MOURA, DINARA J. ; MORÁS, ANA MOIRA ; **STEFFENS, LUIZA** ; DE MOURA, ANA CAROLINA ; GIOVENARDI, MÁRCIA ; RASIA-FILHO, ALBERTO A. . NEUROTROPHIC FACTORS IN THE POSTERODORSAL MEDIAL AMYGDALA OF MALE AND CYCLING FEMALE RATS. BRAIN RESEARCH BULLETIN JCR, v. 155, p. 92-101, 2019.

16. ☆ **STEFFENS, LUIZA**; MORÁS, ANA MOIRA ; ARANTES, PABLO RICARDO ; MASTERSON, KEVIN ; CAO, ZHI ; NUGENT, MICHAEL ; MOURA, DINARA JAQUELINE . Electrospun PVA-Dacarbazine nanofibers as a novel nano brain-implant for treatment of glioblastoma: in silico and in vitro characterization. EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES JCR, v. 1, p. 105183, 2019.

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17. STEINMETZ, ALINE ; **STEFFENS, LUIZA** ; MORÁS, ANA MOIRA ; PREZZI, FLÁVIA ; BRAGANHOL, ELIZANDRA ; SAFFI, JENIFER ; ORTIZ, RAFAEL SCORSATTO ; BARROS, HELENA M.T. ; MOURA, DINARA JAQUELINE . In vitro model to study cocaine and its contaminants. CHEMICO-BIOLOGICAL INTERACTIONS JCR, v. 285, p. 1-7, 2018.

Citações: WEB OF SCIENCE 4

18. FREESE, LUANA ; ALMEIDA, FELIPE BORGES ; HEIDRICH, NUBIA ; HANSEN, ALANA WITT ; **STEFFENS, LUIZA** ; STEINMETZ, ALINE ; MOURA, DINARA JAQUELINE ; GOMEZ, ROSANE ; BARROS, HELENA MARIA TANNHAUSER . Environmental enrichment reduces cocaine neurotoxicity during cocaine-conditioned place preference in male rats. PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR JCR, v. 169, p. 10-15, 2018.

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19. BOHN, DENISE RAQUEL ; LOBATO, FRANCIELLI OLIVEIRA ; THILL, ALISSON STEFFLI ; **STEFFENS, LUIZA** ; RAABE, MARCO ; DONIDA, BRUNA ; VARGAS, CARMEN REGLA ; MOURA, DINARA J. ; BERNARDI, FABIANO ; POLETTO, FERNANDA . Artificial cerium-based proenzymes confined in lyotropic liquid crystal: Synthetic strategy and on-demand activation. Journal of Materials Chemistry B JCR, v. 1, p. 1-9, 2018.

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20. ☆ DE MOURA SPEROTTO, NATHALIA DENISE ; **STEFFENS, LUIZA** ; VERÍSSIMO, RODRIGO MOISÉS ; HENN, JEFERSON GUSTAVO ; PÉRES, VALÉRIA FLORES ; VIANNA, PRISCILA ; CHIES, JOSÉ ARTUR BOGO ; ROEHE, ADRIANA ; SAFFI, JENIFER ; MOURA, DINARA JAQUELINE . Wound healing and anti-inflammatory activities induced by a Plantago australis hydroethanolic extract standardized in verbascoside. JOURNAL OF ETHNOPHARMACOLOGY JCR, v. 225, p. 178-188, 2018.

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21. MARCHETTI, DESIRÈE PADILHA ; **STEFFENS, LUIZA** ; JACQUES, CARLOS E. ; GUERREIRO, GILIAN B. ; MESCKA, CAROLINE P. ; DEON, MARION ; DE COELHO, DANIELLA M. ; MOURA, DINARA J. ; VIARIO, ALICE G. ; POLETTO, FERNANDA ; COITINHO, ADRIANA S. ; JARDIM, LAURA B. ; VARGAS, CARMEN R. . Oxidative Imbalance, Nitrative Stress, and Inflammation in C6 Glial Cells Exposed to Hexacosanoic Acid: Protective Effect of N-acetyl-l-cysteine, Trolox, and Rosuvastatin. CELLULAR AND MOLECULAR NEUROBIOLOGY JCR, v. 1, p. 1-1, 2018.

