Dextran-based hydrogel containing chitosan microparticles loaded with growth factors to be used in wound healing

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ABSTRACT

Skin injuries are traumatic events, which are seldom accompanied by complete structural and functional restoration of the original tissue. Different strategies have been developed in order to make the wound healing process faster and less painful. In the present study in vitro and in vivo assays were carried out to evaluate the applicability of a dextran hydrogel loaded with chitosan microparticles containing epidermal and vascular endothelial growth factors, for the improvement of the wound healing process. The carriers’ morphological characterization by scanning electron microscopy and their cytotoxicity profile and degradation by-products were evaluated through in vitro assays. In vivo experiments were also performed to evaluate their applicability for the treatment of skin burns. The wound healing process was monitored through macroscopic and histological analysis. The macroscopic analysis showed that the period for wound healing occurs in animals treated with microparticle loaded hydrogels containing growth factors that were considerably smaller than that of control groups. Moreover, the histological analysis revealed the absence of reactive or granulomatous inflammatory reaction in skin lesions. The results obtained both in vitro and in vivo disclosed that these systems and their degradation by-products are biocompatible, contributed to the re-establishment of skin architecture and can be used in near future for the controlled delivery of other bioactive agents used in regenerative medicine.

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1. Introduction

Wound healing is an extremely dynamic and interactive biological process [1]. It involves complex interactions of extracellular matrix (ECM) molecules, soluble mediators, various resident cells (fibroblasts and keratinocytes) and infiltrating leukocyte subtypes which, together, act to re-establish the integrity of the damaged tissue and replace the lost one [1]. Usually, three processes are involved in wound healing: (i) contraction of the wound edges; (ii) formation of epithelialized scar and (iii) tissue regeneration [2]. This process is slow and rarely accompanied by a complete structural and functional restoration of skin functions, which has repercussions in the quality of life of millions of people around the world [3]. Skin generally needs to be covered by a wound dressing immediately after it’s damaged in order to improve the odds of survival and to minimize the loss of its functions. The application of skin substitutes is aimed at avoiding inhibition, fluid and protein loss prevention, electrolyte imbalance control as well as improving esthetic appearance of the wound site [4]. An ideal wound dressing must be biocompatible and biodegradable, prevent dehydration and have good mechanical properties to allow cell growth. Besides that, it should also be porous to allow diffusion of wastes and nutrients [5]. The modern dressings are mainly classified, according to the materials used in their production, to hydrocolloids, alginates and hydrogels [1].

Hydrogels are three-dimensional (3D) polymeric networks capable of absorbing high amounts of water and/or biological fluids. Such is fundamental for the absorption of the excess of wound exudates. Moreover, hydrogels protect the wound site from a secondary infection, are malleable and promote the healing process, by providing a moisturized wound healing environment [6]. These materials are non-adherent and contribute for surface cooling of the wound, which may lead to a marked reduction in patient pain and therefore have high host acceptability [1]. These systems have also been applied for the controlled drug delivery of therapeutic agents (antimicrobials or growth factors (GFs)) into the affected area [7].

However, despite their attractive physical properties, the amount of drug loaded into hydrogels is limited and the high water content of most of these 3D polymeric matrices often results in relatively rapid release profiles, which limits their application as drug delivery systems. Furthermore, there is also the risk of harmful side-effects for patient due to the exposure to high drug concentrations [7]. Dextran is a natural glucose-containing polysaccharide that is a very versatile starting polymer for hydrogel synthesis [8]. The oxidation of dextran by sodium periodate is an easy and well-known method
to functionalize dextran with aldehyde moieties. These aldehyde moieties in conjugation with Nucleophile has been tested for the synthesis of pro-drugs, as spacers in enzyme immobilization or for GFs’ controlled release [8]. Dextran hydroydyl are has been used for the stabilization and delivery of fibroblast GFs for tissue regulation [9]. The limitations of hydrogels used in drug delivery can be overcome by the incorporation of different nano- and micro-devices within their polymeric matrix [10]. These systems protect all unstable biological active compounds from degradation, when in contact with the body fluids, and allow a sustained and targeted release of these molecules. This is fundamental to decrease the number of therapeutic doses administrated and also increase the therapy effectiveness [11]. Different natural or synthetic polymers, lipids, surfactants and dendrimers have been used for drug carrier production [10]. Among them, polysaccharides such as chitosan and alginate have attracted huge attention from various researchers due to their outstanding physical and biological properties [12]. Chitosan is a deacetylated derivative of chitin, a natural polysaccharide found primarily in exoskeletons of arthropods and some fungi [13,14]. Chitosan presents characteristics like biocompatibility, biodegradability and pH sensitivity that are fundamental for its application as a drug carrier [11,13]. In recent studies, chitosan has been used to deliver bioactive molecules such as GFs [15].

