Femtosecond laser ablation of 3D-printed PCL Scaffolds as a strategy to enhance bone tissue regeneration efficacy

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Abstract. New photonic techniques need to be developed to improve personalised medicine methods in tissue engineering. In the case of severe bone injuries, difficulties arise when creating platforms where cells required to be efficiently adhered. Femtosecond laser ablation appears as a versatile technique for modifying the surface of materials with high precision and neat outcomes. Thus, a strategy combining 3D printing of biopolymeric scaffolds and femtosecond laser ablation is proposed to design a device with enhanced material properties in terms of cell growth for bone tissue regeneration. Three different patterns were proposed, and it was proven that cell adhesion improvements rely on the pattern profile, assessing that grooved scaffold successfully increased cell adhesion and proliferation in comparison with micropitted samples.

1 Introduction

Laser processing has proven its versatility in the microtexturing of materials of different nature, size, and geometry, being employed in a wide range of fields. Specifically, it finds a promising potential in the medical field, in particular in tissue engineering. Among them, bones are one of the organs that benefit most from this technology, especially in the case of severe fractures, as they lose their capacity for self-regeneration.

To overcome these limitations, biopolymers are proposed as an effective solution for bone regeneration. These can be implanted in the form of scaffolds, 3 dimensional (3D) shape-variable structures which allow to attach the stem cells from the patient to their surface and to be incorporated among the affected bones, acting as a cell regeneration-inducing bridge in the affected areas. However, there is a growing interest in developing mechanisms to increase cell adhesion on these biopolymers, thereby accelerating the regeneration process.

For this purpose, the femtosecond (fs) Pulsed Laser Ablation (PLA) technique is proposed. Provided its short pulse time, high-precision micromachining with minimal thermal effects is possible [1] in contrast to longer temporal regime technologies such as picosecond or nanosecond ablation [2]. This is of great importance in the case of polymers, given their ease to melt and redeposit the material [3], which may lead to poor ablation outcomes.

In this work a combination of photonic technologies with tissue engineering is proposed in two steps: first the design and 3D printing of biopolymers and second the modification of their morphology by femtosecond laser ablation. It will be checked whether preosteoblasts cell adhesion can be enhanced by means of the micropattern on the scaffold surface.

2 Materials and Methods

The layer-by-layer microtextured scaffold manufacture integrates two combined processes. On one hand, a 3D-Bioplotter System (EnvisionTEC, Gladbeck, Germany) allows printing the layers from the Scaffold via Fused Deposition Modelling (FDM). For this purpose, polycaprolactone (PCL) was heated above its melting temperature (121 ºC) and a pressure of 6 bar was applied to deposit 400 µm width PCL fibres to build one layer.

The second process in the scaffold micropatterning comprises fs PLA. This technique was performed with the STELA laser of the Laser Laboratory for Acceleration and Applications (L2A2), a research facility of the University of Santiago de Compostela. Its pulses offer a power of 1 mJ, with a wavelength of 800 nm, a bandwidth of 75 nm, a pulse duration of 35 fs and a repetition rate of 1kHz. Once the layer was printed, the laser was focused on the

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surface of its fibres employing a Mitutoyo M Plan APO NIR 20x objective, as depicted in Fig. 1.

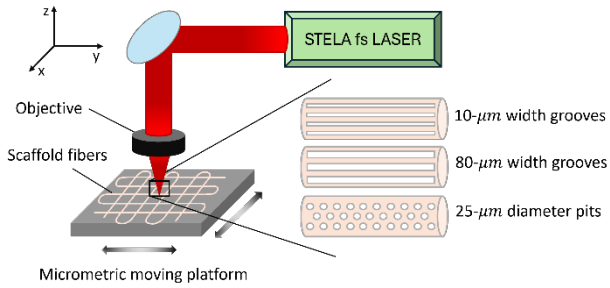

Fig. 1. PLA microtexturing principle and patterns studied

Finally, to assess the efficacy of the microtextured scaffolds, an in vitro stem cell culture was performed. Murine stem cells were attached onto the surface of stylised scaffolds under dynamic conditions. After that, scaffolds were placed in cell wells, where a differentiation assay of murine stem cells into osteoblasts was performed and the cell density and proliferation were analysed by fluorescence confocal microscopy.