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22. ☆ HENN, JEFERSON GUSTAVO ; **STEFFENS, LUIZA** ; SPEROTTO, NATHALIA DENISE DE MOURA ; DE SOUZA PONCE, BETÂNIA ; VERÍSSIMO, RODRIGO MOISÉS ; BOARETTO, FERNANDA BRIÃO MENEZES ; HASSEMER, GUSTAVO ; PÉRES, VALÉRIA FLORES ; SCHIRMER, HELENA ; PICADA, JAQUELINE NASCIMENTO ; SAFFI, JENIFER ; MOURA, DINARA JAQUELINE . Toxicological evaluation of a standardized hydroethanolic extract from leaves of Plantago australis and its major compound, verbascoside. JOURNAL OF ETHNOPHARMACOLOGY JCR, v. 1, p. 1-1, 2018.

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23. DA SILVA, CLAUDIA B ; GIL, EDUARDA S ; SANTOS, FABIANO DA SILVEIRA ; MORAS, ANA M ; **STEFFENS, LUIZA** ; GONCALVES, PAULO F. B. ; MOURA, DINARA J ; LÜDTKE, DIOGO S. ; RODEMBUSCH, FABIANO SEVERO . Proton-transfer based azides with fluorescence off-on response for detection of hydrogen sulfide. An experimental, theoretical and bioimaging study. JOURNAL OF ORGANIC CHEMISTRY JCR, v. 1, p. acs.joc.8b02489-1, 2018.

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24. BIANCINI, GIOVANA BRONDANI ; MORÁS, ANA MOIRA ; **REINHARDT, LUIZA STEFFENS** ; BUSATTO, FRANCIELE FACCIO ; DE MOURA SPEROTTO, NATHALIA DENISE ; SAFFI, JENIFER ; MOURA, DINARA JAQUELINE ; GIUGLIANI, ROBERTO ; VARGAS, CARMEN REGLA . Globotriaosylsphingosine induces oxidative DNA damage in cultured kidney cells. NEPHROLOGY JCR, v. 22, p. 490-493, 2017.

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25. BORILLE, B. T. ; GONZALEZ, M. ; **STEFFENS, L.** ; ORTIZ, R. S. ; LIMBERGER, R. P. . CANNABIS SATIVA: A SYSTEMATIC REVIEW OF PLANT ANALYSIS. Drug Analytical Research, v. 1, p. 1-23, 2017.

26. CALETTI, GREICE ; HERRMANN, ANA P. ; PULCINELLI, RIANNE REMUS ; **STEFFENS, LUIZA** ; MORÁS, ANA MOIRA ; VIANNA, PRISCILA ; CHIES, JOSÉ ARTUR BOGO ; MOURA, DINARA JAQUELINE ; BARROS, HELENA MARIA TANHAUSER ; GOMEZ, ROSANE . Taurine counteracts the neurotoxic effects of streptozotocin-induced diabetes in rats. AMINO ACIDS (WIEN. INTERNET) JCR, v. 1, p. 1, 2017.

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27. DA COSTA E SILVA, LIANA DANTAS ; PEREIRA, PATRÍCIA ; REGNER, GABRIELA GREGORY ; BOARETTO, FERNANDA BRIÃO MENEZES ; HOFFMANN, CLEONICE ; PFLÜGER, PRICILA ; DA SILVA, LUCAS LIMA ; **STEFFENS, LUIZA REINHARDT** ; MORÁS, ANA MOIRA ; MOURA, DINARA JAQUELINE ; PICADA, JAQUELINE NASCIMENTO . DNA damage and oxidative stress induced by seizures are decreased by anticonvulsant and neuroprotective effects of lobeline, a candidate to treat alcoholism. METABOLIC BRAIN DISEASE JCR, v. 1, p. 1, 2017.

