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Development of a new nanofiber scaffold for use with stem cells in a third degree burn animal model

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ABSTRACT

The combination of mesenchymal stem cells (MSCs) and nanotechnology to promote tissue engineering presents a strategy for the creation of new substitutes for tissues. Aiming at the utilization of the scaffolds of poly-D,L-lactic acid (PDLLA) associated or not with *Spirulina* biomass (PDLLA/*Sp*) in skin wounds, MSCs were seeded onto nanofibers produced by electrospinning. These matrices were evaluated for morphology and fiber diameter by scanning electron microscopy and their interaction with the MSCs by confocal microscopy analysis. The biomaterials were implanted in mice with burn imitating skin defects for up to 7 days and five groups were studied for healing characteristics. The scaffolds demonstrated fibrous and porous structures and, when implanted in the animals, they tolerated mechanical stress for up to two weeks. Seven days after the induction of lesions, a similar presence of ulceration, inflammation and fibrosis among all the treatments was observed. No group showed signs of re-epithelization, keratinization or presence of hair follicles on the lesion site. In conclusion, although there was no microscopical difference among all the groups, it is possible that more prolonged analysis would show different results. Moreover, the macroscopic analysis of the groups with the scaffolds showed better cicatrization in comparison with the control group.

1. Introduction

The skin is the largest immunologically competent organ in the body. As it serves as a protective barrier of our body to the outside world, any injury caused to it needs to be quickly and efficiently repaired.

In burns, high temperatures dilate the blood vessels and the liquids therein contained come out, forming bubbles. These bubbles may result in wounds vulnerable to infections.

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The severity of the burn is defined by the extent of the damage occurred to the victim and deaths occur in 5%–7% of cases [1]. Therefore, the greater the depth of the lesion, the greater the effect on the healing process [2].

Skin grafts are commonly used for the treatment of burn victims. However, this type of treatment usually results in scar contraction and this effect is particularly problematic in children because of their body growth [3]. Furthermore, it is becoming more difficult due to the lack of suitable donors as well as low quality of skin. Thus, skin regeneration is an important field for tissue engineering [3,4]. Available treatments, especially in large burns and chronic wounds, are insufficient to prevent scar formation and promote healing of the patient [4,5].

The challenge for researchers is to combine new materials with living cells to produce skin equivalents that are durable and functional, allowing the integration and manipulation of the cell biology of host cells and the multiplicity of signals that control their behavior [6].

There is currently a variety of skin substitutes for clinical use and the materials commonly used as artificial extracellular matrices include those derived from natural sources (collagen and hyaluronic acid, for example) and those manufactured (such as poly-lactic acid and poly-glycolic acid) [6].

However, the vast majority of the available products employ animal sources as raw material for the production of these skin substitutes [6–8]. These substitutes are known as xenografts. Some problems in their use are the possibility of disease transmission and immune rejection [9]. However, there are no materials that completely mimic the anatomy, physiology, biological stability or esthetic nature of healthy skin. Some problems, such as poor vascularization, poor wound healing, the lack of differentiated components, among others, are frequently present in the available equivalents [6,7,10,11]. Thus, viable and safe alternatives are still needed.

The structure of these biomaterials or scaffolds is responsible for determining the transport of nutrients and metabolites and regulatory molecules from the cells to the extracellular environment and vice versa [12]. Therefore, new technologies are being developed to transform raw materials into molds which are porous and have complex internal architecture. Among them is electrospinning [13,14]. This technique works on the electrostatic principle, in which polymeric solutions produced with different solvents and polymers are processed. The fibers obtained range in diameter from a few micrometers to nanometers.

Due to the great need for skin substitutes capable of regenerating large skin loss as well as the lack of an ideal replacement, this scientific work aims to produce scaffolds of nanofibers. They must be able to serve as an adequate support for cell growth for the period of time required for the occurrence of skin tissue regeneration. In addition, it aims to incorporate the microalga *Spirulina* to poly-D,L-lactic acid (PDLLA) scaffolds. This microalga is accepted by the Food and Drug Administration (FDA) for use in humans and possess biological activities, such as antioxidant, anti-bacterial, antiviral, anticancer, anti-inflammatory, anti-allergic and antidiabetic and a plethora of beneficial functions. Antibacterial and anti-inflammatory properties are very important in implants in human bodies, mainly in burn patients when the body's external barrier has been completely lost and another source of protection is necessary.

Thus, the intention is to promote the cultivation of stem cells with these new biomaterials produced (patent pending) for testing in an animal model with skin injury, with the final aim of replacing skin, for example, in burn patients.

2. Materials and methods

2.1. Preparation of polymer solutions

The polymer solution consisting of PDLLA (mol wt 75,000–120,000) (Sigma–Aldrich®) was produced at a concentration of 7% (w/w) using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Sigma–Aldrich®). The solution of PDLLA, which was incorporated in the microalga Spirulina (Arthrospira) (PDLLA/Sp) was prepared at 8% (w/w) of PDLLA in HFIP, plus 2% of the biomass of Spirulina. The microalga was cultivated as standard protocol established by the group [15]. Both solutions were left 24 h under agitation at room temperature and kept refrigerated until their use.

2.2. Electrospinning

The construction of biomaterials was performed by the electrospinning method. The polymer solutions were placed between electrodes, which were connected to a high voltage. The voltage used for the solution of PDLLA was 20 kV, using an inner needle diameter of 0.45 mm and a flow rate of 1.88 mL/h. For the solution of PDLLA/*Sp*, the voltage was 15 kV, the inner needle diameter 0.60 mm with a flow rate of 2 mL/h. The distance between the needle and collector for both solutions was 15 cm. The nanofiber scaffolds were adhered onto coverslips with 2.0 cm diameter and were sterilized with UV light for 2 h on a 24-well plate in a laminar flow hood.

2.3. Analysis of morphology and fiber diameter

The morphology of the nanofiber matrices was evaluated by scanning electron microscopy (SEM) model JOEL – JSM 6060, with acceleration voltage of 10 kV. The diameters of the fibers were evaluated by reading 30 fibers in three different fields of the same sample, in triplicate, resulting in 270 fibers analyzed by ImageJ software.

2.4. Collection and cultivation of mesenchymal stem cells

The cells used for biological experiments were from mice C57/B16N. All the procedures were performed following authorization from the Ethics Committee (Number 14715, approved in 24th June, 2010). For the extraction of kidney cells, adult mice were anesthetized and put down by cervical dislocation. The kidneys were extracted, cut and then washed with Hank's solution and were then left in contact with collagenase type I (Gibco®) to release the cells for 60 min at 37 °C. After the required time, the cells were washed and centrifuged at 400 × *g* for 10 min and suspended in culture plates with 6 wells [16]. They were kept in a humidified incubator with 5% CO_2 at