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Original Article

Bioresorbable scaffold as a dermal substitute

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Abstract: Introduction: Bioresorbable polymers are often used in medical procedures. Since they are biocompatible, this class of materials is a viable alternative for cases in which tissue regeneration is strongly compromised. Bioresorbable synthetic polymers may be used as membranes to support and guide cell growth through the process of tissue repair. Objective: To assess the efficiency of a porous bioresorbable membrane Poly (L-co-DL lactic acid)-co-trimethylene carbonate, PL-co-DLA-co-TMC, as a dermal substitute associated with partial skin graft in rats. Methods: A 1.5×1.5 cm defect was created on the backs of 40 Wistar rats. The rats were divided into a control group, in which the defects were filled with partial skin graft, and a treated group, in which a membrane associated with the graft was implemented. The animals were sacrificed 7 days or 60 days after the procedure and the results were evaluated by macroscopic and microscopic analysis. Results: The polymer was biocompatible and allowed better regeneration of the dermis with less shrinkage, unlike what occurs in second intention healing. Compared to the control group, the treated group showed a thicker and wider dermis with the presence of skin appendages, suggesting partial graft integration and better healing. The skin graft acted as a biological protection of the wound. Conclusion: The study material was shown to act as a biocompatible dermal substitute and promoted less scarring of the dermis. Further studies should be conducted to improve the methodology of the surgical procedure.

Keywords: Cutaneous wound, graft, dermal substitute, bioresorbable polymer, *in vivo* study

Introduction

Situations, such as burns, trauma, chronic and complex wounds can result in complete loss of skin, leading to infection, increased insensible water loss, hypothermia, difficulties in patient management, and increased costs [1]. Autografts have been used as a treatment, but limitations, such as scarcity of donor areas or situations where the recipient is not in favor of coverage with grafts, can occur. There is also great difficulty in revascularization due to the vast amount of macrophages and neutrophils, and the shortage of collagen and elastin, resulting in graft loss and deforming scars [2]. Among the main causes of failure in the integration of autograft are hematoma, infection, and seroma [3].

In 1981, Burke and Yannas leveraged a new line of research in skin substitutes. Until then, many have sought to develop organic polymers as membranes that were biologically inert, controlled loss of fluids, prevented infection, and adhered to the wound. Researchers have aimed to develop a material that has ideal physical-chemical properties and also promotes the migration of fibroblasts and blood vessels to the wound in a non-antigenic and non-inflammatory manner. One product of these studies was a bilaminar membrane, similar to the dermis in terms of it's anatomical structure and chemical composition, that would act as a biodegradable scaffold inducing the synthesis of new dermis [4].

These results have stimulated research into the development of dermal substitutes and dermal matrices and their clinical applicability based on the surgical deployment time [5]. Skin substitutes and dermal matrices consist of a heterogeneous group of biological and/or synthetic elements that support the temporary or permanent occlusion of injuries [6]. However, all commercially available materials are biological or biosynthetic, and their access is not always easy due to the high cost. Other studies investigated different applications for the use of der-

Figure 1. Process for obtaining the porous membrane by solvent evaporation with the addition of sucrose as a leaching agent.

mal matrices beyond the treatment of burn injuries, such as abdominal wall reconstruction, breast reconstruction, reconstruction of oral mucosa, and treatment of chronic wounds [7-10].

The concept behind the development of these new devices is tissue organization. Almost every cell in the human body, except for blood, is anchored to the extracellular matrix (ECM), which contains multiple components required for tissue homeostasis [11, 12]. Therefore, scaffolds, typically developed from polymeric biomaterials for tissue engineering, promote structural support for cell attachment and tissue development, and act as a guide for tissue growth [11]. However, the best scaffolds must be equivalent to the ECM target tissue, and thus are designed to mimic native functions [11, 13].

Accordingly, tissue engineering is presented as a promising field that can synthesize and manufacture a polymer similar to that of the target tissue intended to mimic characteristics. However, despite recent progress in the area, there are several challenging criteria for the development of the ideal scaffold: (1) the surface should allow cell adhesion, promote cell growth, and enable the functions of differentiated cells; (2) the material should be biocompatible, i.e. neither the polymer nor their degradation products should cause inflammation or toxicity *in vivo*; (3) the material should be biodegradable; (4) the porosity must be sufficiently large for cell adhesion and migration, regeneration of the extracellular matrix, and diffusion limitations during culture, and the pore structure must allow a uniform spatial distribution of the cells throughout the scaffold; (5) the material must be in a processable and reproducable form in three-dimensional structure, and mechanically strong in order to promote stability to the injured/defective tissue [14]. Moreover, the size and the degree of interconnection between the pores is an important prerequisite variable between the size of invading cells and the architectural device [15, 16].

To this end, porous scaffolds have been prepared by various techniques and are the focus of many studies. Of all the techniques, rapid prototyping 3D plots [17-20] have been explored extensively and used for purposes in tissue engineering.

Regardless of the technique, the material must have favorable biomechanical properties, fostering an environment that allows the cells to integrate, differentiate, and develop new tissues [21-25]. In this context, the increased flexibility of poly (L, co-D, L lactic acid) was provided by the addition of trimethylene carbonate (TMC) with elastomeric characteristics along the polymer chain and improving the mechanical properties of the vitreous material [26], making it a terpolymer (PL-co-DLA-co-TMC) in 50:50 that can be used in the regeneration of soft tissues such as skin [27].

The terpolymer has all the properties for use as a regenerative matrix. Thus, co-PL-DLA-co-TMC serves as a scaffold for partial skin graft, acting as a dermal substitute and allowing for integration in cases where autografting using a less complex surgical procedure is impracticable.

Objective

Evaluate the efficiency of the PL-co-co-TMC-DLA membrane as a dermal substitute for the surgical treatment of skin wounds in rats.

Materials and methods

Obtaining polymeric membranes (dermal matrix)

The poly (L-co-DL lactic acid-co-trimethylene carbonate), denoted PL-co-DLA-co-TMC (50:50), was synthesized in the Laboratory of Biomaterials at the Pontifical Catholic University of São Paulo.