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28. DE SOUZA, VANESSA PEREIRA ; VENDRUSCULO, VINÍCIUS ; MORÁS, ANA M. ; **STEFFENS, LUIZA** ; SANTOS, FABIANO S ; MOURA, DINARA J. ; RODEMBUSCH, FABIANO SEVERO ; RUSSOWSKY, DENNIS . Synthesis and photophysical study of new fluorescent proton transfer dihydropyrimidinone hybrids as potential candidates for molecular probes. NEW JOURNAL OF CHEMISTRY JCR, v. 1, p. 1-1, 2017.

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Capítulos de livros publicados

1. **STEFFENS, LUIZA**; de Barros Dias, Mabilly Cox Holanda ; ARANTES, PABLO RICARDO ; HENN, JEFERSON GUSTAVO ; NUGENT, MICHAEL ; MOURA, DINARA JAQUELINE . Nanopolymeric systems to improve brain cancer treatment outcomes. Advances and Challenges in Pharmaceutical Technology. 1ed.: Elsevier, 2021, v. , p. 355-394.
2. **REINHARDT, LUIZA STEFFENS**; ARANTES, PABLO RICARDO ; HENN, JEFERSON GUSTAVO ; MOURA, DINARA JAQUELINE . Bionanocomposites for In Situ Drug Delivery in Cancer Therapy: Early and Late Evaluations. Materials Horizons: From Nature to Nanomaterials. 1ed.: Springer Singapore, 2021, v. , p. 145-165.
3. **REINHARDT, L. S.**; DIAS, M. C. H. B. ; GNOATTO, J. ; WAWRUSZAK, A. ; HAŁ ; ARANTES, P. R. ; ROWAN, N. J. ; MOURA, D. J. . Polymeric Nanocomposites for Cancer-Targeted Drug Delivery. In: Hasnain M.S.; Nayak A.K.; Alkahtani S.. (Org.). olymeric and Natural Composites. Advances in Material Research and Technology. 1ed.: Springer, Cham, 2021, v. 1, p. 1-1.
4. **STEFFENS REINHARDT, L.**; DIAS, M.C.H.B. ; ARANTES, P.R. ; GNOATTO, J. ; RAABE, MARCO ; MOURA, D. J. . Modified polysaccharides in wound healing. In: Amit Nayak; Md Saquib Hasnain; Tejraj Aminabhavi. (Org.). Tailor-Made Polysaccharides in Biomedical Applications. 1ed.: Academic Press, 2020, v. 1, p. 1-.
5. **STEFFENS, LUIZA**; de Barros Dias, Mabilly Cox Holanda ; MORÁS, ANA MOIRA ; MOURA, DINARA JAQUELINE ; NUGENT, MICHAEL . Natural polysaccharides for the delivery of anticancer therapeutics. Natural Polysaccharides in Drug Delivery and Biomedical Applications. 1ed.: Elsevier, 2019, v. , p. 441-470.

