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Polycaprolactone hybrid scaffolds combining 3D printing and electrospinning to guide the osteogenic differentiation of MC3T3 preosteoblasts for bone tissue regeneration

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1. Introduction

Polycaprolactone (PCL), a synthetic linear hydrophobic polymer, has been widely used in tissue engineering over the last decade. This FDA approved polymer has been specially applied in the field of bone tissue engineering since it presents interesting features including a high biocompatibility, chemical stability and adequate mechanical properties [1,2].

Different methods can be employed for the fabrication of PCL-based scaffolds for bone tissue regeneration. Among them, electrospinning is a versatile technique that generates a nanofibrous network that resembles the natural extracellular matrix (ECM), thus enhancing cell adhesion and promoting a correct cell function. On the other hand, 3D printed PCL scaffolds have demonstrated to enable cellular migration, vessel formation and in growth of tissue due to its architecture, which resembles the mechanical features of bone tissue [1,3].

Taking advantage of the unique properties of PCL and the important advantages of both scaffold-fabrication techniques above mentioned, in the present work, we developed PCL hybrid scaffolds, comprised of a bioprinted PCL layer covered by electrospun nanofibers of such polymer. Obtained results showed the potential of this platform to drive the differentiation of MC3T3-E1 murine proteosteoblast towards bone tissue.

2. Materials and methods

The hybrid PCL scaffolds were fabricated using domoBIO 2A bioprinter Domotek equipped with an electrospinning module and a filament extruder. The two technologies work in sequential mode. Resulting scaffolds had a diameter of 21 mm and a height of 1 mm. Biocompatibility studies were performed in L929 fibroblasts following ISO guidelines (ISO 10993-5:2009) for in vitro cytotoxic tests [4]. MC3T3-E1 murine preosteoblasts were cultured following cell culture standard conditions. For all experiments, cells were used at passage 4-7. Metabolic activity was assessed by means of the Cell Counting Kit-

8 (CCK-8) and early osteogenic features were measured following the intracellular alkaline phosphatase (ALP) test.

3. Results and Discussion

Hybrid PCL scaffolds demonstrated good biocompatibility, scoring values above 70 % of the positive controls in both direct and indirect cytotoxicity assays (Fig. 2A-B). Furthermore, MC3T3 preosteoblasts were cultured for 21 days on the hybrid PCL scaffolds, showing an increase in proliferation over time (Fig. 3A-B). Moreover, MC3T3-E1 cells cultured in the hybrid PCL scaffolds showed a significantly increased activity of intracellular ALP —a marker of early osteogenic differentiation— as compared to the same cells cultured onto 2D plates (Fig. 3C).

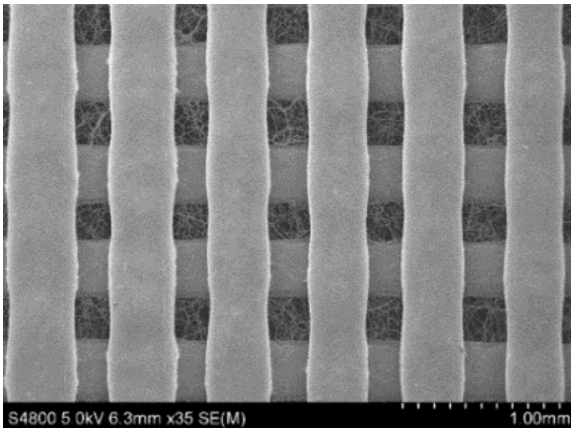

Fig1. SEM image of PCL hybrid scaffold

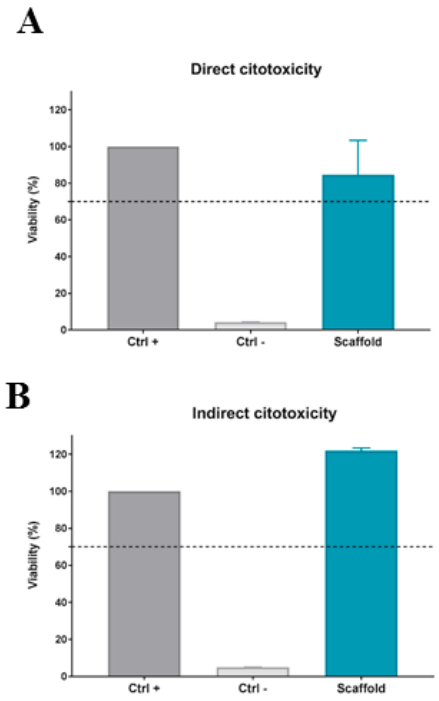

Fig.2. Biocompatibility of hybrid PCL scaffolds. (A) Direct and (B) indirect cytotoxicity evaluation of PCL scaffolds according to ISO 109

4. Conclusions

This new hybrid PCL scaffold was demonstrated biocompatible and enabled the culture and proliferation of MC3T3 preosteoblasts, while promoted their ALP activity. These results indicate the scaffolds developed here as a potential platform to guide MC3T3 preosteoblast through bone tissue differentiation

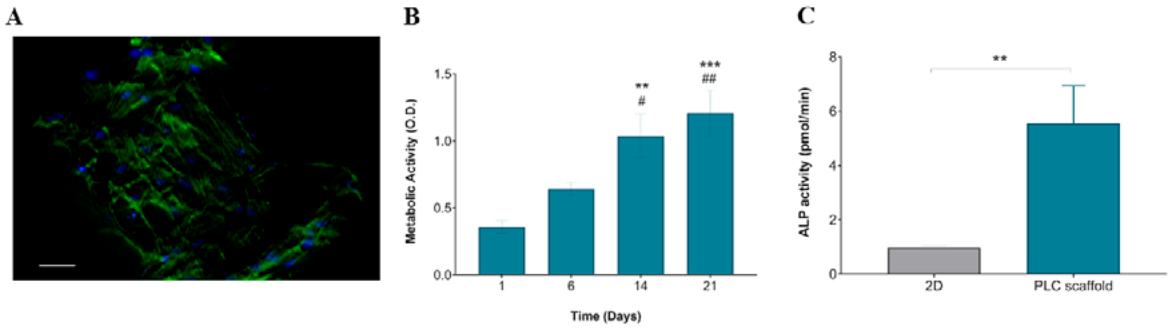

Fig 3. MC3T3-E1 murine preosteoblast culture in hybrid PCL scaffolds. (A-B) Hybrid PCL scaffolds enabled MC3T3 culture and proliferation, with the subsequent increase in metabolic activity over-time (C). Intracellular ALP activity of MC 3T3-E1 on tissue culture plates (2D) or after 21 days on PCL scaffold

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