Effects of a flow perfusion conditions on the viability of cells seeded on anisotropic scaffolds

Ângela Semitela, Gonçalo Ramalho, Paula A.A.P. Marques, António Completo
TEMA, Universidade de Aveiro, Portugal
angela.semitela@ua.pt, g.ramalho@ua.pt, paulam@ua.pt, completo@ua.pt

ABSTRACT
Articular cartilage is a highly organized tissue that it is adapted to the complex mechanical loading in joints. Given the limited self-healing abilities of this tissue, there is an increasing demand for tissue engineering approaches to develop successful cartilage replacements. However, it is difficult to mimic the biochemical and biomechanical microenvironment of the native tissue. Generally, tissue-engineered cartilage does not possess an anisotropic organization, particularly the collagen fibre alignment, which will induce a suitable cell response. The combination of electrospun scaffolds, cells and mechanical stimulation have been reported to develop tissue engineered cartilage with spatially-varying properties. Flow perfusion bioreactors have also been applied to enhance the formation and anisotropy of tissue engineered cartilage, as it imitates the physiological environment of the cartilaginous tissue. A series of anisotropic fibrous/porous electrospun scaffolds of polycaprolactone (PCL), gelatin, collagen and graphene oxide were developed, and their biocompatibility evaluated in static and perfused conditions. The results revealed that these scaffolds could not only allow cell adhesion, but also cell proliferation. The cell-seeded scaffolds subjected to flow perfusion displayed even higher cell viability, suggesting that the dynamic environment was beneficial to cell proliferation, and in the future, to the formation of tissue engineered cartilage.

Palavras-chave: Cartilage tissue engineering, Electrospun scaffolds, Anisotropic, Perfusion bioreactor

INTRODUCTION
Articular cartilage is a highly organized tissue that is adapted to the complex mechanical loading in joints. Though, this tissue doesn't own vascularization or neural networks able to participate on its regeneration [1]. Given these limitations, there is an increasing demand for tissue engineering (TE) approaches to develop successful cartilage replacements. However, it is difficult to mimic the microenvironment of the native tissue, and in the long-term, only tissue replacements with biochemical and biomechanical properties similar to those of the native tissue will resist the loading [1]. The majority of the studies dedicated to TE cartilage doesn't take in account the anisotropic organization of this tissue, particularly the collagen fiber alignment, progressing from parallel in the superficial zone, to random in the middle zone, and finally orientating perpendicular in the deep zone [2]. These topographic indications are essential to induce a suitable cell response because they influence the morphology and function of the chondrocytes and consequently the production and maintenance of the extracellular matrix (ECM) elements [3]. Thus, several factors should be taken in consideration in TE cartilage, namely the already mentioned scaffolds anisotropic organization and also dynamic mechanical environment to recreate the native one. Electrospinning has been extensively used to produce fibrous scaffolds able to resemble the structure of the native cartilaginous ECM [4,5]. Flow perfusion bioreactors have been used in TE: to increase mass transport through the interior of the scaffold, allowing efficient nutrient and oxygen supply and metabolic waste removal; and to apply mechanical stimulation, through shear stress, which will enhance cell proliferation and uniform distribution inside the fibrous scaffold [6,7].

MATERIALS AND METHODS
A series of anisotropic fibrous and porous electrospun scaffolds of polycaprolactone (PCL), gelatin, collagen and graphene oxide (GO) were developed and tested in vitro using a cartilage progenitor cell line, in static and dynamic conditions, which were provided by a flow perfusion system. First, the anisotropic fibrous layers of PCL and gelatin with depth-dependent variations in the fibrillar size and orientation were fabricated via electrospinning as described elsewhere [8]. Gelatin was added to improve the PCL surface hydrophobicity. A sacrificial agent (polyethylene glycol (PEG) particles) was introduced on the scaffolds to increase the inherent small pore size. The PEG particles, which were co-electrospun with PCL and gelatin, were then dissolved in water. These were then assembled and incorporated within a microporous GO-collagen network, already developed in the group [8]. Several anisotropic configurations were produced and characterized. The scaffolds were then sterilized, washed and slightly dried. A cartilage progenitor cell

line was seeded on the top and the bottom of the scaffolds, and their viability, morphology and migration were accessed after 3 weeks of culture under static and perfused conditions (3.75 mL/min).