Apresentações de Trabalho

1. MORAS, A. M. ; **STEFFENS, LUIZA** ; MARTINS, KIANE ; MOURA, D. J. . Investigação do papel da proteína NEK1 no tratamento de células de glioblastoma. 2019. (Apresentação de Trabalho/Congresso).
2. FERRO, MATHEUS ; HENN, J. ; **STEFFENS, LUIZA** ; ALVEZ, GABRIEL ; DA ROSA, RICARDO ; AGUIRRE, TANIRA ; MOURA, D. J. . INFLUÊNCIA DE NANOEMULSÕES DE NÚCLEO LIPÍDICO CONTENDO COMPOSTOS DE FERROCENOS NA VIABILIDADE DE CÉLULAS DE GLIOBLASTOMA TRATADAS COM TEMOZOLOMIDA. 2019. (Apresentação de Trabalho/Congresso).
3. **STEFFENS, LUIZA**; HENN, J. ; MORAS, A. M. ; SPEROTTO, N. ; FERRO, MATHEUS ; CAO, ZHI ; NUGENT, MICHAEL ; MOURA, D. J. . CARACTERIZAÇÃO E AVALIAÇÃO DA ATIVIDADE CICATRIZANTE DE NANOFIBRAS POLIMÉRICAS CONTENDO EXTRATO HIDROETANÓLICO DE PLANTAGO AUSTRALIS. 2019. (Apresentação de Trabalho/Congresso).
4. **STEFFENS, LUIZA**; MORAS, A. M. ; ARANTES, P.R. ; MASTERSON, K. ; CAO, ZHI ; NUGENT, MICHAEL ; MOURA, D. J. . Electrospun PVA-Dacarbazine nanofibers: novel nano brain-implant for treatment of glioblastoma. 2019. (Apresentação de Trabalho/Congresso).
5. **STEFFENS, LUIZA**; MORAS, A. M. ; ARANTES, P.R. ; HENN, J. ; BRAGANHOL, E. ; DIAS, M.C.H.B. ; CAO, ZHI ; NUGENT, MICHAEL ; MOURA, D. J. . Nek1-inhibitor and temozolomide-loaded nanofibers as a co-therapy strategy for glioblastoma treatment. 2019. (Apresentação de Trabalho/Congresso).

Bancas

Participação em bancas de comissões julgadoras

Outras participações

1. **REINHARDT, LUIZA S.**. HCRA Symposium. 2020. Hunter Cancer Research Alliance.
2. **STEFFENS, L.**. SciFest@College 2018. 2018. Athlone Institute of Technology.
3. **STEFFENS, L.**. III Mostra de Trabalhos de Ensino, Pesquisa e Extensão. 2017. Fundação Universidade Federal de Ciências da Saúde de Porto Alegre.

