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Desenvolvimento e avaliação da biocompatibilidade de uma matriz compósita de quitosana e colágeno

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1. Biomateriais. 2. Biocompatibilidade. 3. Medicina Regenerativa. I. Título.

SUMÁRIO

RESUMO

A produção de uma matriz compósita de quitosana e colágeno visa o desenvolvimento de uma alternativa nacional acessível para o tratamento de lesões cutâneas que necessitam de substitutos dérmicos, visto que as opções atuais são importadas e têm alto custo. Propomos neste trabalho a formulação de um substituto dérmico de quitosana e colágeno associado a lipossomas, sua caracterização físico química e a avaliação de sua biocompatibilidade. Sua caracterização se deu por calorimetria exploratória diferencial (DSC), avaliação das características de intumescimento e microscopia eletrônica de varredura. A avaliação da biocompatibilidade se deu através de parâmetros de biologia celular como viabilidade, adesão e morfologia através dos ensaios de MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) com células estromais mesenquimais derivadas de tecido adiposo humano (ADSC) e linhagem de queratinócitos (HaCaT) e cultivo celular direto sobre o material com ADSCs e linhagem de melanócitos (GFP labeled SK MEL 103). O material apresentou características físico químicas homogêneas que favoreceram o aporte celular, sendo adequado para as análises *in vitro*, o que permitiu a verificação de suas características biocompatíveis como ausência de toxicidade e promoção da adesão e da viabilidade celular, não causando alterações morfológicas significativas em ADSC e SK MEL 103. Os resultados obtidos permitem sugerir a continuidade da investigação do filme proposto utilizando modelos pré-clínicos para cobrir lesões dérmicas, a fim de testar a capacidade do filme para a administração de medicamentos, bem como a capacidade de promover o reparo do tecido da pele.

Palavras-chave: Filme de Quitosana, Colágeno, Lipossomas, Biocompatibilidade

ABSTRACT

The production of a composite matrix of chitosan and collagen aims to develop an affordable national alternative for the treatment of skin lesions that require dermal substitutes, since the current options are imported and have a high cost. In this work, we propose a dermal substitute of chitosan and collagen associated with liposomes, in order to obtain a film with potential reparative properties. A chitosan hydrogel was formulated together with collagen and liposomes suitable for *in vitro* analysis for subsequent physicochemical characterization and biocompatibility assessment. It was characterized by differential scanning calorimetry (DSC), evaluation of swelling and scanning electron microscopy. The evaluation of biocompatibility was done through cell biology parameters such as viability, adhesion and morphology through MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with adipose-derived mesenchymal stem cell (ADSC) and keratinocyte lineage (HaCaT) and direct cell culture on the material with ADSC and melanocyte lineage (GFP labeled SK MEL 103). The material showed homogeneous physicochemical characteristics that favored cellular input, being suitable for *in vitro* analyzes, which allowed the verification of its biocompatible characteristics such as absence of toxicity and promotion of cell adhesion and viability, without causing significant morphological changes in ADSC and SK MEL 103. Therefore, these results allow to suggest the continuity of this investigation using preclinical models to cover dermal lesions in order to test the film capacity to deliver drugs as well as the capacity to promote skin tissue repair.

Keywords: Chitosan film, Collagen, Liposomes, Biocompatibility

1 INTRODUÇÃO

1.1 Os Biomateriais na Medicina Regenerativa e Engenharia de Tecidos

A medicina regenerativa e a engenharia de tecidos são campos de estudo que têm extrapolado o âmbito da pesquisa e alcançado a prática clínica. Visando substituir total ou parcialmente tecidos humanos lesionados, essas áreas de pesquisa introduziram inúmeras possibilidades de reparo através do uso isolado ou combinado de recursos como materiais biológicos, sintéticos, células, biomoléculas e outros (NEREM, 2011). Para o desenvolvimento de *scaffolds* visando a engenharia de tecidos são utilizados biomateriais, substâncias projetadas para tomar formas que, sozinhas ou como partes de sistemas complexos, são usadas para direcionar, pelo controle das interações com os componentes dos sistemas vivos, o curso de qualquer procedimento terapêutico ou diagnóstico (BIOMATERIALS - JOURNAL - ELSEVIER, [s.d.]).

Entre os biomateriais mais utilizados estão cerâmicas, metais, vidros e polímeros. Visando sua utilização na medicina regenerativa, os biomateriais disponíveis para a engenharia de tecidos buscam simular a matriz extracelular (FEINBERG; PARKER, 2010). As matrizes tridimensionais formadas pelos biomateriais tendem a evoluir para não somente comportar células, mas também carregar e liberar medicamentos e até mesmo estimular química ou mecanicamente a diferenciação celular (HUEBSCH; MOONEY, 2009). Entre suas aplicações estão a reconstrução de vasos sanguíneos, nervos, ossos, pele e órgãos completos (BERNSTEIN, 2011).

1.2 A Pele Humana e os Substitutos Dérmicos

A barreira entre os ambientes externo e interno formada pela pele proporciona a manutenção da homeostase do corpo humano através da regulação da temperatura, hidratação, síntese de vitamina D e proteção contra agentes externos como químicos e patógenos (NYAME et al., 2015). Lesões a esse órgão, o maior do corpo humano, podem ocasionar tratamentos longos, dolorosos e caros, portanto não surpreende que, entre os tecidos e órgãos desenvolvidos até o momento, a pele foi o primeiro a ir da pesquisa em laboratório ao leito hospitalar (VIG et al., 2017).

Inúmeras opções de substitutos para as diferentes camadas da pele estão disponíveis comercialmente, entre elas estão produtos elaborados com ácido hialurônico, fibrina, membrana amniótica, silicone, plasma rico em plaquetas, submucosa intestinal porcina e muitos outros (HO et al., 2017). Atualmente, a cicatrização de feridas move 12 bilhões de dólares por ano nos Estados Unidos (LEAVITT et al., 2016). No Brasil, onde temos mais de 1 milhão de pessoas sofrendo queimaduras todos os anos, urge o desenvolvimento de alternativas nacionais acessíveis para o tratamento de lesões da pele (BATISTA; CORDOVIL; DE N. M, 2012).

1.3 A Quitosana na Engenharia de Tecidos

A quitosana é um polissacarídeo formado a partir da desacetilação da quitina, que é o segundo biopolímero mais presente na natureza, perdendo apenas para a celulose. A quitina está presente no exoesqueleto de artrópodes como o camarão e o siri (LI et al., 2018). Seu caráter policatiônico contribui para suas características favoráveis ao uso na engenharia de tecidos, como mucoadesividade e capacidade hemostática e antimicrobiana (YOUNES; RINAUDO, 2015). Devido à sua biocompatibilidade, não-toxicidade e biodegradabilidade, a quitosana foi aprovada pela

organização americana *Food and Drug Administration* (FDA) como revestimento dérmico (WAHL et al., 2015).

No estágio inflamatório a quitosana pode ativar a migração de neutrófilos e a fagocitose de macrófagos, estimulando a deposição da matriz extracelular e a reepitelização, além disso, ela pode inibir a ação de metaloproteinases, enzimas super expressas no exsudato de feridas crônicas que podem prejudicar a fase de remodelamento da cicatrização da ferida, exercendo um efeito corrosivo nos componentes da matriz extracelular (VIGANI et al., 2019).

Até 2017, as principais patentes depositadas por universidades e institutos de pesquisa que envolviam quitosana ou colágeno estavam relacionadas a materiais antibacterianos (GWAK; SOHN, 2017). Apesar da prevalência dos antibióticos na produção desses materiais, diferentes estudos testaram outras drogas, incluindo filmes de quitosana preparados por diferentes métodos e carregados com FGF (fator de crescimento de fibroblastos) e EGF (fator de crescimento epidermal) facilitando o reparo tecidual em excisões de pele de porcos e de ratos (HONG et al., 2008; MIZUNO et al., 2003).

Diferentes estudos com análises *in vivo* avaliam filmes compósitos de quitosana (TSAO et al., 2011; XU et al., 2007; ZHANG; YANG; NIE, 2008), porém encontram-se com menos frequência trabalhos com cultivo direto de células sobre o material para avaliação de sua biocompatibilidade, o que pode estar relacionado à dificuldade de transposição de parâmetros de análise. Além das características de superfície do *scaffold*, seu pH e suas condições de esterilidade podem impedir ou facilitar o cultivo celular, que pode ser objetivado tanto para análise de biocompatibilidade quanto para futura utilização do *scaffold* como suporte para administração de células com potencial terapêutico.

Considerando melhorar as propriedades de um filme preparado com quitosana tanto em termos de biocompatibilidade quanto em termos físico químicos, diferentes materiais podem ser associados (PARK et al., 2002). O colágeno tem sido estudado

como componente de matrizes compósitas por ser uma das proteínas fibrosas mais abundantes no corpo humano, demonstrando melhorar a adesão, migração e proliferação celular (HESSE et al., 2010; TOZZI et al., 2016).

1.4 Matrizes compósitas de quitosana como sistemas de liberação controlada

Uma das principais funcionalidades de biomateriais de quitosana é a administração de fármacos e moléculas bioativas ao longo do tempo, sem a necessidade de repetidas aplicações. Para a associação do composto ativo é, muitas vezes, necessária a proteção da substância por nanocarreadores. Vesículas de carreamento como as lipossomas podem proteger as drogas de degradação, aumentar a absorção intracelular, promover a liberação sustentada e melhorar a localização para o sítio de aplicação (MENGONI et al., 2017).