Different GFs like epidermal growth factor (EGF), basic fibroblast GF, granulocyte-macrophage colony-stimulating factor, human growth hormone–insulin-like GF, platelet derived GF, transforming GF β and vascular endothelial growth factor (VEGF) have been described in the literature as being involved in the wound healing process [16]. EGF is a single polypeptide comprised of 53 amino acid residues and it has been described that this GF increases the epithelial cell proliferation and the ECM synthesis, which are fundamental to accelerate the wound healing process [17]. VEGF is a multifunctional molecule with important biological activities that depend on both the stage of development and physiological function of the organ, in which it is expressed. It has potent effects on the vascular system, including the ability to stimulate new vessel growth and to increase vascular permeability [18]. Karakecil et al. reported it as a good candidate to be used in wound healing, due to its specific role in the angiogenesis cascade and its relationship with other GFs and cells [19].

In this study a dextran hydrogel loaded with chitosan microparticles containing VEGF and EGF was produced in order to be used in a near future as a wound dressing.

2. Materials and methods

2.1. Materials

Adipic acid dihydrazide (AAD), amphotericin B, chitosan (medium molecular weight), dialysis membranes (MWCO = 12,000 Da), Dulbecco’s modified Eagle’s medium (DMEM-P12), diethylene glycol, EGF, ethylenediaminetetraacetic acid (EDTA), lactate dehydrogenase (LDH) assay, L-glutamine, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt (MTS), penicillin G, phosphate-buffered saline solution (PBS), sodium periodate, sodium tripolyphosphate (TPP), streptomycin, trypsin and VEGF were purchased from Sigma-Aldrich (Sintra, Portugal). Dextran T500 was purchased from Pharmacia LKB, Sweden. Human fibroblast cells (Normal Human Dermal Fibroblasts adult, cryopreserved cells) were purchased from PromoCell (Labclinics, S.A.; Barcelona, Spain). Fetal bovine serum (FBS) was purchased from Bionichrom AG (Berlin, Germany).

2.2. Methods

2.2.1. Chitosan microparticle preparation

Microparticles were prepared by ionotropic gelation between the positively charged chitosan and the negatively charged TPP ions, as previously reported in the literature [20]. An aqueous solution of chitosan 1.5% (w/v) was prepared by dissolving chitosan in a 1% acetic acid solution [21]. Then, different amounts of EGF, VEGF and EGF + VEGF were dissolved in various chitosan solutions and mixed for 3 h. Microparticle production was performed by using an electrosprinning apparatus. The previously prepared solutions were loaded separately into a 10 ml plastic syringe with a needle of 23 gauge at room temperature. The needle was connected to a high-voltage generator (CZE 1000B, Spellman, UK) at a voltage of 9 kV and an aluminum foil was used as the counter electrode. The solution feed rate was controlled through a syringe pump (KD Scientific, KDS-100, Sigma) at a flow of 10 ml/h [21]. Subsequently, the microparticles were collected and washed with distilled water.