Solutions of PL-co-DLA-co-TMC (50:50) were prepared by diluting the polymer in chloroform (10% w/v). After complete dissolution of the

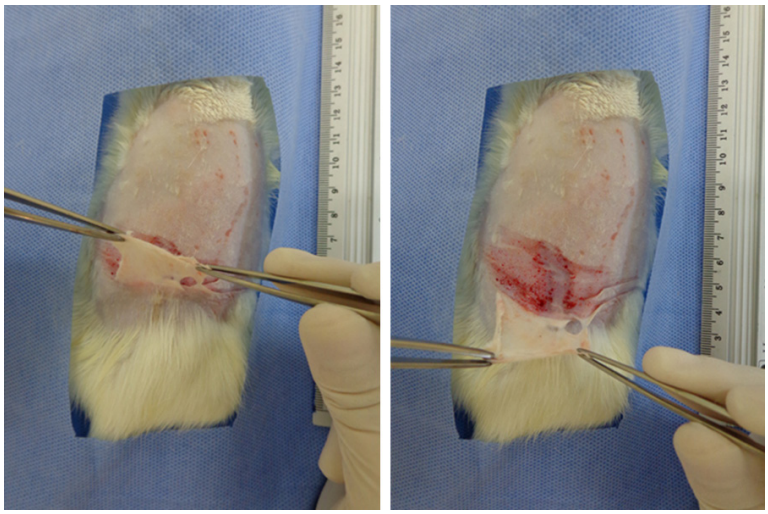

Figure 2. Obtaining the graft still fixed at its lower end.

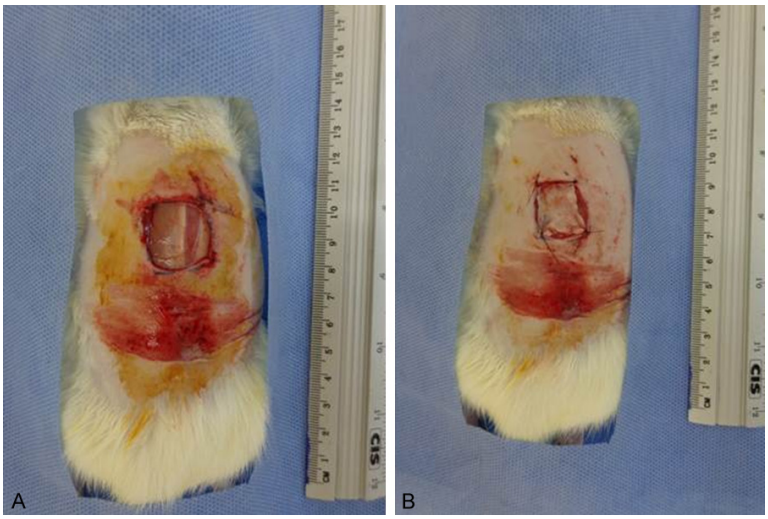

Figure 3. A. Defect of 1.5×1.5 cm created on the back of the animal. B. Control group: filling the defect with partial skin graft and sutures.

polymer, 50% w/v sucrose (Synth®) was added with a particle size between 250 and 500 µm. The solution was poured into a glass mold. **Figure 1** illustrates the process of obtaining the membrane.

After evaporation of the solvent and removal of sucrose in 1% poly vinyl alcohol, the membranes were dried and disinfected with 70% ethanol for later use.

Scanning electron microscopy (SEM)

The samples were fixed on a metallic support and coated with a thin layer of gold using a sputter sample BAL-TEC SCD 050. The materials were observed using SEM JEOL JXA 860A.

Surgical procedure

Forty Wistar rats of both sexes and approximately 3 months of age, weighing an average of 250 g from the animal house of the Health and Medical Sciences Faculty of Pontifical Catholic University of São Paulo (FCMS-PUC/SP) were used. The work was approved by the Ethics Committee on Animal Use of the School of Medical Sciences and the Pontifical Catholic University (PUC-CEUA-FCMS/SP) under No. 2013/01 Health protocol, which is in accordance with the ethical principles followingthe Brazilian Guidelines for the Care and Use of Animals for Sc-ientific Purposes and Teaching. The animals were divided into two groups: control and treated. After intramuscular gluteal anesthesia with a solution of ketamine hydrochloride (90 mg/kg) and xylazine (5 mg/kg), the backs of the animals were shaved near the cervical region. The skin and subcutaneous tissue of a regular quadrangular area of 1.5×1.5 cm was dissected, and the defect was filled with autologous skin obtained with a Blair knife (**Figures 2** and **3**) and secured with sutures. The control group received only the grafted skin and the treated group was used to study the membrane before performing the skin graft (**Figure 4**). To prevent possible dehydration due to the large area of the wound, a subcutaneous infiltration solution of sodium chloride at 0.9% capacity of 10 ml was used [28]. After the procedure, each animal received a three-layer curative with moistened gauze, cotton wool, and a covering of elastic tubular net (Poolfix®) to support and fix the curative (**Figure 5**). The animals remained in the vivarium, receiving commercial feed and water *ad libitum* with analgesics (Paracetamol 1 mg/mL in the drinking water) according to the

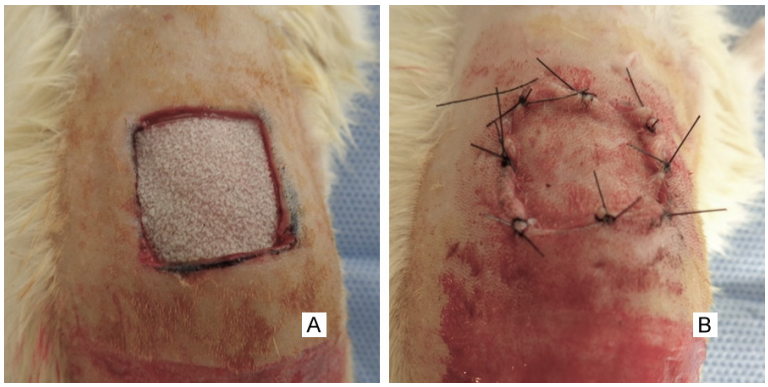

Figure 4. A. Filling the defect with the membrane. B. After skin grafting secured with sutures.