Lipossomas são compostas por fosfolipídios que formam uma bicamada lipídica com característica anfipática, circundando um núcleo aquoso. Essas estruturas são alternativas para a encapsulação de compostos de interesse como peptídeos e macromoléculas e podem transformar revestimentos inertes em revestimentos bioativos e multifuncionais, incorporando agentes terapêuticos (ELOY et al., 2014; VIGANI et al., 2019).

Apesar de não haver estudos de um filme compósito de quitosana e colágeno em associação com lipossomas reportados na literatura, já foi demonstrado que um gel de quitosana contendo fator de crescimento epidermal (EGF), tanto encapsulado em lipossomas quanto livre na formulação, acelera a epitelização de queimaduras de segundo grau em ratos de maneira superior à aplicação das lipossomas com EGF isoladamente (ALEMDAROĞLU et al., 2006; DEĞIM et al., 2011; ALEMDAROĞLU et al., 2008).

2 JUSTIFICATIVA

A capacidade de reparo tecidual mediante feridas crônicas e queimaduras muitas vezes é insuficiente sem intervenção terapêutica. Essa capacidade é prejudicada por fatores em ascensão que diminuem o potencial regenerativo intrínseco da pele, como o aumento da prevalência de diabetes, doenças arteriais e venosas, infecções resistentes e deficiências metabólicas apresentadas pela população idosa também em ascensão. Considerando-se a dependência do Brasil de materiais para substituição dérmica na terapia de lesões cutâneas, os quais são importados e têm alto custo para o Sistema Único de Saúde, há a necessidade de desenvolver alternativas nacionais acessíveis, como a matriz compósita proposta neste trabalho, visando sua futura aplicação em modelos clínicos de regeneração tecidual.

3 OBJETIVOS

Objetivo Geral

Desenvolver um filme compósito de quitosana e colágeno com lipossomas e avaliar sua biocompatibilidade *in vitro*.

Objetivos Específicos

1. Formular um filme compósito de quitosana, colágeno e lipossomas com características que permitam a biocompatibilidade;
2. Caracterizar o filme desenvolvido por calorimetria exploratória diferencial (DSC), microscopia eletrônica de varredura (MEV), intumescimento e umidade;
3. Isolar, cultivar e caracterizar células estromais mesenquimais derivadas de tecido adiposo (ADSC);
4. Avaliar a viabilidade celular após contato com extratos do filme compósito através de ensaio de MTT utilizando ADSC e linhagem de queratinócitos (HaCaT);
5. Avaliar o cultivo e adesão de linhagem fluorescente de melanoma (SK MEL 103) sobre o filme.

4 ARTIGO CIENTÍFICO

Development of chitosan, gelatin and liposome film and analysis of its biocompatibility *in vitro*

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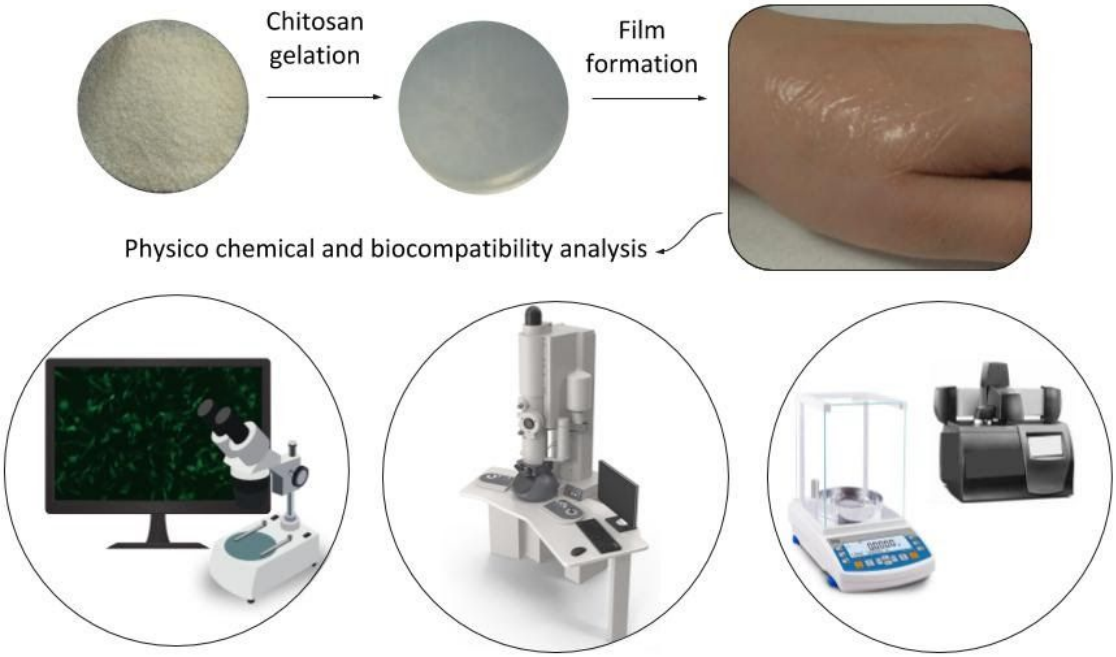
Abstract

A film of chitosan, gelatin and liposome has been designed for dermatological applications. Several adaptations were required throughout development to facilitate *in vitro* analysis, physicochemical characterization and biocompatibility evaluation. The final version of the film was characterized by differential scanning calorimetry, evaluation of swelling and scanning electron microscopy. The biocompatibility of the film was assessed by investigating cellular parameters of three types of human cells by direct contact or through films extracts: I) primary culture of adipose-derived mesenchymal stromal cells (ADCSs) and melanoma cell lines were used to test cell adhesion and morphology by direct cell culture on the material; II) ADSCs and immortalized keratinocytes were used in cell viability assay using different films extracts. The film showed physicochemical characteristics that favored cellular input, being suitable for *in vitro* analysis, which allowed its biocompatible characteristics such as the

absence of toxicity to be verified without causing significant morphological changes in ADSCs and melanoma cell line. Altogether, these results suggest that the material has a potential application for drug delivery and promotion of skin tissue repair and is therefore worthwhile for further investigations using preclinical models to cover dermal lesions.

Keywords: Chitosan, Gelatin, Liposome, Biomaterial, Film, Biocompatibility analysis

Graphical abstract



Highlights:

- Films of chitosan, gelatin and liposome have been developed.
- The films were modified for *in vitro* analysis in terms of integrity and acidity.
- The film is a blend with a homogeneous surface.
- Chitosan, gelatin and liposome films showed biocompatible characteristics.

1. Introduction

The fields of tissue engineering and regenerative medicine have suggested dermal substitutes of different materials aimed at accelerating wound healing. From natural to synthetic, these matrices with pro-regenerative characteristics are developed through a combination of substances that have the capacity to carry drugs and biomolecules to improve their properties [1]. These structures can be designed to release drugs in a controlled manner, through assembling and/or association with carriers of different origins and configurations. Several combinations can be used to provide different strategies to administer biologically active compounds, acting as delivery systems [2].

Chitosan is a cationic polymer derived from chitin, an abundant polysaccharide extracted from invertebrate skeletons [3]. Due to its biocompatibility, non-toxicity, stability, low allergenicity and biodegradability properties, chitosan is a suitable material for dermal substitutes [4]. In addition to the biological functions, chitosan film architecture enables the creation of a structure which guarantees material's integrity while handling [5]. This polymer has previously been used as a scaffold loaded with bioactive molecules with different applications for epithelial and soft tissues, such as vascularization, nerve regeneration, periodontal tissue repair and others [6]. To accomplish sustained drug release in chitosan scaffolds, nanoparticles and liposomes may be employed to carry and deliver the substance of interest [7].

Many studies have shown that chitosan-based materials promote wound healing and can be utilized for drug delivery. For example, Degim et al. (2011) demonstrated that a chitosan gel formulation containing liposomes loaded with epidermal growth factor observed more rapid epithelialization of second-degree burn wounds in treated rats [8]. In another study, Griseofulvin-loaded liposomes in chitosan film were developed with greater efficiency by

Bavarsad et al. (2016) for topical drug delivery in superficial fungal infections [9]. Additionally, a composite chitosan-based vaginal sheet successfully incorporated drugs to treat vaginal clinical conditions characterized by excessive fluid [10]. Chitosan hydrogels have also been used in mucoadhesive semisolid formulations combined with polymeric nanocarriers for the treatment of human papillomavirus infection. In this study the authors found that nanocapsules incorporated into chitosan hydrogel performed better than chitosan-coated nanocapsules incorporated into hydroxyethylcellulose gel [11]. These studies indicate that chitosan-derived materials are a promising tool for wound therapy and other clinical issues affecting the skin and mucosa.

