Scientific Report for Short Term Scientific Mission

COST Action MP 1301

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Micropatterning and Characterization of 3D PCL Scaffolds by Femtosecond Laser Ablation for Tissue Engineering Applications

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BACKGROUND

Three – dimensional (3D) scaffolds fabricated from polymers are used to provide structural support and guidance of cells for tissue regeneration. The created 3D templates mimic the mechanical and biological properties of native extra cellular matrix. The scaffolds have to possess specific properties for enhancing the cell adhesion and proliferation. Generally, the treatment of bone fractures using 3D scaffolds which are osteoconductive and osteoinductive are able to facilitate the osteogenesis process.
Many synthetic and natural polymers are successfully applied as major ingredient for 3D scaffolds fabrication by different methods. One of the main techniques for creation of nanofiber meshes is the electrospinning technique, where the cells tend to adhere mostly on the fibrous surface which is a serious limitation of the method. Many research groups have focused to fabricate 3D structures with smaller diameters abilities to extend their surface area to improve the cell infiltration control [1, 2]. The major limitation of the implementation of 3D PCL scaffolds in regenerative medicine is associated mainly with the problems related to vascularisation of the artificial constructs in volume. The additional laser assisted treatment of surface by inducing additional surface modification could provide enhanced cell infiltration.
Currently, 3D – PCL scaffolds are used for producing artificial structures for hard – tissue replacements material due to its non–toxic nature and good biodegradability. The PCL material is bioresorbable and suitable for bone and cartilage engineering. It is posses' hydrophobic properties. Thus, makes the incorporation of such matrices unsuitable for *in vitro* cell seeding without additional treatment of the surface which can improve significantlly their cytocompatibility
Three – dimensional poly (ε - caprolactone) (PCL) scaffolds as suitable biocompatible material for manufacturing tissue replacements will be designed for tissue engineering purposes. The porous structures will be fabricated by rapid prototyping method based on hypodermic dispensing process. Creation of surface micro, nano- modifications will demonstrate the possibility of the fs laser assisted method to induce additional surface scaffold treatment to improve implant adhesion characteristics. The created 3D PCL matrices with modified surfaces will be tested for improving the cell adhesion and infiltration in volume.

Description of the work performed during the STSM

1. Creation of 3D PCL constructs by Fused Deposition Modelling (FDM)

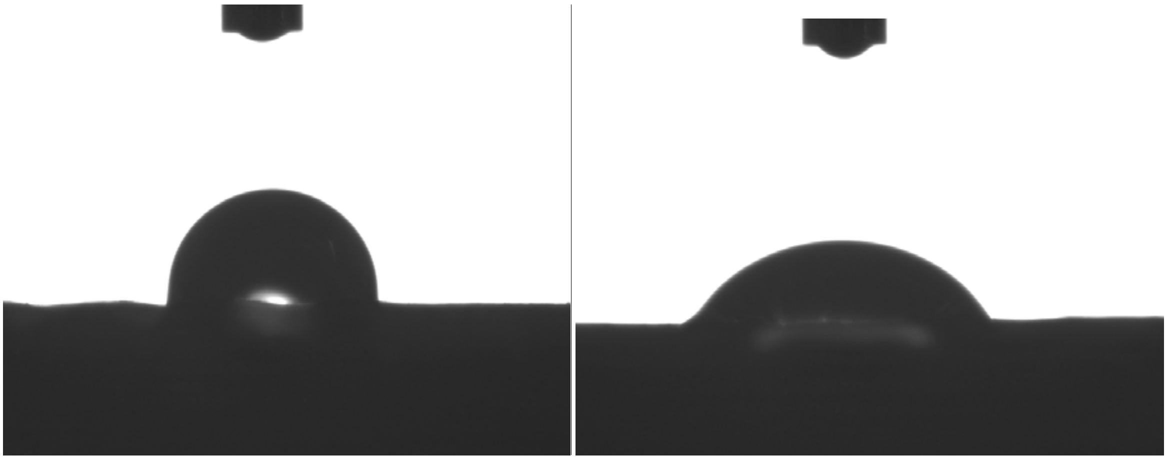
Despite the high level of porosity and high specific surface area of the fiber meshes, the pore size is usually too narrow for some applications in tissue engineering, where cell migration into the inner regions of the fiber-mesh scaffolds is aimed. In this study, we fabricated polycaprolactone scaffolds with interconnecting pores using a 3-D melt plotting system. Scaffold matrices 5x5x3mm from Poly (ε-caprolactone) (average molecular weight (~Mw) 45,000) have been fabricated via rapid prototyping technique.

2. Laser treatment performed at home institution

PCL and chitosan samples were irradiated by Ti:sapphire fs laser in air at central wavelength 800nm, with pulse durations in the range of 150 fs, max. output power 1mJ, repetition rate 25 or 50 Hz, and variation in number of pulses.

2. Examination of hydrophilicity and hydrophobicity of the surface before and after fs laser treatment.

In surface science, an instrument generally called a contact angle goniometer is used to measure the static contact angle. The current generation of contact angle instruments uses cameras and software to capture and analyse the drop shape and are better suited for dynamic and advanced studies. Generally, if the water contact angle is smaller than 90°, the solid surface is considered hydrophilic and if the water contact angle is larger than 90°, the solid surface is considered hydrophobic. Many polymers exhibit hydrophobic surfaces.
For our measurements' a 1μl distilled, deionised water droplet was gently positioned on the surface using a microsyringe and images were captured to measure the angle formed at the liquid–solid interface. It is evident that the fs laser treatment induces change in the surface properties of the films.