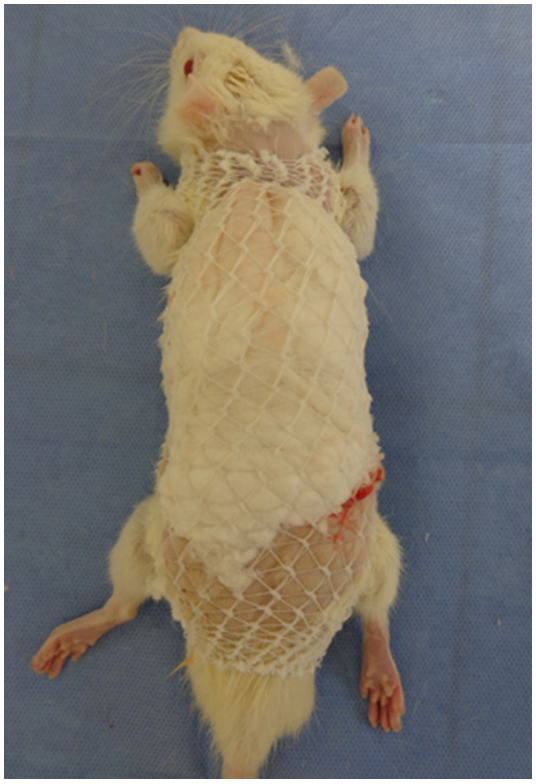

Figure 5. Animal in the immediate postoperative curative with three layers.

protocol established by SB Damy *et al* [29]. The animals were kept in individual cages at 22°C ± 2°C throughout the experimental period.

Ten animals of each group were sacrificed at 7 days or 60 days after the initial surgery using anesthesia with halothane inhalant anesthetic soaked cotton. After shaving the dorsal region, the sample was removed by biopsy from the central area of the operated region. The tissue fragments were fixed in 10% formalin for the histological procedures.

Material processing

The samples were prepared for histological analysis in accordance with techniques used for light microscopy using wax as a means of inclusion. Thus, the tissue sample was cut in half and the two halves processed as follows:

1) dehydration: 70% ethanol (24 h), 80% ethanol (30 min), 95% ethanol (30 min), and 100% ethanol (three times for 30 min); 2) clearing: 100% ethanol + xylene at a ratio of 1:1 (30 min), pure xylene (60 min); 3) coating: xylene + paraffin at a ratio of 1:1 (30 min in the oven), pure paraffin (two times for 90 min); 4) inclusion: ambient temperature in liquid paraffin.

Slide preparation

The cuts were made using a microtome with a thickness of 3 µm. The histological slides were placed in a greenhouse at 40°C to remove excess wax and were stained as follows:

1) dewaxing: xylene (two passes of 20 min); 2) hydration (in sequence): 100% ethanol (5 min), 95% ethanol (5 min), 80% ethanol (5 min), 70% ethanol (5 min), distilled water (multiple washings); 3) staining with hematoxylin-eosin.

For this technique, two dyes were used: hematoxylin, a basic dye that stains the anionic compounds (eg: nucleic acid) purplish-blue, and eosin, an acidic dye that stains the cationic compounds (most proteins, glycoproteins in the art dyes were used 2 acidic, etc.) reddish-pink.

Slides were stained first with hematoxylin (10 min) and then washed well with running water (5 min) and distilled water (5 min). Then, the slides were placed in 0.5% aqueous eosin (5-10 min) and, subsequently, washed quickly with distilled water.

4) dehydration: 95% ethanol (fast passage), 100% ethanol (5 passages of 3 min); 5) clearing: xylene (3 quick passes); 6) permanent assembly.

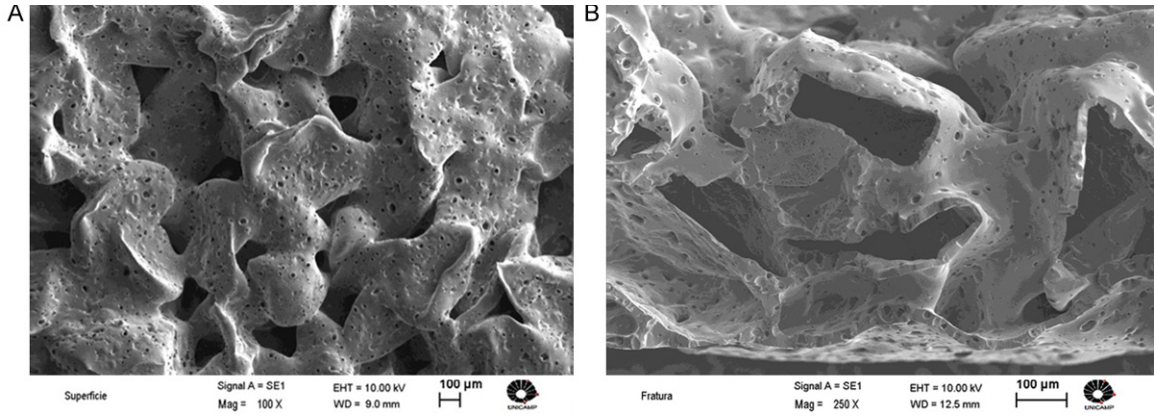

Figure 6. Electron micrographs obtained by SEM of the polymer membrane PL-co-co-TMC-DLA (50:50). A. Surface of the membrane. B. Fracture surface of the membrane.

The sections were fixed between the slide and coverslip using Entelan (Merck®).

Histopathology

Histological tests of sections of neotissue through a double-blind study. The integration between the device and the autograft were evaluated for the following features:

1) Characteristics of the acute inflammatory response, and chronic tissue necrosis; 2) Angiogenesis and collagen fibers; 3) Organization of tissue repair.

Histomorphometric evaluation

Images of the prepared sections were captured using a camera control unit attached to a NIKON (E800) Nikon TS-RI1 microscope. With the use of the objective and 2× aid of the Nis-Elements Advanced Research (version 3.0) software, three measurements (center and side edges) of the thickness (in micrometers) of the blades of the repair tissue in each animal were performed in the treated and control groups.