Previously, Lionzo et al. (2012) ascertained that the addition of nanovesicles has an effect on the porosity, hydrophobicity, flexibility and roughness of chitosan film[5]. Nanovesicles, such as liposomes, contribute to the film's composition by increasing the lipophilicity and malleability of the material. As well as affecting the film structure, liposomes can also modulate the wound healing process because of its ability to adhere strongly to the skin and cell surfaces, biocompatibility and rapid cellular penetration which are useful properties for drug delivery systems [12]. Given that blends containing chitosan as an excipient released more than 80% of the active pharmaceutical ingredient within 30 min, the addition of carriers, such as liposomes, could be an alternative when a more prolonged release is desired [13].

Following the film formulations of Lionzo et al. (2012) and Libio et al. (2016), gelatin was added to improve the flexibility of the film, as gelatin and chitosan have good miscibility and form a wide range of blends [14,15]. Due to its solubility, gelatin functions by modulating the degradation of the film, in addition to providing amino acid residues that favor cell interaction [16]. Gelatin has a relatively low cost and it was added because of its hemostatic activity,

excellent biocompatibility, low antigenicity, controlled biodegradability and cellular attachment and growth stimulation [17,18].

In this work we present a new film formulation composed of chitosan, gelatin and liposome aimed at applications for treating skin injuries. To achieve this, we analyzed the physicochemical properties and adjusted the characteristics of the material to the cell culture environment to be able to evaluate the toxicity *in vitro* and cell biology parameters, including biocompatibility tests. The methodological adaptations needed during the development to allow the *in vitro* analysis are available as separated chapters to the readers. Importantly, here we bring an innovative wound healing application of a new film composition that has not been explored in the field.

2. Materials and methods

2.1. Materials

Medium Molecular Weight Chitosan was purchased from Sigma-Aldrich (Steinheim, Germany) and Gelatin from CRQ Quimica (São Paulo, Brazil). Hydrogenated phosphatidylcholine from soybean (Lipoid S PC-3) and 1,2-Dipalmitoyl-sn-glycero-3-phosphate, Monosodium salt, (DPPA-Na) (Lipoid PA 16:0/16:0) was purchased from LIPOID GMBH (Ludwigshafen, Germany). Dulbecco's modified Eagle medium low and high glucose (DMEM) were obtained from Sigma Chemical Co. (Missouri, USA).

2.2. Production of liposomes

Liposomes (LP) were prepared by reverse-phase evaporation method adapted from Mertins et al., 2006, briefly: 5.4 mg of phosphatidylcholine (PC) and 0.6 mg of phosphatidic acid (PA) were dissolved in 0.5 mL of chloroform; an emulsion was formed by the addition of 200 μL

of phosphate buffer to the solution and then sonicated (2 – 3 min) to obtain the reverse micelles. The solvent was removed under reduced pressure, leading to a viscous organogel. Then, the LP was formed by stirring 2 mL of phosphate buffer in the organogel (3 mg of phospholipids per mL) [19]. The samples were made in triplicate.

2.3. Characterization of liposomes

Liposomes size distribution and zeta potential were evaluated by using dynamic light scattering (DLS) and electrophoretic mobility (Zetasizer Nano ZS, Malvern). For analyses samples were diluted (1:500) using pre-filtered NaCl aqueous solution (10 mM). The analyses were performed at 25 ºC.

2.4. Preparation of chitosan, gelatin and liposomes film

The films were prepared with chitosan (2.6% m/v) hydrated with glacial acetic acid (0.7%, aqueous solution) and methylparaben (0.05%). The chitosan dispersion was left for 24 h for gelification. For film formation the liposomes (0.3% m/v) were mixed with chitosan (2.6% m/v) and gelatin (2% m/v) in the final concentration of 60% chitosan, 15% gelatin and 25% liposomes (v/v). The sample was named as CH-GEL-LP film. All homogenizations were performed by 1 min stirring. The liposome-loaded chitosan-gelatin hydrogel was then poured into plastic petri plates to dry for 24 h at 60 °C in the drying oven (002 CB Fanem, SP, BR). After drying, they were left in a desiccator for 24 h. The dried films were then neutralized with three washes of sodium hydroxide 1 M and three washes of ultrapure water (Milli-Q®). Aiming to avoid contamination during the cell culture tests, after drying, the films were exposed to UV-C radiation (254 nm) for 1 h inside Thermo Scientific™ 1300 Series A2 Biological Safety Cabinet.

2.5. Film microscopy

The films were analyzed by optical microscopy (OM) to verify their homogeneity with a BX-50 Olympus microscope with optical lens (10×/0.30 Ph1-UplanFI) coupled to a Motican 2500 camera (Olympus, Hamburg, Germany). For scanning electron microscopy (SEM) the films were crushed while immersed in liquid nitrogen to expose the internal structure. The film pieces were then attached to aluminum stubs and coated with gold using a Bal-Tec SCD 050 Sample Sputter Coater. Images were acquired with the Jeol JSM-6060 scanning electron microscope (200×and 1000× magnification).

2.6. Differential scanning calorimetry

Thermal analyses of CH-GEL-LP film were determined by differential scanning calorimetry (DSC 60 WS, Shimadzu, Kyoto, Japan). Samples of 5.0 – 9.0 mg from the CH-GEL-LP film and from the pure raw materials used to prepare the films [chitosan, gelatin and phospholipids PC:PA (9:1)] were placed into aluminum crucibles and sealed. Samples were heated from 30 to 200 °C at a heating rate of 5 °C/min with N_2 (atm) in 50 mL/min.

2.7. Degree of hydration and weight Loss

The hydration and weight loss that the films suffered during the immersion time were measured using pieces of 1 cm². Each piece was weighed (Shimadzu AY-220, Tokyo, Japan) before being immersed in the cell culture medium (DMEM high and low glucose 10% FBS) at 32±1 ºC. To determine the hydration of the matrix, the excess of humidity was removed from the pieces with absorbent paper after elapsed time of immersion (0.5; 1.0; 1.5; 18; 24; 48 and 72 h) and then were weighed again. The results were applied in the equation 1:

$$Hydration(\%) = \frac{W_t - W_0}{W_t \times 100} \quad \text{(Eq. 1)}$$

Where: W_0 is the dry weight of the portions before hydration and W_t is the weight after immersion in DMEM.

For weight loss three pieces were separated, left for drying in a desiccator for 48 h and weighed again. The results were used to determine the weight loss during the immersion time, by applying the equation 2:

$$Weight\ Loss\ (\%) = \frac{W_0 - W_t}{W_0 \times 100} \quad \text{(Eq. 2)}$$

Where: W_0 is the dry weight before the hydration test and W_t is the weight of the portions dried in the desiccator after immersion in the buffer. The assay was made in triplicate for each sample.

2.8. Isolation and culturing of ADSC

Adipose-derived mesenchymal stromal cells (ADSC) were isolated from abdominal adipose tissue of a woman donor after consent. Tissue collection was approved by Research Ethics Committees from Federal University of Health Sciences of Porto Alegre (UFCSPA) and Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA) (REC n° 3.734.612 and n° 2.998.992, respectively). ADSCs were isolated as already established by Naasani et al. (2019) and Rodrigues et al. (2019) [20,21]. Briefly, the blood tissue was removed from the adipose tissue by washing with phosphate-buffered saline (PBS) in a separatory funnel. Collagenase solution (Type I, 1 mg/mL solubilized in DMEM) was used for fat digestion in a 30 min water bath (37 °C) with vortex agitation every 10 min. Collagenase inactivation was performed with DMEM 10% FBS. The cell's supernatant was discarded after centrifugation (600 × g; 10 min).

Resuspension with erythrocyte lysis solution (150 mM NH_4Cl, 10 mM $NaHCO_3$ and 1 mM EDTA) diluted in ultrapure water 1:1 (v:v) was performed to remove blood cells. There was simultaneous vortex agitation and then 15 min incubation at room temperature. Cells were resuspended in DMEM 10% FBS after new centrifugation (600 × g, 10 min) and counted using 0.4% trypan blue (Bio-Rad, Hercules, CA, USA) exclusion method. Remaining cells were seeded in 25 cm² cell culture flasks with DMEM 10% FBS (1000 mg/L glucose) at 37 °C in a humidified incubator (5% CO_2). Medium exchanges were realized 48h later and repeated every 3 days.

2.9. Characterization of ADSC

2.9.1. Immunophenotyping

Cell surface marker expression was measured by direct immunofluorescence staining with FACSCalibur™ flow cytometer (488 nm argon laser) (Becton-Dickinson, San Diego, CA, USA) with CellQuest software. Antibodies PE-anti-CD14, FITC-anti-CD34, FITC-anti-CD44, PE-anti-CD45, PE-anti-CD105 (Invitrogen™, Waltham, MA, USA), PE-anti-CD90 (Biolegend®, San Diego, CA, USA) and FITC-anti-CD73 (BD Pharmingen™, San Diego, CA, USA) were incubated with 1×10^5 cells for 30 min (4 °C). A minimum of 10.000 events were collected and data was evaluated with Flowing Software 2.

