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IN VIVO EVALUATION OF A BILAYER SCAFFOLD FROM PLGA/ FIBRIN AND FIBRIN HYDROGEL FOR SKIN REGENERATION

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INTRODUCTION

Skin is the largest organ and its function is to protect the body from physical and chemical attacks (CAO et al., 2017). The incidence of skin wounds requiring clinical treatment represents a public health problem worldwide (VOS & ALLEN, 2016). Scientific literature shows various alternatives from the field of tissue engineering for contributing to the formation of a functional tissue. The present work aims to develop a bilayer scaffold of poly (lactide- co- glycolide) (PLGA)/fibrin electrospun membrane and fibrin hydrogel layer to be tested in vivo as skin substitutes. Fibroblasts were cultivated in the fibrin hydrogel and keratinocytes through electrospun to generate a skin substitute using an air/liquid system. Scaffolds were tested in a full-thickness wound model in 3 month old Wistar Kyoto rats (WKY). Three groups were analyzed macroscopically and microscopically: 1 (bilayer scaffold without cells), 2 (heterotypic skin substitutes), 3 (negative control). Partial results showed a scab formation at day 14 in all animals from groups 1, 2, and 3. No signs of wound infection were presented. On day 14, all wounds were re-epithelialized and granulation tissue was thicker in group 2. It could be concluded that the bilayer scaffold is thus a promising matrix to be used as a skin substitute. However, it will be necessary to complete the sample size for each group and realize histological and immunoenzymatic assays to better understand the tissue regeneration process.

THEORETICAL REFERENCE

Electrospinning is a promising method for the rapid and cost-effective production of nanofibers, with high surface area to volume ratios, having the potential for promoting scar- free wound healing (MULHOLLAND et al., 2020). This method allows for the incorporation of both synthetic and natural polymers to take advantage of their mechanical and biological properties.

Fibrin polymer has been widely used in tissue engineering due to its good biocompatibility and biodegradability, with the advantage of being autologously derived. In addition, this natural polymer is an important signaling molecule, which actively participates in wound healing (SHPICHKA et al., 2020).

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On the other hand, PLGA is a synthetic polymer with good mechanical properties, and spun membranes from this biomaterial have been demonstrated to provide good keratinocyte adhesion and viability, being a good option for epithelial support (AR et al., 2016) (BASTIDAS et al., 2020). Thus, in this study, the spun sheet of the bilayer scaffold will provide an adequate matrix for epithelial formation and the fibrin hydrogel layer will provide an appropriate matrix for dermis formation.

METHODOLOGY

All animal experiments were approved by the Ethical Committee from Universidade Federal do Rio Grande do Sul (number 36484).

Primary cultures of fibroblasts and keratinocytes, and blood samples were obtained from isogenic WKY rats. Fibrin was isolated from rat blood plasma, and for the bilayer scaffold fabrication, a PLGA/fibrin electrospun membrane was produced, as previously reported (BASTIDAS et al., 2020). The electrospinning was carried out with 1% fibrin dissolved in formic acid and 1,1,1,3,3,3-hexafluoro2-propanol at the proportion of 1:1. PLGA was used at a concentration of 40% and PEG at 3%. The electrospinning parameters were high-voltage of 18kV at the positive and 1kV at the negative, with a flow rate of 0.48ml/h and a tip-collector distance of 20cm. The scaffolds were sterilized with UV light for 1 hour at each site in a laminar flow hood.

A plastic ring with 1.2mm internal diameter was then covered with the membrane, and the membrane adhered to the ring edges with the same polymeric solution used for the production of the scaffold in order to avoid contraction of the spun membrane during cell culture. The membranes with the ring were placed in 24-well plates.

For group 1, blood plasma was added to the spun membrane and its coagulation was induced with CaCl2. For group 2, fibroblasts were mixed with the plasma to later induce coagulation. The fibroblasts remained in culture for 1 day, after which, the bilayer scaffold was rotated by placing the spun membrane on the top, where keratinocytes were seeded and cultivated for 14 days in a liquid-air interface.

The scaffolds were tested in a full-thickness wound model in 3 month old WKY rats. The animals were randomly divided into three groups: 1 (bilayer scaffold without cells), 2 (heterotypic skin

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substitutes), 3 (negative control). After an adequate degree of anesthesia was reached, a full-thickness 1,2 mm diameter excisional wound was produced in the animals' dorsum. In groups 1 and 2, the bilayer scaffold was sutured, and a transparent film (Suprasorb® F) was used to dress the wounds. In group 3, the wound without treatment was also covered with the transparent film dressing. For the positive control, samples of normal skin were collected from the same animals. At days 14 and 21 post-treatment, the wounds were photographed, and the animals euthanized.

For histopathological analysis, the wound tissue samples were collected and fixed with formaldehyde. The samples were embedded in paraffin and sectioned (5µm) for Mallory Trichrome staining. In the images taken from the tissue slides, the depth of the neo-epithelia and granulation tissue at three different spots (left, right, and the middle of the wound) were measured using ImageJ software to analyze epithelial thickness and granulation tissue thickness.

The data were expressed as the mean ± standard deviation of epithelial thickness and granulation tissue thickness. Statistical analyses were performed using the Bioestat 5.0 software using the ANOVA method, followed by Tukey; statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

The results showed a scab formation at day 14 in all the animals. No signs of wound infection were presented. Histopathological analysis showed re-epithelization in all the animal wounds at day 14. There was a significant difference at day 14 in epithelial thickness between group 1 and 3 ($p < 0.01$), while no significant difference was seen at day 21 between the groups when compared to the positive control. Additionally, granulation tissue at day 14 was markedly thicker ($p < 0.01$) in group 2 when compared with group 1 or group 3 (negative control), suggesting that the incorporation of cells contributed to the granulation tissue formation of proliferative phase, indicating higher cell proliferation and extracellular matrix deposition. Groups 1 and 2 presented cutaneous appendages at day 21, while no appendages were seen in group 3.

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FINAL CONSIDERATIONS

These partial results suggest that treatment could contribute to the early establishment of the skin barrier function, and the incorporation of cells into the bilayer scaffold could improve the healing process. These results are promising; however, it will be necessary to analyze a greater number of tissue sections as only one section per animal was analyzed until now. The next analyses to be carried out will be the collagen fibers accumulation level, the granulation tissue thickness, blood vessels density, inflammatory cell infiltration and laminin expression. Lipid peroxidation and immunoenzymatic assays for VEGF, EGF, TGFβ and IL-10 will be realized to better understand the tissue regeneration process.

Keywords: Scaffold, Tissue Engineering, Skin, Fibrin, PLGA.

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