Analysis of collagen fibers

The analysis of type I and type III collagen fibers was performed from the observation of three random samples of the histological slides in the dermis region only. Samples were stained using the histochemical technique Tricrômio de Picrossírius and observed with the NIKON (E800) microscope and captured with Nikon TS-RI1 image capture, at 10×, 20×, and 40× magnification. Pixel variation was measured over a range of intensity that covered the peak of the histogram of the image. The Picrossírius trichrome method essentially consisted of the collagen protein staining. The thicker, strongly birefringent collagen fibers are stained in orange, yellow, and red tones and represent type I collagen, while the finer and sparse fibers, which are poorly birefringent, are stained in green, representing collagen type III.

Statistical analysis

The samples were compared using the Student's t-test, and the level of significance between the means of the parameter set was set to $\alpha=0.05$.

Results

Morphological analysis of the PL-co-DLA-co-TMC membrane

The morphology and distribution of the pores of the membranes were investigated by SEM. **Figure 6A** and **6B** represent the surface and fracture surface of the PL-co-DLA-co-TMC (50:50) membrane, respectively. Both surfaces have uneven and rough porous surfaces with pores distributed throughout the membrane. The distribution of heterogeneous pore size results from evaporating the solvent and leaching porogenous agent. The fracture surface reveals the presence of some interconnected pores, which is an important feature for the diffusion of fluids, cell migration, and guided tissue regeneration.

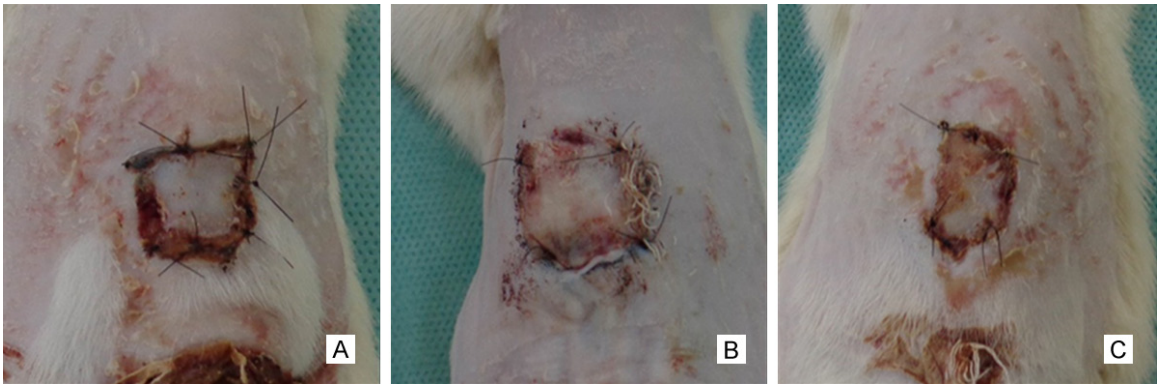

Figure 7. Control group animals sacrificed 7 days after the procedure. Note the heterogeneous graft integration between defects in animals.

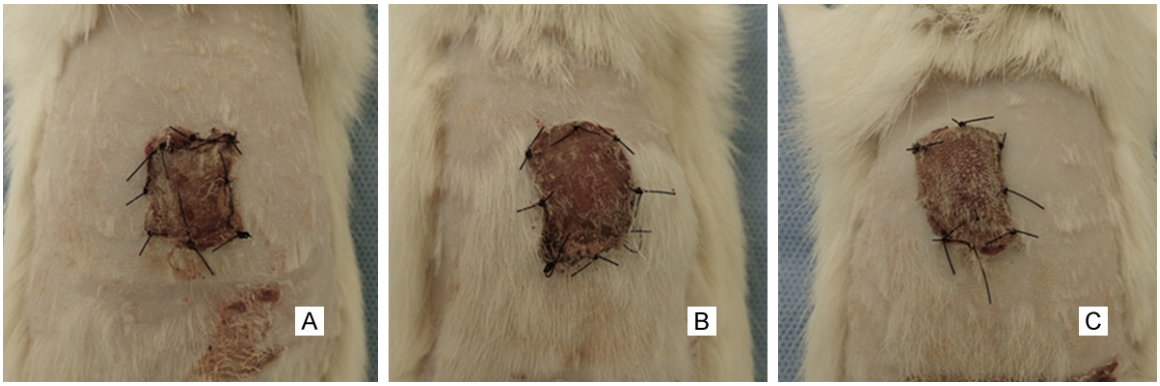

Figure 8. Treated animals sacrificed 7 days after the procedure. Note that the graft did not show signs of good integration compared to the control group.

In vivo analysis

Both the treated and control animals sacrificed 7 days after the procedure showed heterogeneity of the wound. The wound appeared healthier in the animals that received the integrated external curative (**Figure 7**). Compared to the control group, the skin grafts in the treated group appeared dehydrated, formed a thick crust, and showed the presence of serous exudate below the polymer membrane, which revealed signs of degradation (**Figure 8**).

Histologic examination revealed that the lesions of the control group presented with edges and remnants of inflammatory granulation tissue in the dermis. The young granulation tissue presented with mononuclear infiltrates, vessels, hemorrhage, and foreign body granulomas. Some sections showed hyperplasia of myofibroblasts around the deep vessel cells. Collagen was organized in parallel beams at the surface (**Figure 9**). In the treated group, histological sections revealed a similar appearance between all cases and presented with ischemic necrosis of grafted skin with no inflammatory infiltrate in the reticular dermis and epidermis. However, below this layer, the area corresponding to the polymer membrane, we observed dense acute inflammatory infiltration and did not detect polymeric fragments. In some segments, we did not observe the membrane, but the cuts in the membrane were in direct contact with viable tissue. Furthermore, the synthetic material appeared to be associated with an inflammatory reaction, creating a giant cell foreign body (**Figure 10**).