2.2.2. Dextran hydrogel synthesis

An aqueous solution of dextran (1 g; 0.125% w/v) was oxidized with 2 ml of sodium periodate solution (165 mg/ml) at room temperature, in accordance with a procedure previously described in the literature [8]. The reaction was stoped after 4 h, by adding 10% (v/v) of diethylene glycol. The solution was then dialyzed for 3 days against Milli-Q water, using a dialysis membrane and then lyophilized for 172 h (ScanVac ColdSafe Freeze Drying LaboGene, Denmark).

The oxidized dextran (DeOx) at 10% (w/w) was solubilized in PBS. Then, to prepare the hydrogel, 250 µl of DeOx solution was mixed with 250 µl of AAD solution at 15% (w/w) for 30 min [22]. Microparticles loaded within without GFs were added to DeOx samples before their complete reticulation with AAD. The final concentration of each GF was 10 µg/ml in all tested samples [23].

2.2.3. Proliferation of human fibroblast cells in the presence of the carriers

Human fibroblast cells were seeded in T-flasks of 25 cm² with 6 ml of DMEM-P12 supplemented with heat-inactivated PBS (10% v/v) and 1% antibiotic/antimycotic solution. After the cells attained confluency, they were subcultivated by a 3–5 min incubation in 0.18% trypsin (1:250) and 5 mM EDTA. Then, the cells were centrifuged, resuspended in culture medium and then seeded in T-flasks of 75 cm². Hereafter, the cells were kept in culture at 37 °C, in a 5% CO₂ humidified atmosphere inside an incubator [8,14]. To evaluate cell behavior in the presence of the carriers, each formulation of hydrogel with microparticles was added (n = 5) into a 96-well cell culture plate, in amounts that never exceeded 50 µL of hydrogel and 10 µg/ml of different GFs per well. The materials were sterilized by UV exposure for at least 30 min. Then, DMEM-P12 was added to each well and was left in contact with the carriers for 24 h. Meanwhile, human fibroblast cells were cultured in 96-well plates at a density of 5 × 10⁴ cells per well. After 24 h, the cell culture medium was removed and replaced by the one that was in contact with polymers. This procedure was repeated for 3 days. Cell growth was monitored using an Olympus CX41 inverted light microscope (Tokyo, Japan) equipped with an Olympus SP-5000 UZ digital camera.

2.2.4. Characterization of the cytotoxic profile of the carriers

To evaluate the cytotoxicity of the carriers, human fibroblast cells were seeded at a density of 5 × 10⁴ cells per well, and cultured with DMEM-P12. At the same time, in another plate, the culture medium was added to the sterilized polymers, and left there for 24 and 48 h. After, the cell culture medium was removed and replaced with 100 µL of medium that was in contact with the carriers. Then the cells were incubated at 37 °C in a 5% CO₂ humidified atmosphere for another 24 h. Subsequently, cell viability was assessed through the reduction of the MTS into a water-soluble brown formazan product (n = 5), by an adaptation of the method previously described in the literature [8]. The medium of each well was then removed and replaced with a mixture of 100 µL of fresh culture medium and 20 µL of MTS/PMS reagent solution. The cells were incubated for 4 h.
at 37 °C, under a 5% CO₂ humidified atmosphere. The absorbance of the produced formazan was measured at 492 nm using a microplate reader (Sanofi Diagnostics Pasteur). Wells containing cells in the culture medium without materials were used as negative control (live cells). Ethanol 96% was added to wells containing cells as a positive control (dead cells) [14,24,25]. Furthermore, a LDH assay was also performed to evaluate the amount of extracellular LDH released from damaged cells to the extracellular medium [26]. After 24 and 48 h in the presence of carriers, the well plates were shaken briefly and 50 μL of culture medium was transferred into a fresh 96-well plate. Then, the LDH assay mixture (100 μL) was added to each well. After 20–30 min, the enzymatic activity was stopped by the addition of chloride acid (HCl). Then, the absorbance of the samples was measured at 492 nm [27]. Wells containing cells in the culture medium without carriers were used as negative control (live cells). Lysis solution was added to wells containing cells that were used as positive control (dead cells) [28].