Eventos

Participação em eventos, congressos, exposições e feiras

1. ASMR NSW Scientific Meeting. Δ40p53 modulates the doxorubicin-induced DNA damage response by promoting the DNA repair in breast cancer cells. 2021. (Congresso).
2. HCRA Symposium.Δ40p53 modulates the doxorubicin induced DNA damage response by promoting DNA repair in breast cancer cells. 2021. (Simpósio).
3. Sydney Cancer Conference. A high Δ40p53:p53 ratio alters the doxorubicin-induced DNA damage response in breast cancer cells. 2021. (Congresso).
4. 32nd Lorne Conference. d40p53 inhibits the p53-mediated response to DNA damaging therapies in breast cancer cells. 2020. (Congresso).
5. 5th Australia Translation Breast Cancer Symposium. 2020. (Simpósio).
6. AACR Virtual Annual Meeting I. 2020. (Congresso).
7. Garvan's COVID-19 research virtual seminar. 2020. (Seminário).
8. HCRA Symposium.Molecular inhibition of Δ40p53 increases sensitivity to DNA-damaging therapies in Luminal A breast cancer cells. 2020. (Simpósio).
9. Future Leaders Group Early and Mid-Career Annual Workshop. 2019. (Simpósio).
10. HCRA Symposium. 2019. (Simpósio).
11. IV Encontro Biociências.Nek1-inhibitor and temozolomide-loaded nanofibers as a co-therapy strategy for glioblastoma treatment. 2019. (Encontro).
12. XI Congreso de Mutagénesis, Carcinogénesis y Teratogénesis Ambiental,. Electrospun PVA-Dacarbazine nanofibers: novel nano brain-implant for treatment of glioblastoma. 2019. (Congresso).
13. 26th EORS Annual Meeting. ELECTROSPUN PVA NANOFIBERS FOR BONE DISEASE THERAPY USING MESENCHYMAL STEM CELL. 2018. (Congresso).
14. 43rd Institute of Chemistry of Ireland Congress. Eletrospun PVA-Dacarbazine nanoparticles: a novel drug delivery system for cancer treatment. 2018. (Congresso).
15. ECP4 The European Composites, Plastics and Polymer Processing Platform a. Eletrospun PVA-Dacarbazine nanoparticles: a novel drug delivery system for brain cancer treatment. 2018. (Congresso).
16. Research Presentations & Poster Day.Eletrospun PVA-Dacarbazine nanoparticles: a novel drug delivery system. 2018. (Encontro).
17. Social & Ethical Responsibility of Third Level Colleges towards Community Ey Engagement across the Life Spectrum. 2018. (Congresso).
18. II Encontro do PPG Biociências & Encontro em Fisiologia do RS.ANÁLISE DE PARÂMETROS BIOQUÍMICOS, NO SANGUE E TECIDOS METABÓLICOS, DE FÊMEAS QUE RECE- BERAM DIETA HIPERCALÓRICA OU RESTRITIVA NA GESTAÇÃO E LACTAÇÃO. 2017. (Encontro).
19. II Encontro do PPG Biociências & Encontro em Fisiologia do RS.NIMA-RELATED KINASE 1 E SEU PAPEL NA RESISTÊNCIA TUMORAL. 2017. (Encontro).
20. II Encontro do PPG Biociências & Encontro em Fisiologia do RS.O TRATAMENTO COM ÔMEGA-3 PROMOVE A MELHORA DE PARÂMETROS METABÓLICOS E DO ESTRESSE OXIDATIVO E INFLAMAÇÃO INTESTINAL EM MODELO EXPERIMENTAL DE OBESIDADE INDUZIDO POR HIGH FAT DIET. 2017. (Encontro).
21. I Encontro do Programa de Pós-Graduação em Biociências, XIX Encontro de Geneticistas do Rio Grande do Sul e I Encontro Regional Sul da Sociedade Brasileira de Genética Médica.Estresse oxidativo em linfoblastos de pacientes com Anemia de Fanconi em resposta à cisplatina. 2016. (Encontro).
22. II Mostra de Trabalhos de Ensino, Pesquisa & Extensão da UFCSPA.Extresse Oxidativo em Linfoblastos de Pacientes com Anemia de Fanconi em Resposta à Cisplatina. 2016. (Outra).
23. Toxicologia em Debate: A polêmica dos efeitos da maconha. 2016. (Outra).
24. Toxicologia em Debate: Desastre em Mariana e suas consequências. 2016. (Outra).
25. Toxicologia em Debate: Direção veicular e drogas de abuso, isso combina?. 2016. (Outra).
26. XII Congresso da MutaGen-Brasil. 2016. (Congresso).
27. XXXI Reunião Anual da Federação de Sociedades de Biologia Experimental - FeSBE. Fanconi Anemia: Recent Remarks and Model of Study Using Lymphoblastoid Cells from Patients. 2016. (Congresso).
28. Cellularised Scaffolds, Co-cultures and Bioreactors for Engineering the Vascular Wall: Potential for Tissue Regeneration. 2015. (Outra).
29. Formando Opiniões. 2015. (Outra).
30. GE DAY UFRGS. 2015. (Outra).
31. I Mostra de Trabalhos de Ensino, Pesquisa & Extensão da UFCSPA.Caracterização de linhagens linfoblásticas derivadas de pacientes com Anemia de Fanconi em resposta a agentes quimioterápicos. 2015. (Outra).
32. Soluções para Preparo de Amostras e Separações Cromatográficas. 2015. (Outra).
33. Toxicologia em Debate: Acidentes em Massa. 2015. (Outra).
34. Toxicologia em Debate: Discutindo CSI. 2015. (Outra).
35. X Jornada do Programa de Pós-Gradução em Patologia. 2015. (Outra).
36. III Semana Acadêmica da Universidade Federal de Ciências da Saúde de Porto Alegre. 2014. (Outra).