RESULTS AND DISCUSSION

The results revealed that these new scaffolds could not only allow cell adhesion, but also cell proliferation, as cell viability increased through the culture period in static conditions. The phenomenon can be attributed to the following factors: the anisotropic configuration, which has been already reported as beneficial to cell proliferation [4]; and the gelatin addition and enlarged porosity [9,10], that improve the bio-inductive properties and pore size of the PCL electrospun anisotropic scaffolds previously developed by our group [8]. This trend suggests that these new scaffolds are a promising alternative solution for cartilage TE. The scaffolds were able to withstand flow perfusion without significant degradation, particularly on the fragile GO-collagen network. The dynamic environment seemed to be beneficial to cell proliferation and migration, as the cell-seeded scaffolds subjected to flow perfusion displayed higher cell viability and more uniform distribution on the inner of the scaffolds. As expected, flow perfusion increased mass transport through the scaffolds, allowing cells to migrate, which could be why in static conditions the cells did not migrate to the interior of the scaffolds [11]. The shear stress applied to the cells should be also a factor to take in consideration, and in the future, several flow rates should be investigated to further improve the beneficial influence of the flow perfusion on the formation of cartilage TE constructs.

CONCLUSIONS

The anisotropic scaffolds developed promoted cell attachment and proliferation, but flow perfusion conditions generated an even higher degree of cell proliferation, confirming that culture dynamic conditions provided by a perfusion bioreactor have a beneficial impact on cell behavior, and consequently, tissue formation.

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REFERENCES

[1] Sophia Fox, A. J., Bedi, A., & Rodeo, S. A. (2009). The basic science of articular cartilage: Structure, composition, and function. Sports Health, 1(6), 461–468.
[2] Johnstone, B., Alini, M., Cucchiarini, M., Dodge, G. R., Eglin, D., Guilak, F., Madry, H., et al. (2013). Tissue engineering for articular cartilage repair – the state of the art. European Cells and Materials, 25, 248–267.
[3] Lu, L., R., O. A., D., T. S., T., B. C., & J., K. D. (2017). Engineering zonal cartilaginous tissue by modulating oxygen levels and mechanical cues through the depth of infrapatellar fat pad stem cell laden hydrogels. Journal of Tissue Engineering and Regenerative Medicine, 11(9), 2613–2628. Wiley-Blackwell.
[4] McCullen, S. D., Autefage, H., Callanan, A., Gentleman, E., & Stevens, M. M. (2012). Anisotropic fibrous scaffolds for articular cartilage regeneration. Tissue Engineering Part A, 18(19–20), 2073–2083.
[5] Reboredo, J. W., Weigel, T., Steinert, A., Rackwitz, L., Rudert, M., & Walles, H. (2016). Investigation of Migration and Differentiation of Human Mesenchymal Stem Cells on Five-Layered Collagenous Electrospun Scaffold Mimicking Native Cartilage Structure. Advanced Healthcare Materials, 5(17), 2191–2198.
[6] Dahlin, R. L., Meretoja, V. V, Ni, M., Kasper, F. K., & Mikos, A. G. (2012). Design of a high-throughput flow perfusion bioreactor system for tissue engineering. Tissue Engineering Part C: Methods, 18(10), 817–820.
[7] Chen, G., Xu, R., Zhang, C., & Lv, Y. (2017). Responses of MSCs to 3D scaffold matrix mechanical properties under oscillatory perfusion culture. ACS Applied Materials and Interfaces, 9(2), 1207–1218.
[8] Girão, A. F., Semitela, Â., Ramalho, G., Completo, A., & Marques, P. A. A. P. (2018). Mimicking nature: Fabrication of 3D anisotropic electrospun polycaprolactone scaffolds for cartilage tissue engineering applications. Composites Part B: Engineering, 154, 99–107.
[9] Wang, K., Zhu, M., Li, T., Zheng, W., Li, L., Xu, M., Zhao, Q., et al. (2014). Improvement of cell infiltration in electrospun polycaprolactone scaffolds for the construction of vascular grafts. Journal of Biomedical Nanotechnology, 10(8), 1–11.
[10] He, X., Feng, B., Huang, C., Wang, H., Ge, Y., Hu, R., Yin, M., et al. (2015). Electrospun gelatin/polycaprolactone nanofibrous membranes combined with a coculture of bone marrow stromal cells and chondrocytes for cartilage engineering. International Journal of Nanomedicine, 10, 2089–2099.
[11] Ravichandran, A., Liu, Y., & Teoh, S. H. (2018). Review: bioreactor design towards generation of relevant engineered tissues: focus on clinical translation. Journal of Tissue Engineering and Regenerative Medicine, 12(1), e7–e22.