3 Results and Discussion

Three different ablation patterns were studied: 25$-\mu m$ pits (Fig. 2b), 10$-\mu m$ grooves (Fig. 2c) and 80$-\mu m$ grooves (Fig. 2d)

3.1 Optical characterization

A morphological characterization of the ablated scaffolds was performed employing different optical techniques. SEM (Fig. 2) enabled to check the ablation outcome on the different patterns. In all cases, a clean texturization is achieved, showcasing the lack of thermal defects and stress provided by femtosecond laser technologies.

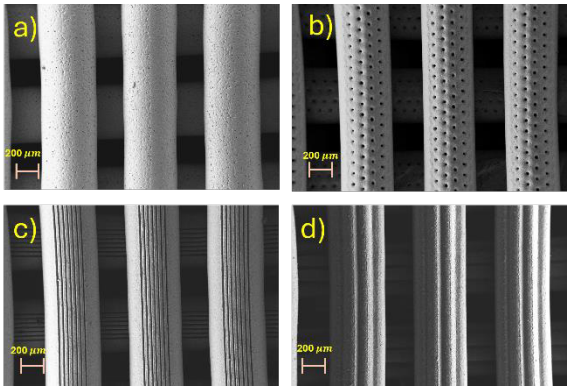

Fig. 2. Scanning electron microscopy (SEM) of scaffolds with a) no texturing, b) pits, c) 10-μm grooves and d) 80-μm grooves

3.2 Stem cell differentiation assay

Fig. 3 shows the fluorescent confocal images for the different micropatterns. Compared to the plain (control) scaffolds (Fig. 3a), texturized scaffolds showed an enhancement of cell adhesion. In particular, grooved scaffolds (Fig. 3c,d) led to a substantial improvement in cell attachment, as noticed in their major fluorescent signal. The scaffolds texturized with micropits (Fig. 3b) offered a noticeable adhesion of cells on the pits.

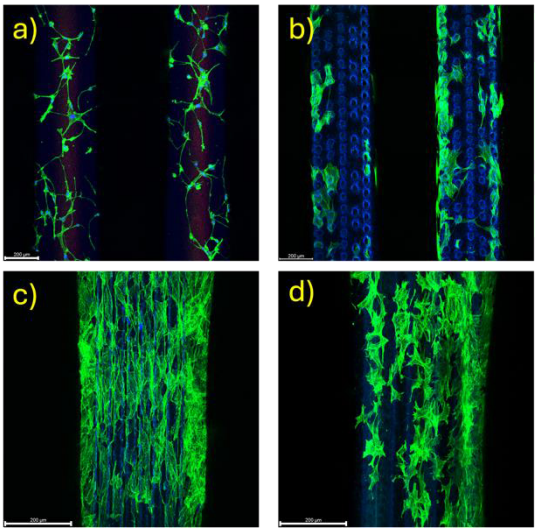

Fig. 3. Differentiation cell assay on a) plain, b) pits, c) 10$-\mu m$ and d) 80$-\mu m$ grooved scaffolds

4 Conclusions

The enhancement in cell adhesion and proliferation for bone tissue regeneration is clear when a printed scaffold is texturized on its surface by femtosecond laser ablation. In the case of grooves, it is worth mentioning the orientation change of the preostheoblastic cells in the direction where the ablation occurred, leading to a noticeable cell density enhancement. On the other hand, micropitted scaffolds led to results no much better than the control case, provided that cells show a tendency to scarcely adhere to the pits. These results showcase the dependence of cell adhesion and proliferation on the structure of the ablated micropattern.

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