BIO-ELECTROSPRAYING OF CHONDROCYTES FOR CARTILAGE TISSUE ENGINNERING APPLICATIONS

Ângela Semitela (1), Gonçalo Ramalho (1), Cátia Sousa (2), Soraia Silva (2), Paula A.A.P. Marques (1),
Alexandrina F. Mendes (2) and António Completo (1)

1. TEMA, University of Aveiro, Portugal; 2. CNC, University of Coimbra, Portugal

Introduction

Electrospinning has been widely employed to produce fibrous scaffolds for cartilage repair [1,2]. Despite the potential of the fibrous scaffolds for cartilage tissue repair, a significant limitation is their inherent small pore size, limiting cell infiltration, leading to inhomogeneities in tissue formation. To overcome this limitation, methodologies to increase their pore have been developed, however these manipulations generally degrade the mechanical properties the final threedimensional (3D) scaffolds [3,4]. So, a direct incorporation of cells into the fibres during electrospinning can be a promising approach to produce functional and homogeneous tissue constructs, as it overcomes the challenges of cell infiltration into small pore sizes by literally surrounding cells with the fibre matrix as it is produced. This can be achieved using bioelectrospraying (BES), a concept first introduced in 2005 by Jayasinghe, and it enables deposition of living cells onto specific targets by exposing the cell suspension to an external high intensity electric field [5,6]. Since cell exposure to the electric field, as well as the shear stress of passing through the BES apparatus may affect cell viability and function, the viability of post-electrosprayed cells was assessed for several cell types, and it was found that cell viability was mostly not significantly reduced by the process [7]. To our knowledge the electrospraying of chondrocytes has not vet been performed. So, in this work, chondrocytes were electrosprayed and their viability assessed afterwards to ensure that the BES process did not affected cell viability and function.

Methods

Several chondrocyte electrospraying experiments were performed by adjusting various process parameters, such as voltage (10-25 kV), flow rate (1.5-5 mL/h), working distance (5-12.5 cm) between the needle and the collector and needle gauge (27-28G). These post-electrosprayed cells will then be cultured for 24 hours and their viability assessed by measuring the cell metabolic activity using a resazurin assay.

Results

Post-electrosprayed chondrocytes possessed considerable viability, suggesting that a substantial number of cells survived to the electrospraying process. It should be noted that the percentage of viability was calculated as a ratio of the metabolic activity of the electrosprayed chondrocytes and the metabolic activity of chondrocytes that did not underwent any process. So,

it is possible that some chondrocytes may have been lost in the electrospraying chamber as a result of the high voltages used.

The different parameters employed did not generate significant differences in chondrocyte viability, however the high voltage applied using the 28G needle (24) led to a significant reduction of viable chondrocytes (figure 1).

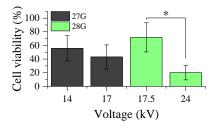


Figure 1: Influence of the applied voltage on chondrocyte viability. Statistical analysis by One-way ANOVA followed by post hoc Tukey's test; *p < 0.05.

Discussion

According to the results obtained, it is possible to infer that a considerable number of chondrocytes were able to survive to the BES process, regardless of the process parameters used, suggesting that this technique is a promising solution for cellular incorporation into the fibres during the electrospinning of 3D scaffolds. Voltages higher than 20 kV should be avoided in the future.

References

- McCullen et al, Tissue Eng. Part A, 18, 2073–2083, 2012
- 2. Steele et al, *Acta Biomater.*, **10**: 2065–2075, 2014.
- 3. Baker et al, *Biomaterials*, **29:** 2348–2358, 2008.
- Wang et al, *J. Biomed. Nanotechnol.*, 10: 1–11, 2014.
 Jayasinghe et al, *Biotechnol. J.*, 1: 86–94, 2006.
- 6. Jayasinghe et al, *Lab Chip*, **6**: 1086–1090, 2006.
- 7. Jayasinghe, *Adv. Biosyst.*, **1**: 1700067, 2017.

Acknowledgements

This work was supported by the Portuguese funding of Program COMPETE-FEDER, Programa Operacional Competitividade e Internacionalização through the projects POCI-01-0145-FEDER-016574, POCI-01-0145-FEDER-028424, and CENTRO-01-0145-FEDER-022083. Also, by Fundação para a Ciência e Tecnologia I.P. (FCT, IP) through the projects PTDC/EMSTEC/3263/2014, IF/00917/2013/CP1162/CT0016 and UID/EMS/00481/2013. The authors thank to FCT for the PhD grant SFRH/BD/133129/2017 and for the Investigator Program IF/00917/2013.