2.2.5. Scanning electron microscopy analysis

The morphology of the microparticles and hydrogel with/without adhered human fibroblast cells was characterized by scanning electron microscopy (SEM). Cells (6 × 10⁴ cells/well) were seeded with sterilized chitosan microparticles and DeOx with/without microparticles in 48-well plates, over a coverslip, for 48 h. Samples were fixed with 2.5% glutaraldehyde overnight and then frozen in a glass container using liquid nitrogen and freeze-dried for 3 h. Finally, the carriers were mounted on an aluminium board using a double-sided adhesive tape and covered with gold using an Emitech K550 (London, England) sputter coater. The samples were then analyzed using a Hitachi S-2700 (Tokyo, Japan) scanning electron microscope operated at an accelerating voltage of 20 kV and at various amplifications [14,29].

2.2.6. In vivo assays

A total of 30 Wistar rats (8–10 weeks) weighing between 150 and 200 g were used in wound healing studies. The animal protocols

![Fig. 1. Images of freeze-drying oxidized dextran (a) and dextran hydrogel with microparticles incorporated (b).](image)

![Fig. 2. SEM images of surface of oxidized dextran 50× (a), chitosan microparticles 400× and (b) chitosan microparticles 2000× (c).](image)

![Fig. 3. Microscopic photographs of human fibroblast cells after being seeded in the presence of the carriers during 24 h and 48 h. DeOx + VEGF: oxidized dextran loaded with chitosan microparticles with VEGF incorporated; DeOx + EGF: oxidized dextran loaded with chitosan microparticles with EGF incorporated; DeOx + (EGF + VEGF): oxidized dextran loaded with chitosan microparticles with VEGF and EGF incorporated; VEGF + EGF: VEGF and EGF dissolved in cultured medium; DeOx + Ch: oxidized dextran loaded with chitosan microparticles; negative control (live cells); positive control (death cells). Original magnification 100×.](image)
followed in the present study were performed according to the guidelines set forth in the National Institutes of Health Guide for the care and use of laboratory animals. The animals were individually anesthetized with an intra-peritoneal injection of ketamine (40 mg/kg) and xylazine (5 mg/kg) for surgery and induction of the burn wound. The operative area of skin was shaved and disinfected using ethanol (96%) and the dorsal skin of the animals was exposed to water at 95 ± 1 °C for 10 s. Wounds of 2 cm diameter were created with no visible bleeding [14]. The animals were divided into six groups: in group 1 wounds were filled with EGF + VEGF dissolved in PBS; in group 2 wounds were filled with DeOx loaded with chitosan microparticles (DeOx + Ch) without GFs; in group 3 wounds

![MTS Assay](image1)

**MTS Assay**

- Positive Control
- Negative Control
- EGF + VEGF
- DeOx + Ch
- DeOx + VEGF + EGF
- DeOx + EGF
- DeOx + VEGF

% Viable Cells

![LDH Assay](image2)

**LDH Assay**

- Positive Control
- Negative Control
- EGF + VEGF
- DeOx + Ch
- DeOx + VEGF + EGF
- DeOx + EGF
- DeOx + VEGF

% Dead Cells

Fig. 5. Cellular activities measured by the MTS assay (a) and cellular integrity measured by the LDH assay (b) after 24 h and 48 h. Positive control (devoid cells); negative control (live cells); VEGF and EGF (VEGF + EGF); oxidized dextran loaded with chitosan microparticles (DeOx + Ch); oxidized dextran loaded with chitosan microparticles with VEGF and EGF incorporated (DeOx + VEGF + EGF); oxidized dextran loaded with chitosan microparticles with VEGF incorporated (DeOx + VEGF); oxidized dextran loaded with chitosan microparticles with EGF incorporated (DeOx + EGF). Each result is the mean ± standard error of the mean of at least three independent experiments. Statistical analysis was performed using one-way ANOVA with Dunnett's post hoc test (*p < 0.05; **p < 0.005).
were filled with DeOx loaded with microparticles with VEGF incorporated (DeOx + VEGF); in group 4 wounds were filled with DeOx with microparticles loaded with EGF (DeOx + EGF); group 5 was used as control and wounds were covered with PBS; the wounds in group 6 were filled with DeOx loaded with microparticles containing EGF + VEGF (DeOx + (EGF + VEGF)). Group 1 was treated every two days while the others were treated every 7 days. Then, the animals were kept in separate cages and were fed with commercial rat food and water ad libitum. All the animals showed good general health condition throughout the study, as assessed by their weight gain. The animals were sacrificed after 7, 14 and 21 days [14].

