Design of a novel electrospinning setup for the fabrication of biomimetic scaffolds for meniscus tissue engineering applications

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ABSTRACT

The tissue engineering field has provided great efforts towards the development of potential treatments for meniscal injuries. The success of these strategies are linked to the creation of scaffolds that are able to mimic the extracellular matrix architecture of the native meniscus. However, most conventional electrospinning setups can only produce either 2D aligned fibrous structures or 3D fibrous scaffolds with fibers randomly distributed. Herein, we designed a novel electrospinning setup, which consisted of two metallic devices as collectors: an external cylindrical hollow piece with a central pin and a mobile internal hollow cylinder. A feasible approach to create single-layer scaffolds with both circumferentially and radially aligned ultrathin fibers was developed. Then, this investigation demonstrated a great potential for the application of these scaffolds towards meniscus tissue engineering, once they are able to reproduce the orientation of the main collagen fibers present in the extracellular matrix of the knee meniscus.

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1. Introduction

The knee menisci are intra-articular fibrocartilaginous structures that play an essential role in the knee function [1]. Due to its unique complex structure, poor intrinsic repair and limited regenerative capacity, damages in meniscus are difficult to treat [1,2]. Unfortunately, meniscal injuries are very common and the therapeutic options available in practice clinical have shown controversial results. To date, these treatments are unable to provide long-term protection of cartilage articular and do not prevent early osteoarthritis [3–5].

Many approaches in tissue engineering have been reported in order to provide potential treatments for meniscal lesions [6,7]. However, the success of these strategies are linked to the development of appropriate biomaterial scaffolds. For instance, an ideal scaffold must be able to mimic the extracellular matrix architecture of the target native tissue, especially in the case of knee menisci, which are composed of highly aligned fibers and have anisotropic mechanical properties [8–11].

A trend in tissue engineering is the application of nanotechnology to fabricate scaffolds [12]. In this context, the electrospinning is an inexpensive, versatile and powerful tool to produce polymeric nano and ultrathin fibers as mimeticscaffolds to the extracellular matrix components [13]. Consequently, several authors have developed methods to control the alignment of electrospun nanofibers [8,14–17]. However, the creation of an electrospinning system capable of producing aligned nanofibers according to the complex extracellular matrix organization of the meniscus still remains a challenge.

Herein, our aim was to develop a novel electrospinning setup in order to fabricate single-layer scaffolds containing both circumferentially and radially aligned nanofibers. In this way, we would be able to reproduce the orientation of main collagen fibers present in the extracellular matrix of the knee meniscus.

2. Materials and methods

Our electrospinning setup consisted of a syringe pump to deliver the polymer solution, a high voltage power supply and a modified cylindrical fiber collector coupled to a motor. The designed fiber collector included two metallic devices: an external cylindrical hollow piece with a central pin and a mobile internal hollow cylinder. The setup is shown in more details in Fig. 1A. The useful area used to collect the fibers had compatible dimensions with the average size of the human medial meniscus [18].

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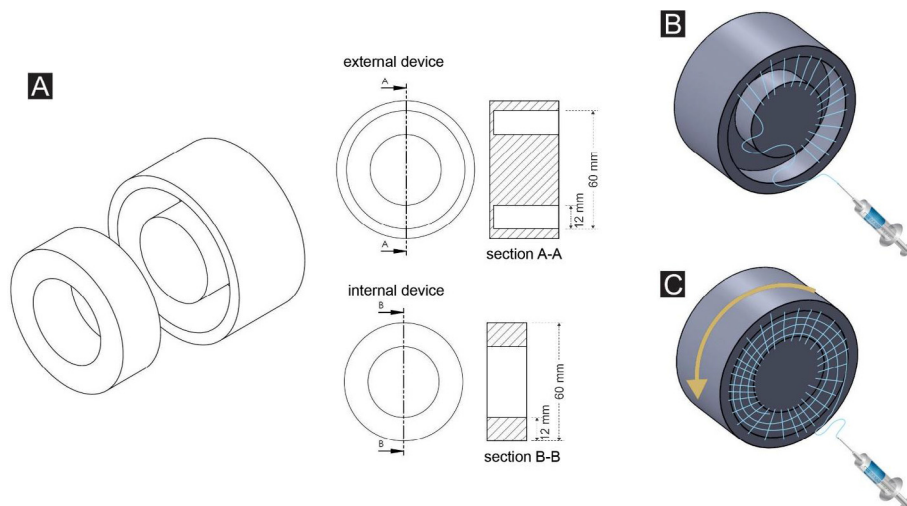 E-mail addresses: aolobo@pq.cnpq.br, lobo.aol@gmail.com (A.O. Lobo).