2.9.2. Adipogenic, osteogenic and chondrogenic differentiation of ADSCs

ADSCs between 5 - 10 passages were seeded (1×10^4 cells/cm²) into 12-well plates (Nest Biotech). Control cells were maintained with standard growth medium (DMEM low glucose 10% FBS) and changed every three days. Three days after seeding, the growth medium was changed for the differentiation medium. StemPro® Chondrocyte Differentiation Basal Medium

(Gibco) was used as described by Naasani et al. (2017) [22]. Osteogenic and adipogenic mediums were prepared as described by Beckenkamp et al. (2018), briefly: adipogenic medium consisting of DMEM high glucose 10% FBS, 1 μM dexamethasone, 200 μM indomethacin and 10 μg/mL insulin for 21 days; osteogenic medium consisting of high-glucose DMEM 10% FBS, 0.1 μM dexamethasone, 200 μM L-ascorbic acid and 10mM β-glycerophosphate for 45 days [23]. Samples were fixed in formaldehyde 10% (60 min) and then washed in isopropanol 60% (5 min). Chondrocyte differentiation samples were stained with 1% Alcian Blue prepared in 0.1 N HCl for proteoglycan visualization. For lipid vesicles staining the cells were incubated with Oil red-O (60 min). To stain calcium phosphate deposits, cells were incubated for 45 min with 2% Alizarin Red S solution (pH 4.2). Stained differentiated cultures were compared with stained controls. Image analysis was performed with the BX-50 Olympus microscope with optical lens (10×/0.30 Ph1-UplanFI) coupled to a Motican 2500 camera (Olympus, Hamburg, Germany).

2.10. Cell culture on chitosan, gelatin and liposome film

2.10.1. Scanning electron microscopy (SEM)

ADSCs were seeded on chitosan, gelatin and liposome films (4×10^4 cells/cm²) for 24 h. The films with ADSCs were washed with PBS and fixed with 2.5% glutaraldehyde (96h, 4 °C). Then they were washed three times with PBS (30 min each). Samples dehydration was performed with propanone in ascending concentrations (30, 50 and 70% for 10 min; 90 and 100% for 20 min). Desiccation was accomplished with Balzers CPD030 critical point dryer. Samples were then attached to aluminum stubs and coated with gold using Bal-Tec SCD 050 Sample Sputter Coater. Images were acquired with a Jeol JSM-6060 scanning electron microscope.

2.10.2. GFP labeled SK-MEL-103

GFP labeled melanoma cell line, SK-MEL-103 (a kind gift from Dr. Silvya Stuchi Maria-Engler, USP), was cultured on chitosan, gelatin and liposome films (2 cm × 2 cm) at a concentration of 2×10^5 cell/well (6 well Nest Biotech cell culture plate) for 24 h and visualized with Invitrogen™ EVOS™ FL Cell Imaging System.

2.11. Viability assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed with different films extracts (chitosan film; chitosan and gelatin film; chitosan, gelatin and liposome film) of 24 h and 72 h as recommended by the International Organization for Standardization 10993-5 and 10993-12 for Biological Evaluation of Medical Devices. Adipose-derived mesenchymal stromal cells and human immortalized keratinocytes cell line (HaCaT) were seeded in 96 well cell culture plates at a density of 3×10^3 cells/well and 10×10^3, respectively. After 24 h of incubation, they were treated with film extracts for 24 h. MTT incubation was carried out for 3 h, then dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. SpectraMax Microplate Reader (Molecular Devices Corporation, USA) at 450 nm was used for measuring optical density. Results are presented in percentages and analyzed by two-way ANOVA.

3. Results and discussion

Chitosan-based hydrogels have been used as occlusive dressings for wound healing because of their ability to contract the wound [1]. In this paper, a chitosan film associated with gelatin and liposomes was developed for wound healing. There are specific requirements that need to be met so that the material can be subjected to testing with cells. For example, films

with a different pH level to the physiological one, fast disintegration and/or a lack of sterility, are difficult to evaluate *in vitro* cellular biology parameters. For this reason, in order to generate an adequate material for *in vitro* analysis, it was determined: i) chitosan and gelatin ratio; ii) films acidification; iii) preservative concentration; iv) hydrogel drying process to obtain the films; v) films dimensions for extracts and vi) films contamination prevention. These adjustments are carefully described in the supplementary material, briefly: gelatin reduction to 15% (v/v) of the final formulation; chitosan establishment at 2.6% (m/v); selection of acetic acid (0.7%) and methylparaben (0.05%); mild drying (desiccator > oven 60ºC > desiccator); neutralization with NaOH (1 M) and purified water; settlement of film thickness at 0.5 mm and adoption of UV-C exposition for 1 h. After the adjustments and development of the film, its physicochemical characteristics and biocompatible properties were evaluated.

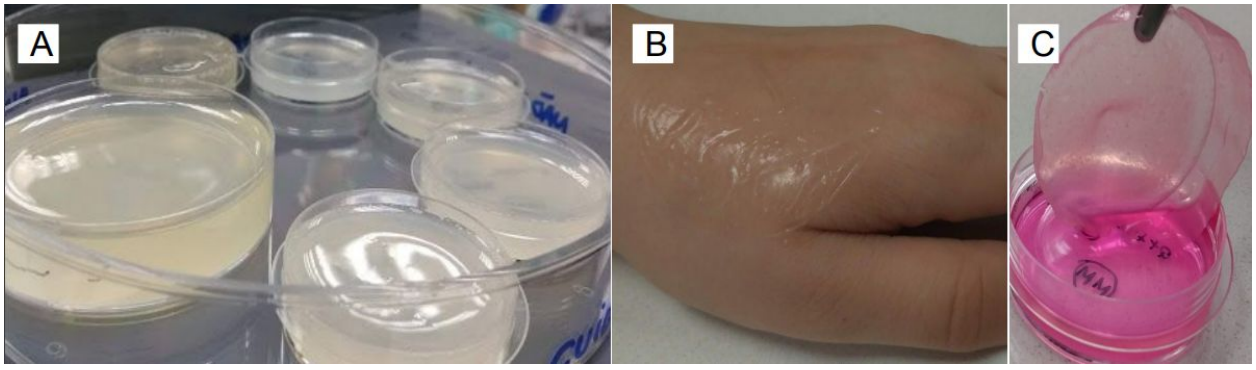


Figure 1. (A) Initial aspect of chitosan and gelatin hydrogel with liposome. **(B)** Final aspect of chitosan and gelatin film with liposome. **(C)** Film after 2 weeks immersion in the cell culture medium (DMEM high glucose 10% FBS).

3.1. Chitosan, gelatin and liposome film characterization

The CH-GEL-LP film with its initial and then final aspect, as well as the appearance after two weeks in the cell culture medium (DMEM high glucose 10% FBS) are shown in Figure 1.

OM and SEM analysis presented a homogeneous surface with birefringence from the lipids, a characteristic of liposomes, and it also showed that the structure was not altered by the cell culture medium (Figure 2). The films were transparent but became more opaque with the addition of liposomes. Disintegration was not observed when films were immersed in DMEM and a medium neutral pH was maintained.

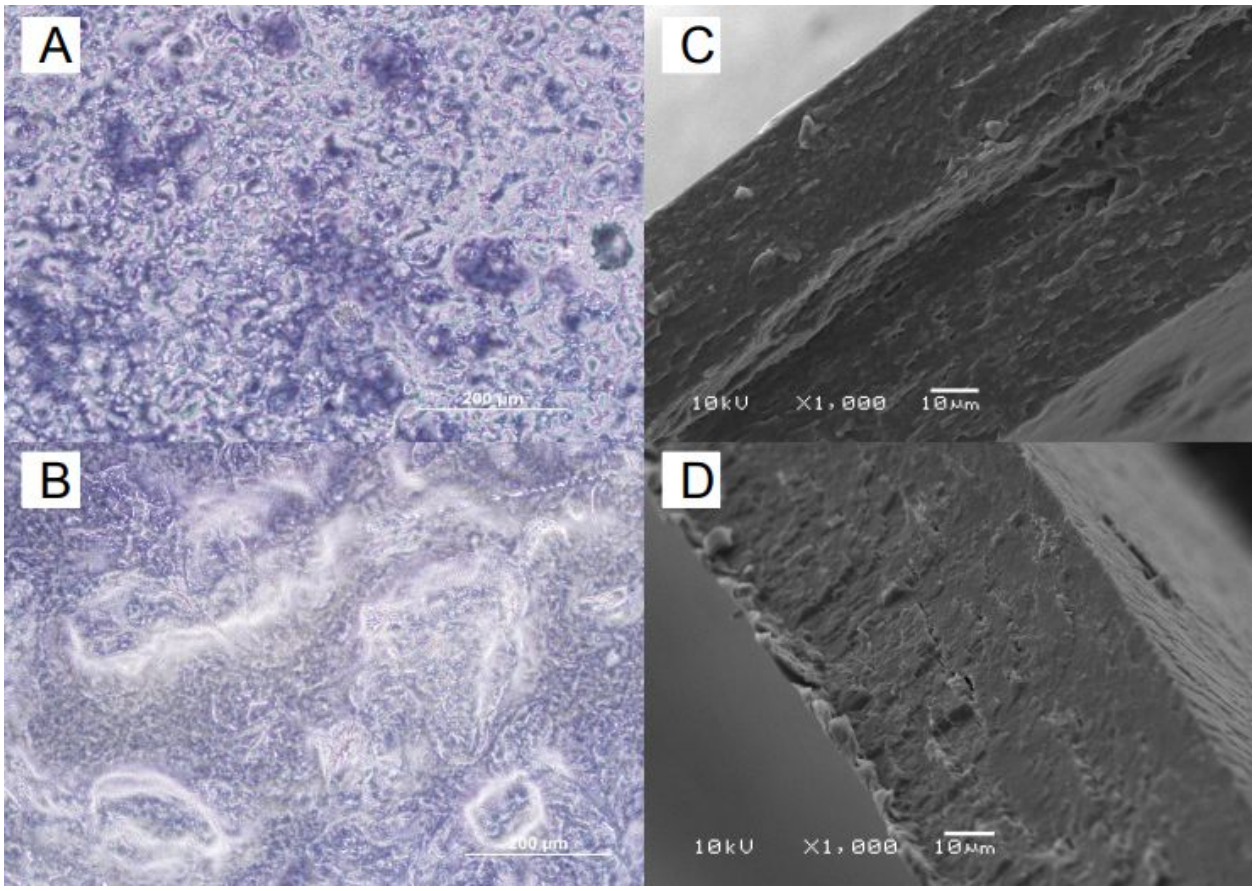


Figure 2. Optical microscopy images showing the surface of CH-GEL-LP films before (A) and after (B) immersion in the cell culture medium for 10 days (20×). Scanning electron microscopy images of lateral sections of CH-GEL-LP films before (C) and after (D) immersion in cell culture medium for 10 days. (1000×). The bar corresponds to 10 µm.