2.2.7. Histological study

Tissue specimens were obtained from the wounded area by sharp dissection at days 7, 14 and 21. The samples from skin lesions and organs (brain, heart, lung, liver, spleen and kidney) were obtained by necropsy and were formalin fixed and paraffin embedded for routine histological processing. A 3 μm section obtained from each paraffin block was stained with hematoxylin and eosin (H&E) and evaluated using a light microscope with specific image analysis software from Zeiss. Skin fragments with no carriers and GPs were used as control. The assessment of the brain, lung, liver, spleen and kidney samples was performed in order to check for any morphological alteration [14].

2.2.8. Evaluation of the wound size

Images of the wound area were taken with a digital camera (Nikon DS5) and analyzed with image analysis software ImageJ (Scion Corp., Frederick, MD). Measurement of the wound closure area was defined by the limits of grossly evident epithelialization, with all surface areas in a two-dimensional plane calibrated against the adjacent metric ruler. The percentage of wound size (WS) was calculated using the following formula (1):

\[
WS = \frac{D_w}{D_0} \times 100\% \tag{1}
\]

where \(D_0\) is the dimension of the full thickness circular skin wound area (2 cm diameter) on day 0, and \(D_w\) is the dimension of the wound area on the indicated day [14].

2.2.9. Statistical analysis

Statistical analysis was performed using one-way ANOVA with Dunnett's post hoc test. Computations were performed using a MYSSTAT 12 statistical package (Systat Software, a subsidiary of Cranes Software International Ltd.).

3. Results

3.1. Characterization of the morphology of the carriers

Freeze-dried DeOx showed a spongy morphology similar to that of cotton, as can be observed in Fig. 1(a). Subsequently the hydrogel was prepared in Teflon molds and impregnated with microparticles (Fig. 1(b)). SEM analysis of DeOx hydrogel (Fig. 2(a)) revealed a highly porous and interconnected surface. Chitosan microparticles...
produced by electrospraying presented a spherical shape and an average diameter of approximately 255 ± 0.5 μm (Fig. 2(b), Fig. 2(c)) shows that the chitosan carriers produced had a slightly smoother surface, in accordance with what was previously reported in the literature [30,31].

3.2. Evaluation of the cytotoxic profile of the carriers

To assess the applicability of our hydrogel for the envisioned biomedical application, the cytocompatibility of dextran hydrogel loaded with chitosan microparticles with/without GFs incorporated was first characterized through in vitro studies. Cell adhesion and proliferation were observed in wells where cells were in contact with different carriers (Fig. 3) and in the negative control (cells without biomaterials), at the predetermined time points. Dead cells with their typical spherical shape were visualized in the positive control (ethanol treated cells). The observation of cell adhesion and proliferation in the presence of the carriers showed that all of them are biocompatible.

SEM images were also acquired to further examine and characterize cell adhesion to the materials. Cell growth and filopodia were observed, indicating that cells adhered and grew on hydrogel surface after 48 h (Fig. 4).

To further assess the biocompatibility of the carriers, MTS and LDH assays were also performed. Both of these assays showed that cells remained viable in contact with all tested samples (with and without the GFs) after 24 and 48 h of incubation (Fig. 5). These results clearly demonstrate that these vehicles are biocompatible and may be used for GF delivery systems for wound healing.