Fig. 1. (A) Illustration of the modified collector device for the novel electrospinning setup; Schematic diagram showing a simulation of the biomimetic scaffold production: (B) step 1 (RadAN) (B) and (C) step 2 (CircAN).

For the electrospinning experiments, we used a solution of polycaprolactone (PCL, MW 80 kDa, Sigma–Aldrich) at 12 wt% using chloroform (Sigma–Aldrich)/DMF (dimethylformamide, Sigma–Aldrich) as solvent system (3:1). The solution was driven through a glass syringe equipped with a metallic needle (19G) coupled to a syringe pump (KDS100, KD Scientific). The needle was linked to a high voltage power supply.

The construction of the scaffold is shown in the schematic diagram of Fig. 1B and C. In order to produce radially aligned nanofibers (RadAN), we used the grounded stationary collector without the internal device, in such a way to create a structure like a peripheral ring and a center point electrode (Fig. 1B). Next, the internal piece was used to form a solid cylinder as rotating fiber collector (angular velocity of 3000 rpm) to generate circumferentially aligned nanofibers (CircAN) on the radially aligned nanofibers (Fig. 1C). In this study, all electrospinning parameters were optimized as follows: for RadAN, 23 kV, 10 cm as needle-collector distance and flow rate of 0.4 mL h^{-1}; for CircAN, 20 kV, 14 cm as needle-collector distance and flow rate of 0.8 mL h^{-1}. For both experiments, we controlled the temperature (23–25 °C) and humidity (30–35%).

The fibers' morphology and diameter distribution were assessed by Scanning Electron Microscopy (SEM, EVO-MA10, Zeiss) after gold-sputtering onto the samples. SEM micrographs were analyzed using *ImageJ* software for determining the average fiber diameter [19].

Fast Fourier Transform (FFT) analysis was used to quantify the alignment of the ultrathin fibers, as described elsewhere [20,21].

For our analysis, CircAN and RadAN were singly collected on glass slides and analyzed in a bright-field light microscopic (DM2700 M, Leica). Digitized images were obtained from 5 different regions of both orientations, with a center distance of 24 mm and processed using *ImageJ* software supported by an Oval Profile plugin (authored by William O'Connell). Alignment plots were then generated and the position of the peak reported the principal angle of fibers' orientation. The FFT data were normalized to a baseline value of 0 and plotted in arbitrary units, allowing for a direct comparison between different data sets. Additionally, we evaluated the fiber coherency using the *OrientationJ*, which is an ImageJ plugin [22]. A Coherency coefficient closes to 1 represents a strongly coherent orientation of the fibers whereas coherency coefficient closes to 0 indicates no preferential orientation.

3. Results and discussion

Fig. 2A shows a SEM micrograph of the electrospun scaffold produced in our electrospinning setup. In general, we observed significant differences between the most superficial (RadAN) and the deepest fibers (CircAN). The average fiber diameters of RadAN and CircAN were 239 ± 32 nm and 485 ± 122 nm, respectively. Such differences may be attributed to the higher voltage and lower flow rate used for RadAN production. The overall morphology of the ultrathin fibers can be controlled by simply modifying electrospinning parameters such as applied voltage, solution flow rate and needle-collector distance [23,24]. This different morphology between electrospun ultrathin fibers was purposely chosen, since
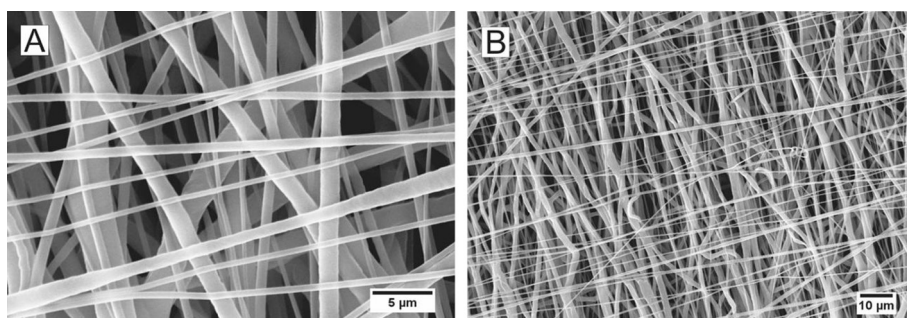

Fig. 2. SEM micrographs of PCL electrospun scaffolds at (A) high and (B) low magnifications.