The transition area of the implanted skin into the normal skin was not observed macroscopically in control animals sacrificed 60 days after surgery, except for some areas with no hair follicles (**Figure 11**). Similarly, the treated group showed complete repair of wounds and, in

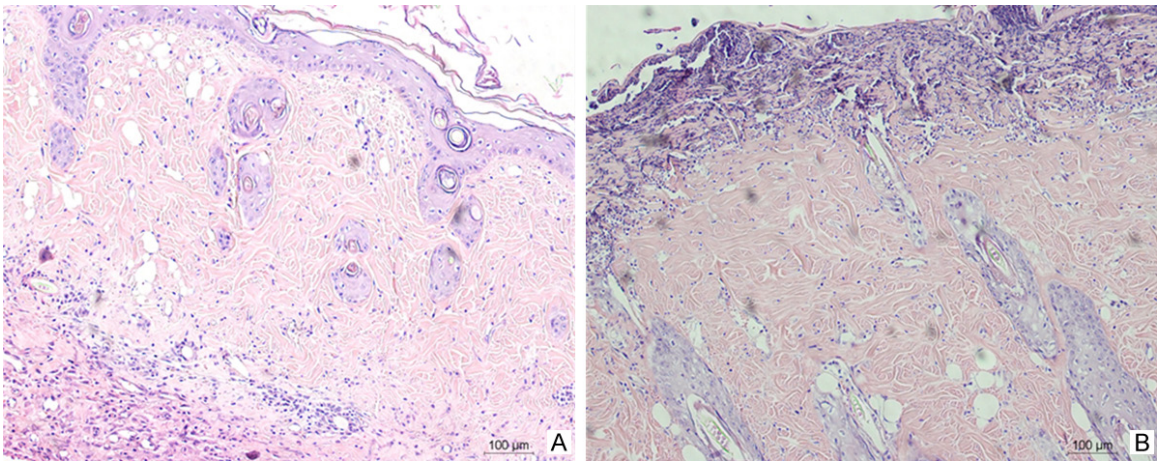

Figure 9. Histological analysis of the control group 7 days after surgery (10×). A. Note the presence of young collagen organized with delicate collagen bundles parallel to the epidermal surface vessels, fibroblasts, mast cells, and mononuclear infiltrate. B. Surface crust and acute inflammation in the area has not yet re-epithelized.

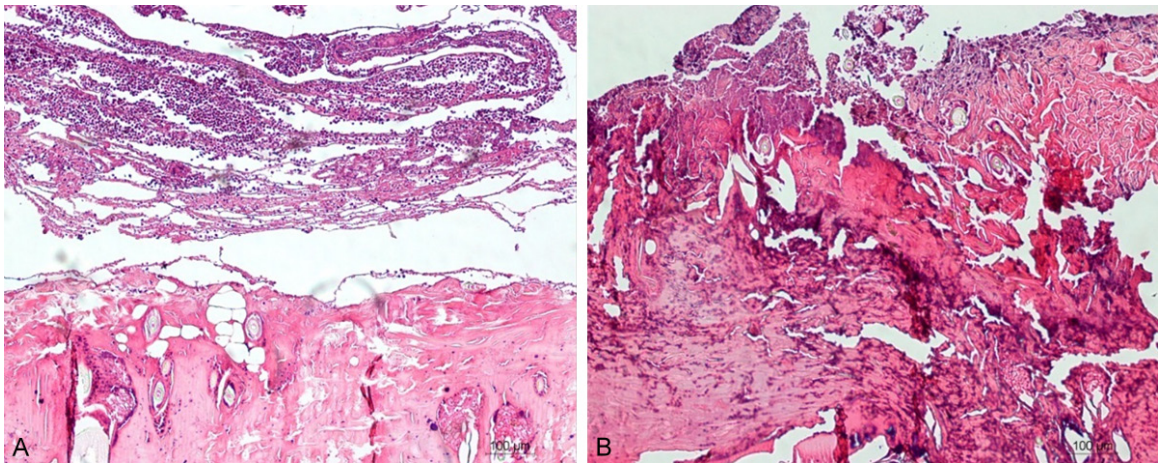

Figure 10. Histological analysis of the treatment groups 7 days after surgery (10×). A. Dense acute inflammatory infiltrate. B. Segment of skin showing ischemic necrosis.

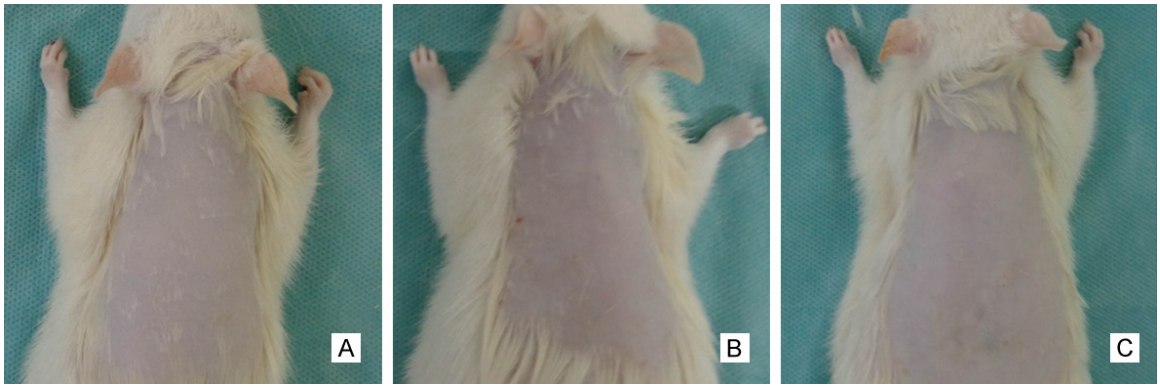

Figure 11. Animals in the control group sacrificed 60 days after the procedure. Note the homogeneous appearance of the skin between animals and integration of the graft to the recipient bed.