3.3. in vivo evaluation of the wound healing process

For the evaluation of the in vivo wound healing process, Wistar rats were used and divided into six groups, as previously described in Section 2.2.6. Groups 1-5 were set as controls. Group 1 was used to check if skin regeneration was correlated with the direct application of multiple GFs (EGF + VEGF). The application of dextran hydrogel loaded with chitosan microparticles without GFs (Group 2) was used to study whether the effect on wound healing was due to the use of GFs or to the biomaterials used for carrier production. Groups 3 and 4 were used to compare the application of GFs alone or in a combined mode. Group 5, where the wounds were only treated with PBS, was used to determine whether wound contraction occurred due to the combined use of biomaterial/GFs. Finally, group 6 was set as a test group where a synergistic combination of dextran hydrogel and chitosan microparticles loaded with EGF + VEGF was used to study the influence of the system loaded with two GFs in the wound healing process. In vivo experiments showed that hydrogel carriers promoted moist healing, as previously reported in the literature [1]. Fig. 6 shows a set of typical wound beds after the surgical procedure and application of the hydrogels. The healing patterns were observed after 2, 5, 9, 12, 16 and 21 days. In Fig. 7, the evolution of the wound size for the different groups over time is presented. From the analysis of this figure, it can be inferred that the best results were obtained for the group treated with DeOx loaded with microparticles containing EGF + VEGF, since the wound closure occurred before.

3.4. Histological analysis

Fig. 8 presents the histological data obtained in this study. From its observation, it can be concluded that the granulation tissue and epithelial layer thickness increased progressively from days 7 to 21. No specific inflammation or reactive granulomas were observed due to the presence of DeOx, chitosan and GFs in all groups. No pathological abnormalities were observed in the brain, lung, liver, spleen or kidney samples (data not shown).

4. Discussion

Skin engineering methodologies require biomaterials that promote the reconstruction of the architecture of native skin, which is sometimes irreversibly destroyed by injuries or diseases [32]. In our study, a DeOx-based hydrogel was produced to be used in a near future as a skin substitute.

Dextran is biocompatible and can be degraded through the action of dextranases in various organs of the human body, including the liver, spleen, kidney and colon [8]. Keeping in mind the wound dressing application, the porous section of DeOx (Fig. 2(c)) promotes drainage of the wound, prevents the build-up of exudates, and may be an optimum wound bed for autografting. It can also increase the surface area-to-volume ratio of hydrogel scaffolds promoting cell growth, tissue invasion, and local angiogenesis and facilitate nutrient transport, which is fundamental for the wound-healing process [14].
These results are in agreement with experimental data obtained by other researchers [33,34].

In order to increase the period during which GFs were released from the DeOx-based hydrogels, chitosan microparticles loaded with these bioactive molecules were incorporated within this polymeric matrix, since first GFs have to be released in a controlled form, by chitosan microparticles to the hydrogel and then from the hydrogel to the surrounding environment. Such strategy allowed to
increase the period among which the GFs are released as previously described for other polymers and other particle-based delivery systems [35,36].

GFs (EGF and VEGF) were chosen since they are actively involved in the natural skin regeneration process [16]. In the present study, their overall contribution for the healing process was evaluated when they were used alone or both at the same time, within drug delivery systems. Chitosan microparticles were produced by an electroporation method (Fig. 2(b)), which is a slightly modified form of the electrosprining process. It allows the production of particles with smaller diameters, from micrometers to nanometers [35]. The chitosan microparticles produced presented a spherical shape, an average diameter of approximately $255 \pm 0.9 \mu m$, and a slightly smoother surface when compared with microparticles produced through traditional methods [37]. The combination of these two systems (hydrogel and microparticles) was advantageous since it allows wound protection against toxins and microorganisms and also avoids dehydration of the patient. Furthermore, the hydrogel acted as a support for the carriers incorporated in its polymeric matrix and increased the period over which GFs were released. The DeOx hydrogel produced showed a highly porous internal structure, with a pore size sufficiently large to accommodate fibroblasts, which is crucial for skin regeneration, as previously described by Weng and collaborators [34].