Organização de eventos, congressos, exposições e feiras

1. MOURA, D. J. ; **STEFFENS, L.** ; BRAGANHOL, E. . II Encontro do PPG Biociências da UFCSPA e Encontro de Pesquisa em Fisiologia do RS. 2017. (Outro).
2. MOURA, D. J. ; **STEFFENS, L.** . Curso Básico de Papiloscopia. 2016. (Outro).
3. **STEFFENS, L.**; MOURA, D. J. . II Jornada Acadêmica da Toxicologia Analítica. 2016. (Outro).

4. MOURA, D. J. ; SAFFI, J. ; MORAS, A. M. ; SILVA, H. T. R. ; **STEFFENS, L.** . I Curso de Toxicidade Genética: Causas, Consequências e Ensaios Pré-clínicos. 2015. (Outro).
5. MOURA, D. J. ; **STEFFENS, L.** . I Jornada Acadêmica do curso de Toxicologia Analítica da UFCSPA. 2015. (Outro).

Orientações

Orientações e supervisões concluídas

Iniciação científica

1. Corentin Labbe. Investigation of the swelling effects of polyvinyl alcohol and polyacrylic acid to mimic the reaction of a human muscle. 2018. Iniciação Científica - Athlone Institute of Technology. Orientador: Luiza Steffens Reinhardt.

Orientações de outra natureza

1. Vinh Nguyen. Conductive Hydrogel. 2019. Orientação de outra natureza - Athlone Institute of Technology. Orientador: Luiza Steffens Reinhardt.

Inovação

Projetos de pesquisa

2013 - Atual

Desenvolvimento de um extrato padronizado de Plantago major L. em ácidos triterpênicos e determinação do potencial cicatrizante, anti-inflamatório e da sua segurança toxicológica
Descrição: Este projeto tem como objetivo elaborar um extrato padronizado de P. major, em ácidos triterpênicos, e determinar o potencial cicatrizante e anti-inflamatório deste extrato em modelos in vitro e in vivo. Também serão conduzidos os ensaios biológicos in vitro com células T-helper (TH-1), para avaliação da atividade anti-inflamatória, com células de queratinócitos (HaCaT), para avaliação da atividade cicatrizante. Paralelamente serão realizados ensaios in vitro para determinação do potencial mutagênico. Os ensaios toxicológicos in vivo complementarão os ensaios biológicos e incluirão as avaliações farmacológicas (anti-inflamatória e cicatrizante) e os ensaios de toxicidade aguda, toxicidade de doses repetidas e genotoxicidade utilizando ratos Wistar. Adicionalmente, este projeto prevê a identificação e a caracterização morfoanatômica desta espécie, visando à elaboração de uma descrição detalhada de suas características macroscópicas e microscópicas..
Situação: Em andamento; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (2) / Mestrado acadêmico: (2) .

Integrantes: Luiza Steffens Reinhardt - Integrante / Dinara Jaqueline Moura - Coordenador / Jenifer Saffi - Integrante / Jeferson henn - Integrante / Nathalia Sperotto - Integrante / Valéria Peres - Integrante / Rodrigo Veríssimo - Integrante / Eliane Dallegrave - Integrante.
Financiador(es): Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul - Auxílio financeiro.

Educação e Popularização de C & T

Organização de eventos, congressos, exposições e feiras

1. MOURA, D. J. ; SAFFI, J. ; MORAS, A. M. ; SILVA, H. T. R. ; **STEFFENS, L.** . I Curso de Toxicidade Genética: Causas, Consequências e Ensaios Pré-clínicos. 2015. (Outro).
2. MOURA, D. J. ; **STEFFENS, L.** . I Jornada Acadêmica do curso de Toxicologia Analítica da UFCSPA. 2015. (Outro).
3. MOURA, D. J. ; **STEFFENS, L.** . Curso Básico de Papiloscopia. 2016. (Outro).
4. MOURA, D. J. ; **STEFFENS, L.** ; BRAGANHOL, E. . II Encontro do PPG Biociências da UFCSPA e Encontro de Pesquisa em Fisiologia do RS. 2017. (Outro).
5. **STEFFENS, L.**; MOURA, D. J. . II Jornada Acadêmica da Toxicologia Analítica. 2016. (Outro).