Liposome characterization by dynamic light scattering and electrophoretic mobility (Zetasizer Nano ZS, Malvern) was performed and showed a mean size of 320±16 nm. The polydispersity index was 0.408±14 and zeta potential was -14.4±3 mV. Thermal analysis of CH-GEL-LP film showed a broad transition (Figure 3. A), probably related to the dehydration that occurs around 80 ºC. By analyzing the raw materials thermograms, it was noted that pure chitosan and pure gelatin exhibited water desorption between 80 ºC and 100 ºC [24]. The main peak observed on pure CH, pure GEL and CH-GEL-LP film thermograms were similar, which is characteristic of an amorphous material. Transitions between 70 and 90 ºC are generally associated with chitosan-related phenomena, and between 160 and 200 ºC, the transitions are related to gelatin [25,26]. This profile predominantly represents the characteristics of chitosan. The presence of gelatin was weakly observed, but it is important to note that it is in the solid state as there is evidence of different thermal events when comparing gelatin in the solid and hydrated states [27]. Moreover, other[25,26][28]. The transitions seen on the thermogram of the phospholipids physical mixture (9:1, PC:PA) were well defined. DPPC may present peaks at 50, 70 or 180 ºC and a band at 100 ºC, as well as a peak > 200 ºC related to melting and decomposition [29,30]. In turn, DPPA may present T_m at 58 ºC and at 67 ºC while liposomes present transitions between 0 ºC < T_m < 100 ºC [31]. No transition related to the phospholipids or liposomes was observed on the CH-GEL-LP film. This finding indicates that the film is structured as a blend, meaning that all of the components are mixed, leading to a combination of all individual transitions. However, a miscible blend can only be confirmed by applying additional X-ray or neutron diffraction/scattering analysis, not performed here [32]. The organizational structure of the film was observed by OM and SEM analysis which showed a smooth and regular surface (Figure 2).

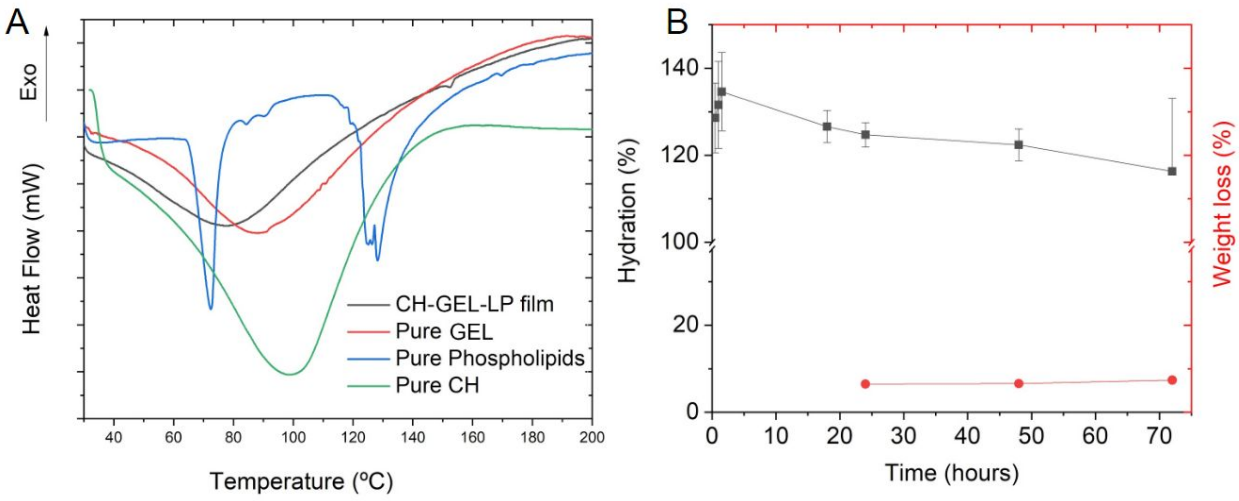


Figure 3. (A) Thermograms from the thermal analysis by Differential Scanning Calorimetry (DSC). **(B)** Percentage of swelling in DMEM at 37 °C and percentage of weight loss of CH-GEL-LP films (5 °C/min heating rate), n = 3.

To estimate the swelling of the CH-GEL-LP film, samples were submitted to similar conditions to those sustained during the assays on cells. The film showed high hydration (over 130%) in the first 2 h (Figure 3. B). However, after 24 h it was noted that the swelling process had stabilized and that there had been some weight loss (6-7%). The film is mainly composed of neutralized chitosan, its insoluble form, but the gelatin is soluble, and some parts of this polypeptide chain can be released from the matrix. This process could lead to water diffusion, swelling and a subsequent detachment of components entangled inside the film (gelatin, as well as liposomes). Despite the changes observed in the weight of the samples during the exposure to the cell culture medium, the topography of the surface and the lateral fractures of the films are similar, whether before the incubation time or after (Figure 2). To increase the degradation by weight loss, the augmentation of gelatin proportion in the film is a possibility, especially when the delivery of components entrapped inside the film is desired. However, when the gelatin

proportion is increased (results not shown), the degradation of the film makes it impossible to perform assays that require cell interaction with the material. Another approach to modulate the swelling and weight loss is to add a plasticizer, i.e. glycerol, maltitol, xylitol or sorbitol [14,33,34].

3.2. ADSC characterization

Cells isolated from human adipose tissue were adherent and presented elongated fusiform morphology as expected. Figure 4 indicates their capacity to differentiate, confirmed by the presence of adipocytes, osteoblasts and chondrocytes features after treatment with differentiation medium. Immunophenotyping of ADSCs showed expressions of CD44+, CD105+, CD90+ and CD73+. The hematopoietic markers CD14, CD34 and CD45 were not expressed (Figure 4.D). These results confirmed the acceptable parameters for ADSCs phenotype identity [35].

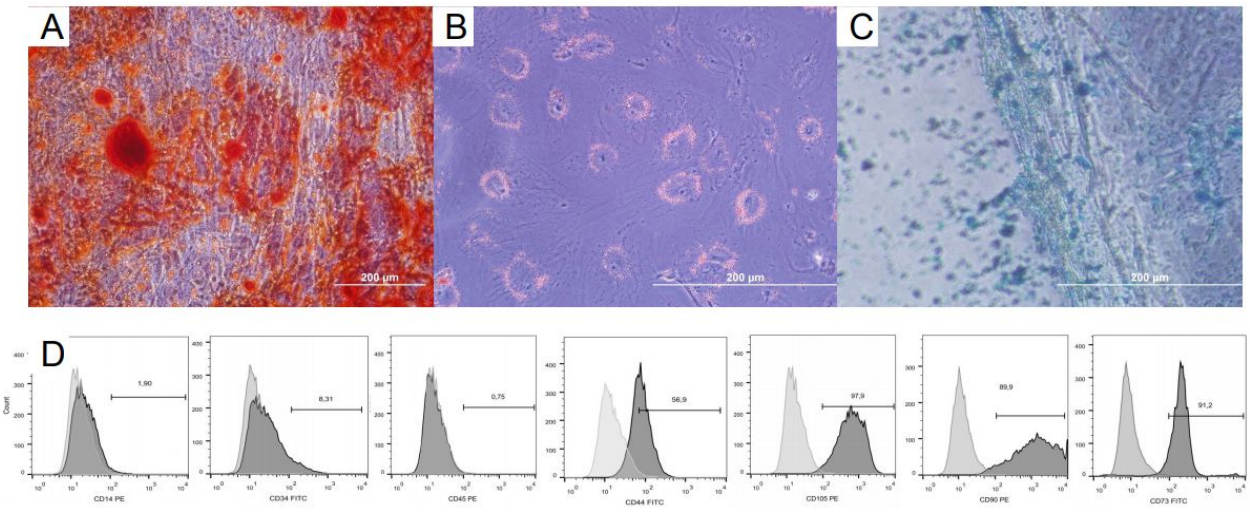


Figure 4. ADSCs characterization. Differentiated ADSCs showing **(A)** calcium phosphate deposits characteristic of osteogenic differentiation stained by Alizarin Red (100×), **(B)** lipid vacuoles stained by Oil Red indicating adipocytes (200×) and **(C)** Alcian Blue staining for proteoglycans visualization proper to chondrogenic differentiation (200×). **(D**) Histograms of Immunophenotyping analysis of ADSCs surface

markers expression by flow cytometry (CD14−, CD34−, CD45−, CD44+, CD105+, CD90+ and CD73+).