The results of the in vitro studies showed that cell adhered and proliferated after 48 h of being seeded in the presence of the carriers. These results were corroborated with those obtained in the MTS and LDH assays. Furthermore, the LDH results demonstrated that human fibroblast membrane integrity was not affected when in contact with carriers. These results were expected since the different components of the system developed have been previously tested individually in other studies [8,14]. Subsequently, these carriers were further characterized through in vivo studies. The wound area of animals treated with PBS (group 5) increased during the first days, while for other groups it did not. Such result emphasizes the importance of an initial covering of the damaged area, as already described in literature [38].

The healing process was slower for animals from group 1, which received several doses of GFs every two days, than for those of group 6 (treated with a single dose per week). These results show an asset for the use of this system since it can, simultaneously, reduce costs and pain associated with skin regeneration. Furthermore, the granulation tissue layer and the epithelial layer thickness increased faster for these two groups, which can be explained by the formation of new blood vessels in dermis layer of these animals (Fig. 8). These findings are in accordance with previous studies, describing that this set of biomaterials may aid in the re-establishment of native tissue architecture [34].

In group 2, in which animals were treated with DeOx loaded with microparticles without GFs, the wound healing was slower than for groups 1 and 6. Such result was expected, since GFs play key roles in the regulation on skin regeneration [19]. On the other hand, hydrogel avoids tissue dehydration and bacterial contamination and also circumvents exuberant inflammatory response.

Groups 3 (DeOx + VEGF) and 4 (DeOx + EGF) presented similar results. The healing process for these groups occurred at a slower rate than that observed for group 6. Such demonstrations that the combined use of GFs improves the establishment of the regenerative cascade in order to produce new extracellular matrix and promote angiogenesis that are fundamental for skin regeneration [39].

In studies of skin regeneration, the analogy between experimental model and human skin is important and relevant [40]. Like for the case of human burns, the thermal injury in rat’s skin destroys the epidermis, dermis and hypodermis [41]. In our study, the lack of a reactive or a granulomatous inflammatory reaction in skin lesions treated with biomaterials and the absence of pathological abnormalities in the organs obtained by necropsy supported the local and systemic histocompatibility of the biomaterials.

In this work, a versatile, non-toxic, in situ crosslinkable biodegradable dextran hydrogel was produced to be used as a wound dressing in the first phase of skin regeneration. The results obtained both in the in vitro and in vivo assays demonstrated the biocompatibility of the synthesized vehicles, thus, suggesting that AAD can be used as a crosslinking agent for DeOx hydrogel production, as previously reported by Maia et al. [8]. The in vivo studies demonstrated that the application of this system improves the mechanical, chemical and biological protection of the damaged skin. Moreover, the incorporation and spatiotemporally controlled release of VEGF and EGF also improve angiogenesis (VEGF), and re-epithelialization (EGF) that are crucial for the reestablishment of native tissue architecture [42]. Further studies are currently being undertaken to evaluate the applicability of these systems as skin substitutes in diabetic rats.

5. Conclusion

A versatile, non-toxic, in situ crosslinkable, biodegradable hydrogel has been successfully prepared with DeOx in order to be used as a wound dressing. The in vivo assays revealed that hydrogel loaded with microparticles both with and without the GFs is non-cytotoxic. The in vivo assays suggested that dextran hydrogel and chitosan microparticles with the two GFs encapsulated promote faster wound healing with no signs of local or systemic inflammatory response. The results obtained here support the simultaneous application of the two GFs, with synergic roles in wound healing mechanism. Moreover, chitosan microparticles were considered good vehicles to deliver the GFs studied, since a unique application per week of DeOx loaded with GFs helps to reduce the wound area faster than when free EGF + VEGF was applied every two days. Furthermore, dextran hydrogel could be adapted to be used as an in situ gelable wound dressing.

In the near future these two systems (hydrogel and microparticles) will also be used as carriers for other GFs or for cell encapsulation, widening the applicability of these devices to other areas of regenerative medicine.

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