Chondrocytes electrospraying: parameters optimization towards in situ cell laden scaffolds design

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ABSTRACT
Cartilage tissue engineering (TE) is offering suitable therapeutic solutions for articular cartilage degenerative diseases, such as osteoarthritis, a big problem in contemporary society. TE is able to combine biomaterials and cells to create biomimetic scaffolds, that can mimic the depth dependent nanostructural organization of the fibrous collagen network of the native articular cartilage. This work uses the cell electrospinning technique, a recently discovered technique that could combine the electrospinning of a polymer with the electrospinning/electrospraying of cells. The objective was to find the optimal operational parameters for cell electrospraying a concentrated cell suspension of chondrocytes, that could be then coupled with concurrent electrospinning of a biopolymer. The results showed to be promising, indicating that the chondrocyte cells were resisting the electrospraying conditions and that were able to be cultured after the process.

Key-words: Cartilage Tissue Engineering, Cell Electrospraying, Scaffolds, Electrospinning.

INTRODUCTION
Cartilage tissue engineering (TE) is offering suitable therapeutic solutions for articular cartilage degenerative diseases, such as osteoarthritis, a big problem in contemporary society. By using convenient fabrication techniques, cartilage TE can combine biomaterials and cells to create biomimetic scaffolds, that can mimic the depth dependent nanostructural organization of the fibrous collagen network of the native articular cartilage [1].
One of these techniques is electrospinning, a technology capable of producing fibrous scaffolds through the application of an electric field to a determined suspension [2]. These produced scaffolds could mimic natural biological environments on which cells can be seeded on [3]. Although being a very promising technique for TE, it has major setbacks, those being the limited infiltration of cells throughout the thickness of the scaffolds and the poor penetration and accessibility of nutrients for the cells inside the obtained scaffold [3].
In 2006, Jayasinghe et al., [4], pioneered with a new technique called Cell Electrospinning (CE). This new procedure proved possible the ability to produce scaffolds containing living organisms in its structure through a method of co-axial electrospinning, in which the cells were directly electrospun under stable threading conditions inside of a biopolymer that was electrospun at the same time [4].
CE, as well as bio-electrospraying, have the possibility of ejecting high cell densities to form a controlled and desired architecture and have even been proved capable of handling whole organisms (Danio rerio, Xenapus tropicalis, etc) [5]. These technologies showed the capacity of handling living organisms/cells without cell damage or death being caused, while cells maintained their functions post-electrospinning, and at the same time forming viable structures that could be used for TE and clinical applications [6, 7].
This study is a preliminary effort to find the optimal operational parameters for cell electrospraying a concentrated cell suspension of chondrocytes, that could be then coupled with concurrent electrospinning of a biopolymer. After cell electrospraying, the cells were seeded on polycrapolactone/gelatin/graphene oxide (PCL/gel/GO) microporous scaffolds to measure their viability. The intended final product is a scaffold with expanded and proliferated cells in its structure for use in cartilage TE.

MATERIALS AND METHODS
A chondrocyte cellular suspension (1.24x106 cells) with DMEM/F-12+1% penicillin/streptomycin + 10%FBS (FETAL BOVINE SERUM) was prepared for the experiments. The experiments consisted in testing several parameters in the cell electrospraying process in order to find the optimal ones for a good cell viability. Different needle sizes (0.36mm x 12mm and 0.4mm x 15mm), different voltages (range: 9-24 kV) for set working distances (5 cm; 10 cm; 12.5cm) and different flow rates (1.5 mL/h; 2 mL/h; 3.5 mL/h; 5

mL/h) for the electrosprayed cellular suspension were tested. For this, a NANON 01 electrospinning equipment and a syringe with a diameter of 13 mm were used. The collector was a petri dish (90x20 mm) containing 6 mL of medium equal to the one used in the cellular suspension. After cell electrospray, the samples were collected and centrifuged (200g, 5 min.) to reduce total volume for 1 mL (containing the electrosprayed cells) and were seeded on a 24-well plate.
The day after, cell viability was measured through the resazurin method to assess cell metabolic activity. The electrosprayed cells with the best cell viability values were chosen to be seeded into PCL/gel/GO microporous scaffolds, and their viability was measured throughout a period of 21 days in static conditions.

RESULTS AND DISCUSSION

After the process of cell electrospraying, cell viability was measured and the results obtained showed promising outcomes for the conditions used. Especially in the specific case of the experiment at which the following conditions were used: needle = 0.36mm x 12mm; flow rate = 2 mL/h; voltage = 17.5kV; working distance = 12.5 cm. Under these conditions a high cell viability was obtained. Apart from having high cell viability, the obtained conditions are compatible with the conditions used for a concurrent electrospinning of biopolymers, such as collagen [8, 9].
These electrosprayed cells showed a good viability, that trusted their use for seeding a PCL/gel/GO microporous scaffold. The viability results from the seeded scaffolds, were measured at day 1, 3, 7, 14 and 21 and showed a progressive increase in viability throughout the 21 days, revealing even higher viability when compared to the cells that were not electrosprayed. This shows that the electrosprayed cells successfully proliferated in the scaffolds which indicates that the chondrocyte cells were resisting the electrospraying conditions and were able to be cultured after this process.

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