3.3. Film's biocompatibility

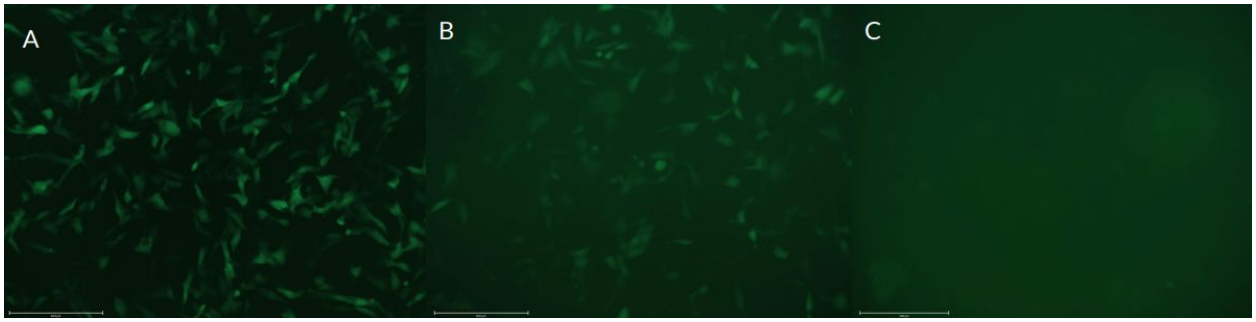


Figure 5. Fluorescent microscopy images (20×) of GFP labeled SK-MEL-103 cultured on CH-GEL-LP film. **(A)** Cell control on standard cell culture plastic plate; **(B)** Cell culture on CH-GEL-LP film; **(C)** CH-GEL-LP film control without cells showing its own inherent fluorescence.

The absence of toxicity in CH-GEL-LP films was verified through biocompatibility and morphology analysis. Adipose-derived mesenchymal stromal cells and melanoma cells (GFP labeled SK-MEL-103) showed adherence to CH-GEL-LP films (Figures 5 and 6). Cell counting by image analysis of melanoma cells showed no difference between the number of cells cultured above the film and control with cells in the culture plate. The SEM images showed that the ADSCs expressed cell extensions like filopodia and lamellipodia protrusion when in contact with the material (Figure 6).

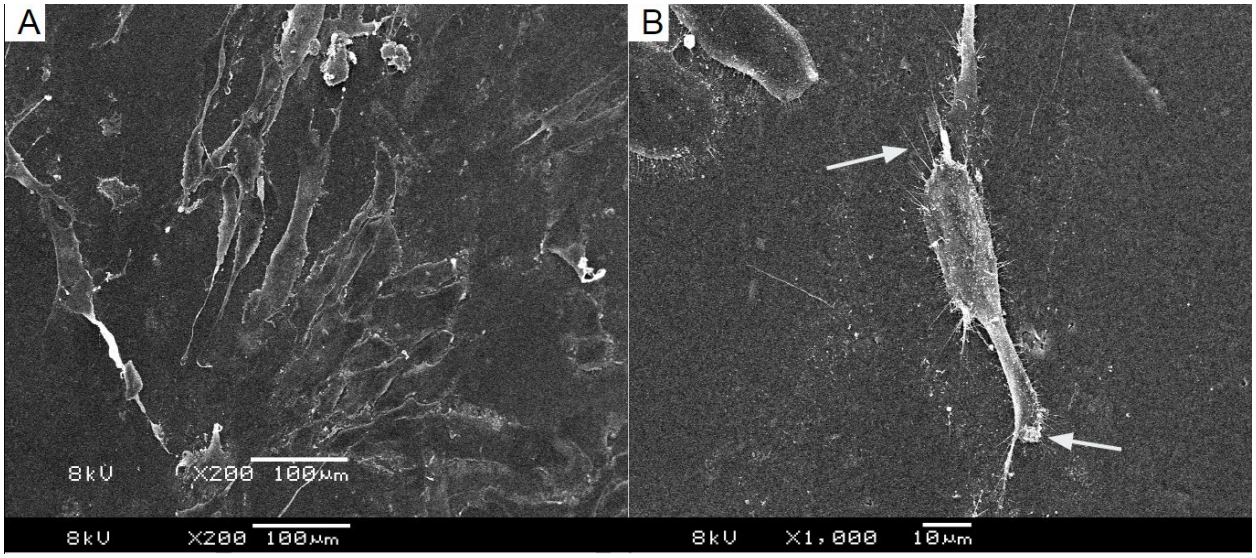


Figure 6. SEM images of ADSC cultured on CH-GEL-LP film (A and B). Cytoplasmic extensions like filopodia (above) and lamellipodia protrusion (below) are indicated by arrows (B). (A) 200×; (B) 1000×.

In addition, there was no statistically significant loss of cell viability when ADSCs and HaCaTs were in contact with the extracts of chitosan, gelatin and liposome films (Figure 7). Interestingly, the MTT assay also showed that, for the immortalized keratinocyte cells (HaCaT), the addition of gelatin to the chitosan film increased cell viability when compared with the CH-GEL-LP film extracts and control (Figure 7).

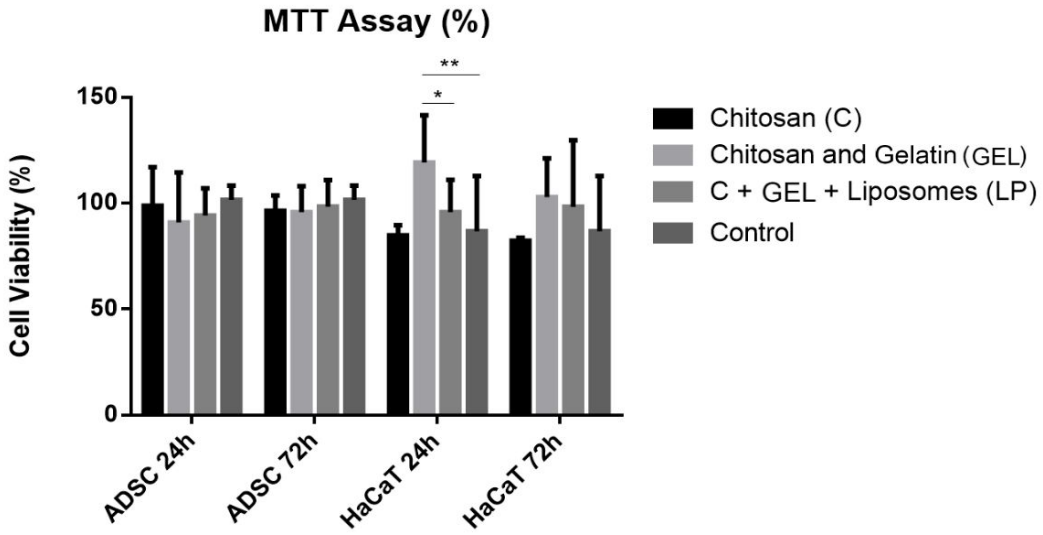


Figure 7. Viability assay (MTT) graph showing cell viability (%) of ADSCs and HaCaT seeded with CH-GEL-LP films extracts made with cell culture medium for 24 and 72 h compared with the control cells (seeded with standard culture medium) and extracts of films from the isolated components. Two-way ANOVA was performed for statistical analysis.

After analyzing the results, the cell interaction with this new material became apparent. Although there are several studies that propose using films composed by chitosan and gelatin for wound healing, there is no previous work proposing this association with liposomes [36–39]. The inclusion of liposomes proposed in this study turned the film into a system that is capable of serving as a platform for the incorporation of numerous bioactive substances within the liposomes, including labile molecules that require encapsulation, such as protein and peptide-based drugs, among others, for use as a biomaterial and as a drug delivery system.

Advanced treatment strategies, including the addition of liposomes for transdermal drug delivery, aim to improve the quality of wound healing due to its superior penetration into the skin [40]. It is also known that the inclusion in polymeric matrices, such as the one presented in this study, improves the stability of liposomes [41]. Although there are no studies of a chitosan and

gelatin film associated with liposomes, the work of Ciobanu et al. (2014) presented liposomes entrapped in a chitosan/gelatin hydrogel, creating a complex system capable of prolonged drug release [41]. Similar works have demonstrated that chitosan gels containing epidermal growth factor (EGF), both encapsulated in liposomes and free in the formulation, accelerate the epithelialization of second-degree burns in rats faster than the application of liposomes with EGF alone [8,42,43]. These findings indicate that chitosan could play an effective role in skin injuries when combined with liposomes, creating a synergistic effect with the drug of interest to enhance the repair of dermal lesions.

Recently, a bilayer collagen sponge/chitosan matrix was produced by electrospinning and is a promising solution for chronic wound management. However electrospinning parameters need further improvements for the chitosan layer [44]. It is important to highlight that the method of film production presented here mitigates complications from electrospinning and the need for expensive fabrication techniques.