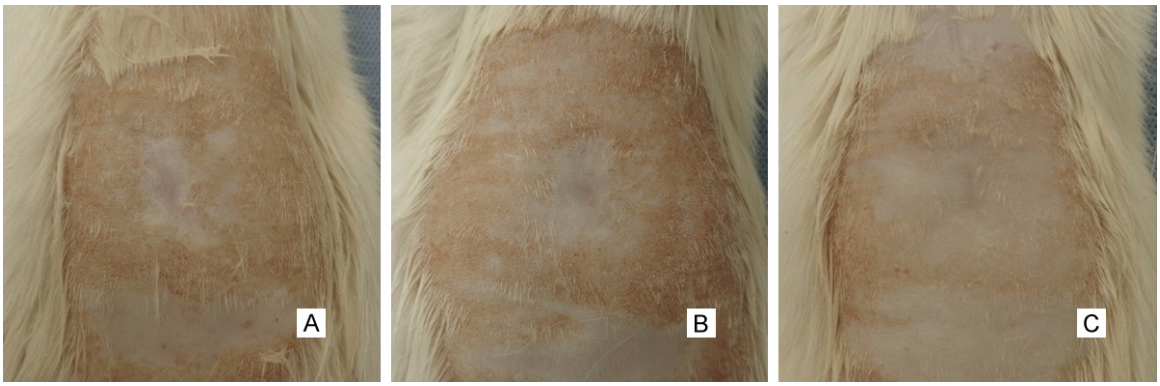

Figure 12. Treated animals 60 days after the procedure. There is delineation of neighboring grafted skin with good elasticity of the graft area.

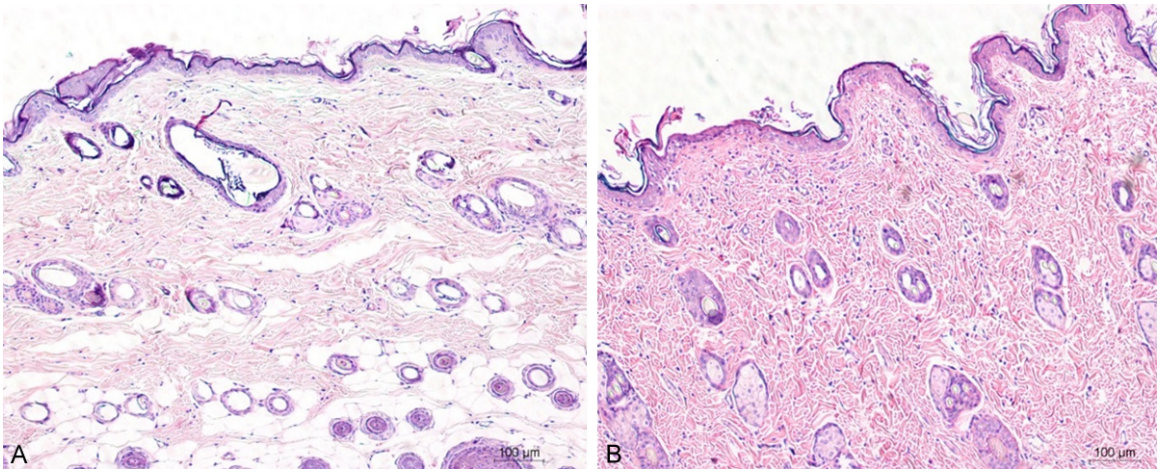

Figure 13. Histological analysis of the control group 60 days after surgery (10×). A. The skin graft is thinner in the surrounding dermis and panniculus carnosus in the absence of skin depth. B. The normal range of the grafted area.

some animals, a debris crust covering the epidermis. Notably, the skin graft site showed a similar elasticity to intact neighboring areas (**Figure 12**). There were no signs of the polymer after removal of material for histopathological study.

In the control group, total graft integration and the presence of some attachments were observed microscopically in the operated area. At the edges of the graft, scarce and small foreign-body granulomas were observed. The operated area, however, did not show the presence of the fleshy panniculus with muscular bundles compared to the surrounding skin. This finding was important because it allowed us to detect the grafted area (**Figure 13**). On the other hand, the animals that received a membrane near the graft had whole skin with the presence of dermal scars characterized by fibroblasts and elastic bundles parallel to the surface, with some irregular and atrophic appendages. In this group we observed a broad and thick dermal scar that suggested a slight retraction (**Figure 14**). We also noticed that the histological architecture of the skin of rats treated with a membrane was similar to the animal's healthy skin. It is evident that both the control and treated groups show an absence of a fleshy paniculus in the original areas of the grafts. This observation was useful for delineating repaired areas.

Thickness of dermis

Taken together, these results suggest that the regeneration of the polymer allowed for good dermis formation with less shrinkage, unlike what occurs in a scar by secondary intention. The polymer also provided greater thickness of the dermis in the control group, which was con-

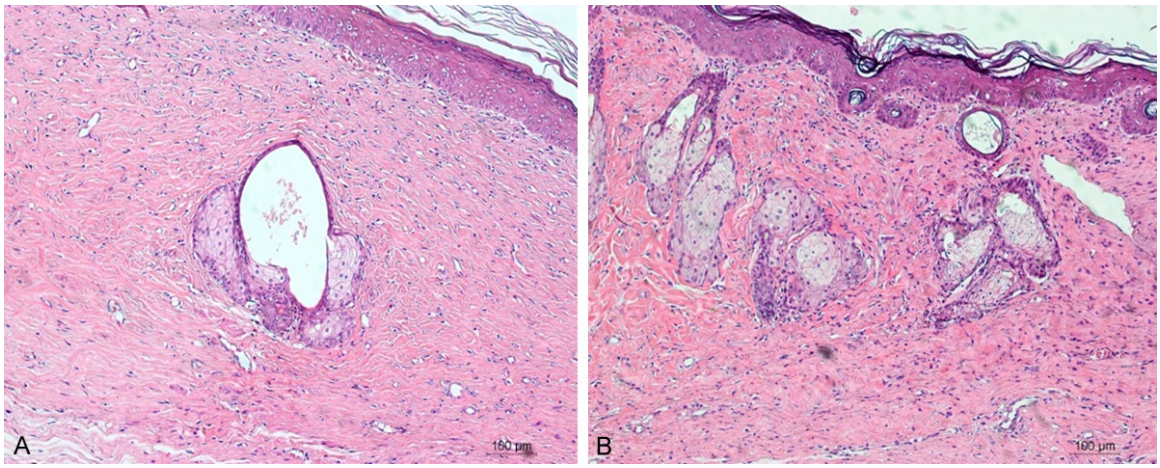

Figure 14. Histological analysis of the treatment group after 60 days of surgery (10×). A. Note the thick dermis tissue organization. B. Presence of skin scarring in the area of attachment.