In a similar fashion, a biocomposite film of chitosan, gelatin and allantoin was produced with a solution casting technique and exhibited good antioxidant and anti-inflammatory properties. These features indicate the potential of the chitosan, gelatin and liposome films for wound healing. [45]. Moreover, hydrogel sheets composed of chitosan and gelatin loaded with Manuka honey extract were proposed as dressings for chronically infected wounds, having a related formulation to ours, and showed evidence of wound healing acceleration with promotion of exudate absorption [46].

The proposed film of chitosan, gelatin and liposome was designed with a focus on manufacturing feasibility, low cost, and the ability to load liposomes with drugs of interest. In comparison with the aforementioned hydrogels, the CH-GEL-LP films presented have the

advantage of not requiring daily applications to the injured area which is necessary when administering hydrogels. The film's properties allowed different cells to grow and its extracts did not alter cell viability. The demonstration of cell support capacity is also important when considering the possibility of cell-based therapies, due to the potential to deliver cells for therapeutic purposes. Thus, the chitosan, gelatin and liposome film can be applied prospectively to chronic and infected wounds other than burn injuries, becoming more suitable for specific applications specific to liposome-loading. In addition, depending on the desired purpose, adaptations can be performed using the supplementary material described in detail.

Conclusion

The adjustments performed during the development of the formulation of chitosan, gelatin and liposome films allowed swelling and degradation levels to be controlled making the films suitable for cell culture assays. The films demonstrated physicochemical characteristics of a homogeneous and resistant material before and after *in vitro* analysis. Biocompatibility analysis through cell viability assessment and cell adhesion to the material showed that there was no significant change in cell morphology. In addition, the films have the advantage of manufacturing feasibility, low cost and the potential to load liposomes with drugs of interest. Altogether, these results suggest that the material has a potential application for drug delivery and promotion of skin tissue repair and is therefore worthwhile for further investigations using preclinical models to cover dermal lesions.

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Declaration of competing Interest

The authors declare no conflict of interests regarding this publication.

Author's contribution

Samlai Vedovatto: Project administration, Writing - Original Draft, Visualization, Conceptualization, Investigation. Jordano Cichelero Facchini: Investigation. Raquel Kiszewski Batista: Investigation. Thaís Casagrande Paim: Writing - Review & Editing, Investigation. Maria Ismenia Zulian Lionzo: Funding acquisition, Writing - Review & Editing, Conceptualization, Supervision. Márcia Rosângela Wink: Funding acquisition, Writing - Review & Editing, Conceptualization, Supervision.

Appendix A. Supplementary data: Chitosan, gelatin and liposome film development

Several adjustments were necessary to adapt the proposed film to *in vitro* analyses, as follows:

Chitosan and gelatin ratio

The initial film composition was a mixture (1:1, v/v) of the polymers dispersions, chitosan (3.25% m/v) and gelatin (2% m/v), in addition with the liposome suspension (0.3% m/v) in a concentration of 25% liposomes, 37.5% gelatin and 37.5% chitosan (v/v). After the drying process, this film formulation had no stability when immersed in the cell culture medium, disintegrating after 48 h. The physical stability is important because the film's requirements were (1°) to support the cells for microscopic analysis and (2°) to be immersed in an aqueous medium during a long period to obtain extracts. In order to establish these parameters, the concentrations were changed until the optimal concentrations (v/v) of 15% gelatin (2% m/v), 60% chitosan (2.6% m/v) and 25% liposomes (0.3% m/v) were reached. The optimal conditions were established when the film remained whole and stable under cell culture medium submersion for at least 2 weeks.

Films acidification

Another issue addressed was the film's acidity. Citric acid was chosen based on a previous work by Libio et al. (2016) [14]. Acid concentration in the initial formulation consisted of 4.7% citric acid in chitosan dispersion. This high concentration was first employed because citric acid is a weak acid, which could be suitable for the intended application. High citric acid concentration led the cell culture medium to acidify almost immediately after film immersion

which was not feasible, as it is well known that pH ~ 7 is crucial for cell culture. In addition to the cell culture medium acidification, another problem with citric acid was due to the remaining microscopic crystals in the films which hindered film neutralization. Citric acid was substituted for acetic acid with the aim of mitigating the crystal formations. To address the medium acidification, a decreasing acetic acid concentration was used starting from 4.7% to 0.7%, to ensure the minimum amount required for the chitosan's complete hydration, which was reached at 0.7% acetic acid.

Preservative concentration

Beyond the preservative function, the concentration of parabens was important for aspects of the film, such as color and transparency, because of its antioxidant activity. Unfortunately, it is known that the chosen preservative, methylparaben, induces cell toxicity. To decrease its effects on the cells, decreasing concentrations of methylparaben were tested starting from 0.16% until 0.05%. The film's coloring and transparency was maintained at 0.05%, without negatively affecting cell viability and morphology.

Hydrogel drying process to obtain the films

The drying process was also adjusted as the films turned dark yellow and retracted when kept in the drying oven for too long (60ºC). Different time intervals in the oven were tested until the optimal period was determined (16 h, maximum). Then the films were desiccated for 24 h then neutralized with NaOH 1 M and ultrapure water, with a further 24 h in the desiccator after neutralization.

Films dimensions for extracts

It was necessary to produce films with adequate dimensions to obtain extracts at 24 and 72 h from the cell culture medium. The extracts were used to treat the cells for MTT assay, in accordance with the International Organization for Standardization 10993-5 and 10993-12 for Biological Evaluation of Medical Devices. To accomplish this, the hydrogel was placed in plastic petri plates for bacterial culture (18 cm^2) to avoid adherence to the plate's surface. Hydrogel thickness was adjusted with 10 mL of chitosan dispersion with gelatin and liposome after observing the formation of a thin film after drying (< 0.5 mm). Following the established extraction ratio (10%) of 6 cm^2/mL, as recommended by ISO 10993-12, 3 mL of DMEM low and high glucose were added, separately, over the film pieces. The extracts were used to treat the cells in the viability assay.

Films contamination prevention

In order to reduce the contamination of the extracts, a protocol of UV-C radiation exposure was tested. Different samples of the dried and neutralized films were exposed to UV-C radiation (254 nm) for 0.25, 0.5 and 1 h inside a Thermo Scientific™ 1300 Series A2 Biological Safety Cabinet. There was no contamination of the cell culture medium when in contact with the film pieces submitted to 1 h UV exposure after 2 weeks of film immersion, thus this step was included in the film's production protocol.

The mentioned adjustments permitted the film to accomplish the *in vitro* analyses proposed. These adaptations can serve as a guide for new tests, enabling the modification of the film as required.

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5 PERSPECTIVAS

O filme de quitosana, colágeno e lipossomas proposto possibilita o carreamento de moléculas bioativas vesiculadas, oferecendo uma camada física protetiva que pode servir como sistema de liberação controlada. Visando a aplicação do filme compósito para o tratamento de lesões da pele, pretendemos associar ao material o fator de crescimento epidermal (EGF). O EGF é conhecido por estimular a proliferação celular devido a indução de respostas mitogênicas (CARPENTER; COHEN, 1990). Uma formulação de quitosana preparada com lipossomas carregadas com EGF foi desenvolvida por Degim et al., 2011, demonstrando crescimento significativo da proliferação celular e aceleração da epitelização. A proposta de Degim e colaboradores, porém, era da aplicação do material como hidrogel, portanto necessitando de reaplicação diária (DEĞIM et al., 2011). Com o objetivo de utilizar o filme desenvolvido neste trabalho para a liberação controlada de EGF, evitando assim a necessidade de reaplicação, temos como perspectivas o encapsulamento do EGF no material, após reformulação com maior proporção de colágeno para melhor destacamento dos componentes emaranhados no filme. Para tanto, prevê-se somar às análises anteriores os testes de eficiência de encapsulamento e de taxa de liberação ao longo do tempo.

6 CONCLUSÃO

Foi formulado, após adequações, um filme de quitosana e colágeno com lipossomas que apresentou características físico químicas de um material homogêneo e resistente, adequado para a transposição às análises *in vitro*. Sua análise físico química indicou uma estrutura miscível com baixo grau de inchamento. Foram verificadas propriedades biocompatíveis que possibilitaram o aporte de células, demonstrando adesão e viabilidade celular nos testes com o material sem modificar significativamente a morfologia celular, o que permite sugerir a continuidade de sua análise em modelos pré-clínicos para posterior uso na terapia de reparo tecidual da pele.

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ANEXO I

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Isolamento de células a partir de material de descarte humano para estudos em medicina regenerativa

Pesquisador: Marcia Rosangela Wink

Área Temática:

Versão: 4

CAAE: 98290318.5.0000.5335

Instituição Proponente: ISCMPA

Patrocinador Principal: Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul

DADOS DO PARECER

Número do Parecer: 3.734.612

Apresentação do Projeto:

Projeto de pesquisa a ser realizado em parceria com o Laboratório de Biologia Celular da Universidade Federal de Ciências da Saúde de Porto Alegre e as Unidades de Obstetrícia, Oftalmologia e Cirurgia Plástica da Santa Casa de Porto Alegre, utilizando material de descarte hospitalar como lipoaspirado, pele, limbo esclero-corneal, membrana amniótica e cordão umbilical, serão extraídos diferentes tipos celulares humanos (queratinócitos, melanócitos, células estromais mesenquimais e células endoteliais) para o desenvolvimento de novas terapias celulares para medicina regenerativa, como a aplicação de células estromais mesenquimais em sítios lesionados e também para a verificação da biocompatibilidade de materiais propostos para uso clínico, tais como filmes de quitosana e colágeno, stents cardíacos de ferro e geopolímeros.