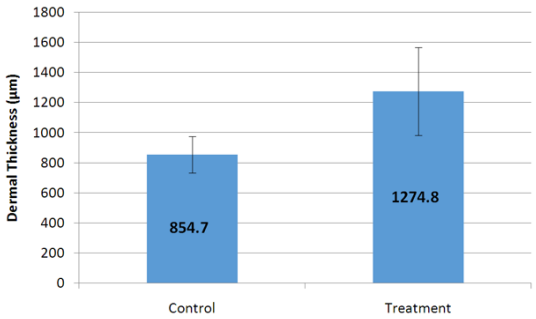

Figure 15. Graph illustrating the comparison of dermal thickness between the control group and the treated group. Values represent the mean ± standard deviation. The means were statistically different. ($P<0.05$).

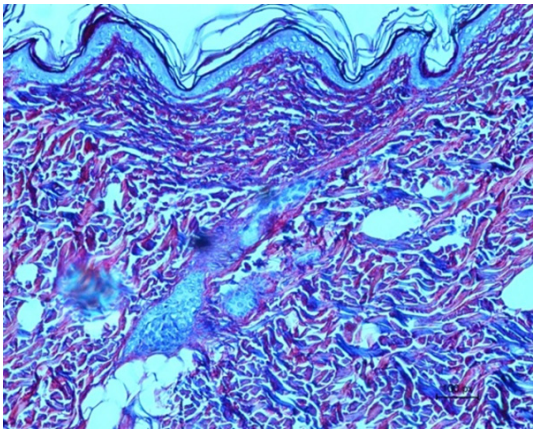

Figure 16. 20× magnification. Type I collagen demonstrating the appearance of normal skin.

firmed by histomorphometry (**Figure 15**). The skin graft, although devitalized, served as biological protection of the wound curative.

The microscopic appearance of the control group shows normal skin with distribution of short and crisscrossing beams of dermal collagen with no set pattern. The area of the scar cannot be recognized.

In the treated group, we observed an extensive scar area with thick collagen bundles parallel to the surface. The limits of the normal dermis scars were evident. We also observed that the blades on the scar dermis area are smaller than that in the normal area. Furthermore, there was delicate and thin fibrosis with irregular bundles near the hypodermis. This analysis showed that the control group had a higher amount of collagen fibers, but the fibers were thinner, whereas as the treated group showed a smaller number of collagen fibers that were thicker. The **Figure 21** shows the comparison of the collagen's area in both groups.

In summary, the material was biocompatible with the skin and had a smaller cicatricial retraction, the presence of collagen fibers, and greater thickness in relation to the control group, providing a scar of better quality. All these results suggest that the use of the studied material as a dermal substitute is favorable.

In the treated group, we observed an extensive scar area with thick collagen bundles parallel to the surface (**Figures 16**-**18**). The limits of the normal dermis scars were evident. We also observed that the blades on the scar dermis area are smaller than that in the normal area (**Figure 19**). Furthermore, there was delicate and thin fibrosis with irregular bundles near the hypodermis (**Figure 20**). This analysis showed

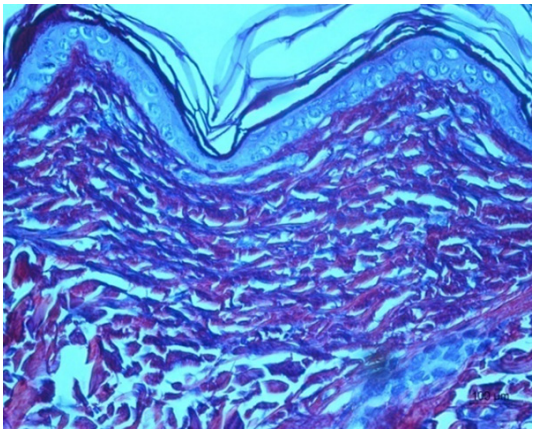

Figure 17. 40× magnification. The short and interwoven type 1 collagen bundles are highlighted.

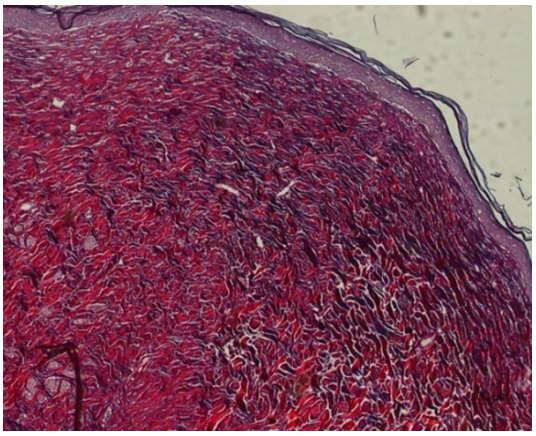

Figure 18. 10× magnification. Presence of collagen type 1 in parallel beams on the surface.

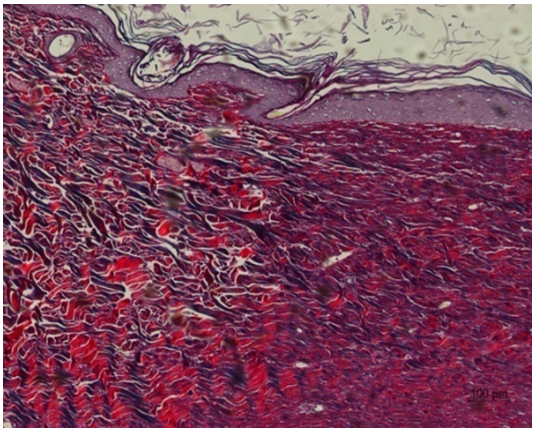

Figure 19. 10× magnification. Transition area between the dermis and scar region.

that the control group had a higher amount of collagen fibers, but the fibers were thinner,