Objetivo da Pesquisa:

Já referido em parecer anteriormente emitido.

Avaliação dos Riscos e Benefícios:

Já referido em parecer anteriormente emitid

Comentários e Considerações sobre a Pesquisa:

Já referido em parecer anteriormente emitid

Endereço: R. Profº Annes Dias,295 Hosp.Dom Vicente Scherer
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Considerações sobre os Termos de apresentação obrigatória:

Já referido em parecer anteriormente emitid

Recomendações:

Não aplicável.

Conclusões ou Pendências e Lista de Inadequações:

As solicitações de adequação do Termo de Assentimento e do TCLE foram atendidas.

Considerações Finais a critério do CEP:

Após avaliação das alterações efetuadas no estudo acima descrito, o presente Comitê não encontrou óbices quanto à implementação das mesmas.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1401744_E1.pdf	21/11/2019 15:02:29		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo_Assentimento_Pele_de_Prepucio_Versao_1_1.pdf	21/11/2019 14:55:24	THAIS CASAGRANDE PAIM	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_Prepucio__Versao_1_1.pdf	21/11/2019 14:52:48	THAIS CASAGRANDE PAIM	Aceito
Outros	Carta_Justificativa_Emenda.pdf	15/08/2019 20:33:27	THAIS CASAGRANDE	Aceito
Outros	Declaracao_de_autorizacao_da_chefia_responsavel_emenda.pdf	09/08/2019 10:48:08	THAIS CASAGRANDE	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_pele_de_prepucio.pdf	07/08/2019 13:55:25	THAIS CASAGRANDE PAIM	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo_Assentimento_Pele_de_Prepucio.pdf	07/08/2019 13:52:44	THAIS CASAGRANDE PAIM	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Materiais_de_Descarte_EMENDA.pdf	07/08/2019 13:47:54	THAIS CASAGRANDE PAIM	Aceito
Recurso Anexado pelo Pesquisador	CARTA_RESPOSTA_AO_CEP.pdf	12/11/2018 16:53:24	Samlai Vedovatto	Aceito

Projeto Detalhado / Brochura Investigador	PROJETO_MATERIAIS_DE_DESCARTE.pdf	12/11/2018 16:50:59	Samlai Vedovatto	Aceito
Folha de Rosto	FOLHA_DE_ROSTO.pdf	12/11/2018 16:49:40	Samlai Vedovatto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEs_materiais_descarte_e_justificativas.pdf	12/09/2018 17:57:45	THAIS CASAGRANDE PAIM	Aceito
Outros	Formulario_Cadastro_Projetos_Unidade_Pesquisa.pdf	16/08/2018 16:02:38	Samlai Vedovatto	Aceito
Declaração de Instituição e Infraestrutura	Termo_responsavel_setor_onde_realizada_pesquisa.pdf	16/08/2018 16:00:56	Samlai Vedovatto	Aceito
Declaração de Pesquisadores	Termo_entrega_relatorios.pdf	16/08/2018 15:59:54	Samlai Vedovatto	Aceito
Outros	Declaracao_de_autorizacao_da_chefia_responsavel.pdf	16/08/2018 15:56:09	Samlai Vedovatto	Aceito
Outros	Formulario_Inscricao_Projetos_pesquisa.pdf	16/08/2018 15:52:48	Samlai Vedovatto	Aceito
Declaração de Pesquisadores	Declaracao_utilizacao_dados_prontuario.pdf	16/08/2018 15:51:29	Samlai Vedovatto	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_isencao_onus_instituicao.pdf	16/08/2018 15:50:45	Samlai Vedovatto	Aceito
Declaração de Pesquisadores	Declaracao_confidencialidade_sujeito_estudo.pdf	16/08/2018 15:49:41	Samlai Vedovatto	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Declaracao_utilizacao_dados_material_biologico.pdf	16/08/2018 15:48:11	Samlai Vedovatto	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 29 de Novembro de 2019

Assinado por:
Claudio Marcel Berdún Stadñik
(Coordenador(a))

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ANEXO II

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Isolamento de células a partir de material de descarte humano para estudos em medicina regenerativa

Pesquisador: Marcia Rosangela Wink

Área Temática:

Versão: 1

CAAE: 98290318.5.3001.5345

Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre

Patrocinador Principal: Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul

DADOS DO PARECER

Número do Parecer: 3.214.091

Apresentação do Projeto:

Projeto de pesquisa a ser realizado em parceria com o Laboratório de Biologia Celular da Universidade Federal de Ciências da Saúde de Porto Alegre e as Unidades de Obstetrícia, Oftalmologia e Cirurgia Plástica da Santa Casa de Porto Alegre, utilizando material de descarte hospitalar como lipoaspirado, pele, limbo esclero-corneal, membrana amniótica e cordão umbilical, serão extraídos diferentes tipos celulares humanos (queratinócitos, melanócitos, células estromais mesenquimais e células endoteliais) para o desenvolvimento de novas terapias celulares para medicina regenerativa, como a aplicação de células estromais mesenquimais em sítios lesionados e também para a verificação da biocompatibilidade de materiais propostos para uso clínico, tais como filmes de quitosana e colágeno, stents cardíacos de ferro e geopolímeros.

Objetivo da Pesquisa:

Objetivo Primário:

Isolar células de tecidos humanos, que seriam descartados, para estudos que gerem novas terapias com potencial uso em medicina regenerativa.

Objetivo Secundário:

-Isolar e caracterizar células estromais mesenquimais de tecidos humanos de descarte: pele, cordão umbilical, gordura e limbo esclerocorneal para serem estudadas e testadas em biomateriais

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utilizados em regeneração tecidual;-Isolar e caracterizar queratinócitos e melanócitos de pele humana e limbo esclerocorneal, para aplicação em biomateriais;
-Isolar e caracterizar Células Endoteliais da Veia Umbilical Humana (HUVEC) obtidas de cordão umbilical (proveniente de partos a termo) para avaliar a biocompatibilidade in vitro de amostras de ferro, micro moldado por injeção, para uso em stents cardíacos;
-Estudar in vitro a biocompatibilidade de metais biocorrosíveis com células estromais mesenquimais humanas (MSCs);
-Avaliar a biocompatibilidade da membrana amniótica humana e de uma matriz compósita de quitosana e colágeno com células estromais mesenquimais
humanas.

Avaliação dos Riscos e Benefícios:

Riscos: O único risco ao doador de material de descarte é não ser respeitada a confidencialidade das suas informações pessoais, para prevenção deste problema os pesquisadores assinarão a declaração de compromisso para utilização de dados de material biológico e a declaração de confidencialidade do sujeito no estudo.

Benefícios:
Provavelmente não haverá benefício imediato para o paciente doador. A doação dos materiais poderá vir a beneficiar futuramente a população como um todo gerando novas opções de tratamento para a medicina regenerativa.

Comentários e Considerações sobre a Pesquisa:

O projeto foi iniciado conforme parecer emitido anteriormente.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos apresentados.

Recomendações:

A entrega do relatório final está prevista para março/2019,no entanto está data deverá ser corrigida pois o projeto está sendo aprovado neste mesmo mês/ano.
Lembramos que o projeto deve iniciar apenas após a aprovação do CEP.

Conclusões ou Pendências e Lista de Inadequações:

Projeto em andamento.
Aprovar

Considerações Finais a critério do CEP:

De acordo com o parecer do Relator.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1261653.pdf	15/01/2019 16:57:14		Aceito
Outros	termo.pdf	15/01/2019 16:51:23	Marcia Rosangela Wink	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_MATERIAIS_DE_DESCARTE.pdf	12/11/2018 16:50:59	Samlai Vedovatto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEs_materiais_descarte_e_justificativas.pdf	12/09/2018 17:57:45	THAIS CASAGRANDE PAIM	Aceito
Outros	Formulario_Cadastro_Projetos_Unidade_Pesquisa.pdf	16/08/2018 16:02:38	Samlai Vedovatto	Aceito
Outros	Declaracao_de_autorizacao_da_chefia_responsavel.pdf	16/08/2018 15:56:09	Samlai Vedovatto	Aceito
Outros	Formulario_Inscricao_Projetos_pesquisa.pdf	16/08/2018 15:52:48	Samlai Vedovatto	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Declaracao_utilizacao_dados_material_biologico.pdf	16/08/2018 15:48:11	Samlai Vedovatto	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

PORTO ALEGRE, 21 de Março de 2019

Assinado por:
Fernanda Bordignon Nunes
(Coordenador(a))

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ANEXO III

Normas da Revista

Seguindo as recomendações da coordenação do PPG - Ciências da Saúde, optou-se por inserir apenas o link para as normas online da revista (Guia para Autores).

International Journal of Biological Macromolecules

https://www.elsevier.com/journals/international-journal-of-biological-macromolecules/0141-8130/guide-for-authors