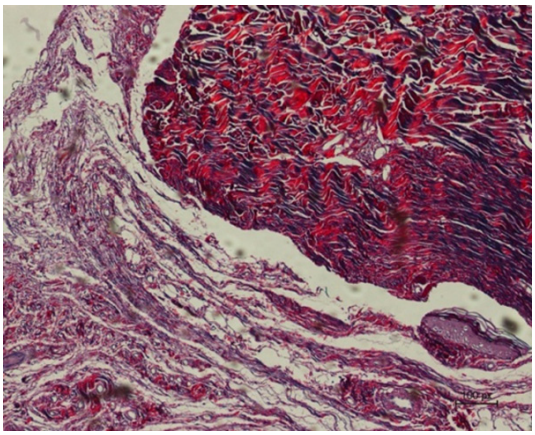

Figure 20. 10× magnification. It is possible to note the area of transition between the area of the scar and the normal dermis. There is fibrosis near the hypodermis.

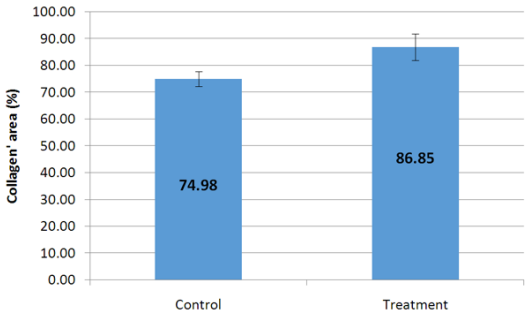

Figure 21. Graph illustrating the comparison of the collagen's area in the control group and the treated group. Values represent the mean ± standard deviation. The means were statistically different. ($P<0.05$).

whereas as the treated group showed a smaller number of collagen fibers that were thicker. The **Figure 21** shows the comparison of the collagen's area in both groups.

Discussion

Since the study of Burke & Yannas in 1981 [4], there have been many studies addressing similar devices that can promote better integration of the graft to the recipient bed, known as the dermal matrix. However, these studies differ in the composition of the graft, the length of surgery, and the ideal time of grafting. They promote similar effectiveness of the autograft meshed skin and are crucial for regaining lost barrier function, as well as reducing donor tissue thickness and promoting superior aesthetic results [30]. Many studies have used tissue engineering to treat skin defects to develop

materials with similar mechanical properties to native skin without triggering immune rejection or toxicity [31-33]. In this study, tissue engineering was used to produce a device that acts as a dermal substitute with similar results to existing biological materials.

An example is the dermal matrix obtained from the skin of human cadavers that are immunologically inert by the removal of cellular components. This is a costly process, which has shown good results in regards to wound contraction, but both the graft and matrix require revascularization and they occasionally end up acting as a barrier and preventing revascularization and graft survival [5]. The material analyzed in this study acted in a similar way. The porous membrane eventually acted as a barrier to the vascularization of the graft. The devitalized graft acted as a biological dressing and the membrane acted as a framework to accelerate the healing process of the wound. This result requires the adaptation of the membrane research methodology with the execution of cutaneous grafting in the second operative period.

In order to overcome the adverse effects of the matrix acting as a barrier to grafting, some materials are commercialized to be implanted in two separate surgeries. This was the case for the material developed by Burke & Yannas, which was a bilaminar dermal matrix obtained from bovine collagen. Although a two-surgery technique was originally described, positive results were reported for the application of this material in a single surgery in rats. This study showed that the mesh graft could be performed in a single surgical procedure and was superior in relation to the time of closure and wound contraction when compared to late grafting [34], a methodology that guided the design of this study.

To date, all dermal matrices available on the market are organic membranes (of biological origin) with similar composition and high costs of treatment [35]. However, the use of dermal matrices has been associated with shorter hospital stays for patients [36].

In this sense, bioresorbable biomaterials, which initially act as a functional bridge or mechanical reinforcement, also act as a biological scaffold for the host tissue after implantation. This increases the applicability of biomaterials and should increase research of biomaterials even further [37]. Biomaterials are capable of reproducing all the requirements for the development of the ideal scaffold intissue engineering. These same requirements are needed for making a dermal matrix [38].

In recent years the number of publications on the use of biomaterals as cutaneous substitutes has increased. Authors have associated these materials with the use of platelet-rich plasma [39], culture of keratinocytes [40], and deposition of stem cells [41], and sought to develop a substitute for the dermis [42], the hypodermis [43], and even total skin [44]. The in *vivo* and *in vitro* results addressing the biocompatibility and mechanical properties of the materials have been promising, and may be an excellent alternative to materials already available in the market. However, all the steps that are involved for development of biomaterials make the final cost high, and none of the studies evaluated the membrane alone without comparing it to another technology, as was done in our study. What motivated this study was the search for a material whose cost was significantly lower than those available in the market, so that it can be a viable alternative in countries with budget constraints.

It is already widely described in the literature that surface amplification through pores allows a greater number of cells per volume compared to commonly used implant types [22-24]. Thus, the histopathological results obtained are attributed to the technique of obtaining the membranes. As a result, the material did not act as a dermal replacement per se since it does not interfere with the total integration of the graft into the receptor bed.

However, the results demonstrate the biocompatibility of the biomaterial PL-co_DLA-co-TMC (50:50), which allowed regeneration of the upper dermis with less contraction and with a histological appearance similar to normal skin, except for the presence of appendages even with the use of the polymer. The material also did not trigger a foreign body-type reaction when implanted, which is different from what was observed with bioresorbable polymers in other studies. These findings favor the use of this material as a dermal substitute, independent of association with stem cells or culture of keratinocytes [35, 45].

This is the first study of the use of PL-co-DLA-co-TMC polymer (50:50) as a dermal substitute, demonstrating great potential for development as a dermal substitute. Future studies will aim to improve the methodology to demonstrate its clinical applicability.

Conclusion

The PL-co-DLA-co-TMC polymer (50:50) showed adequate biocompatibility and good response as a dermal substitute. The use of this biomaterial for skin grafting requires additional studies with improved methodology to demonstrate its efficacy.

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Disclosure of conflict of interest

None.

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