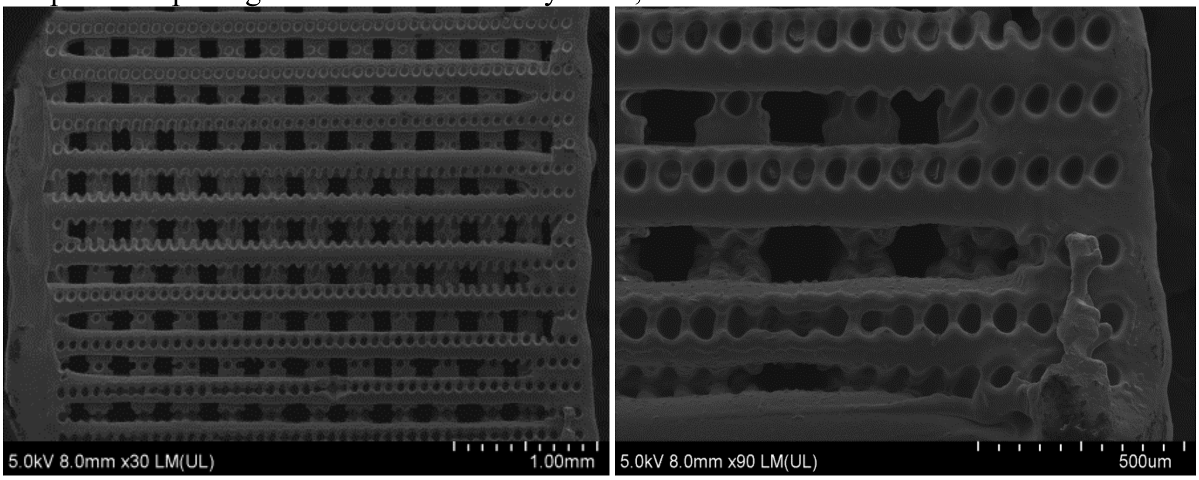
a) b)

Fig. 1. Photographs of water droplets on the: a) laser non-modified and b) laser modidied PCL sample surfaces

A treatment of PCL samples with fs laser turns a hydrophobic surface to a hydrophilic one. It was monitored a decrease in the contact angles from 96,7º to 59,2º.

3. Morphological characterization by SEM of laser modified and non-modified PCL matrices

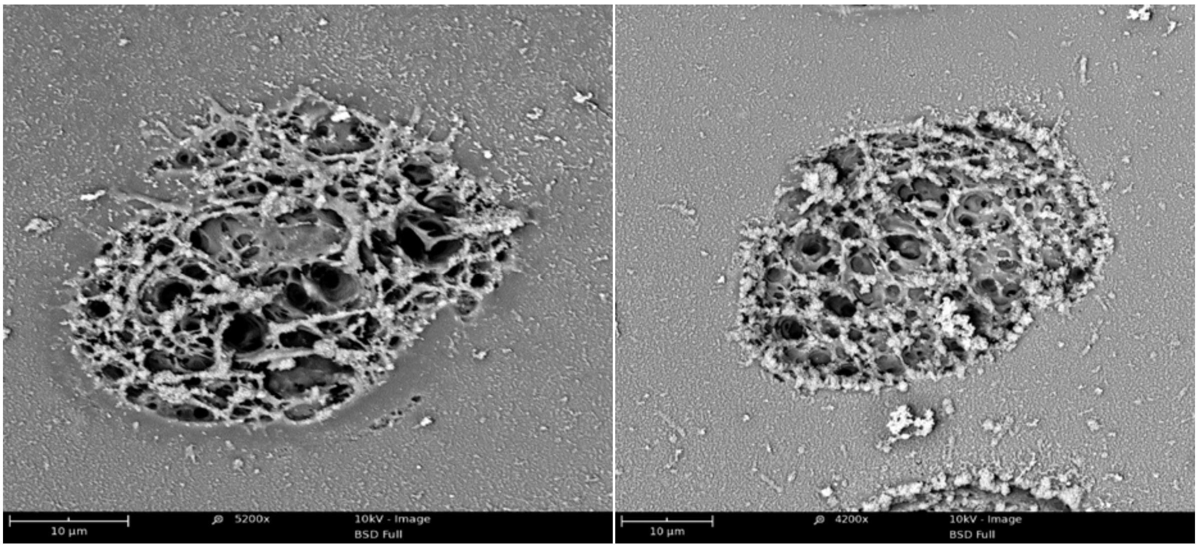
Morphological analysis of rapid prototyped PCL scaffold was done by a scanning electron microscope. Characterization of the properties of 3D scaffolds before and after fs laser treatment could contribute the optimization of the processing parameters and to perform a complete morphological characterization by SEM, of ultra-short laser created modifications.



a) b)

Fig. 2. a) General view of PCL sample after laser irradiation and b) with magnification

4. Morhological examination of fs laser processed chitosan thin film.



a) b)

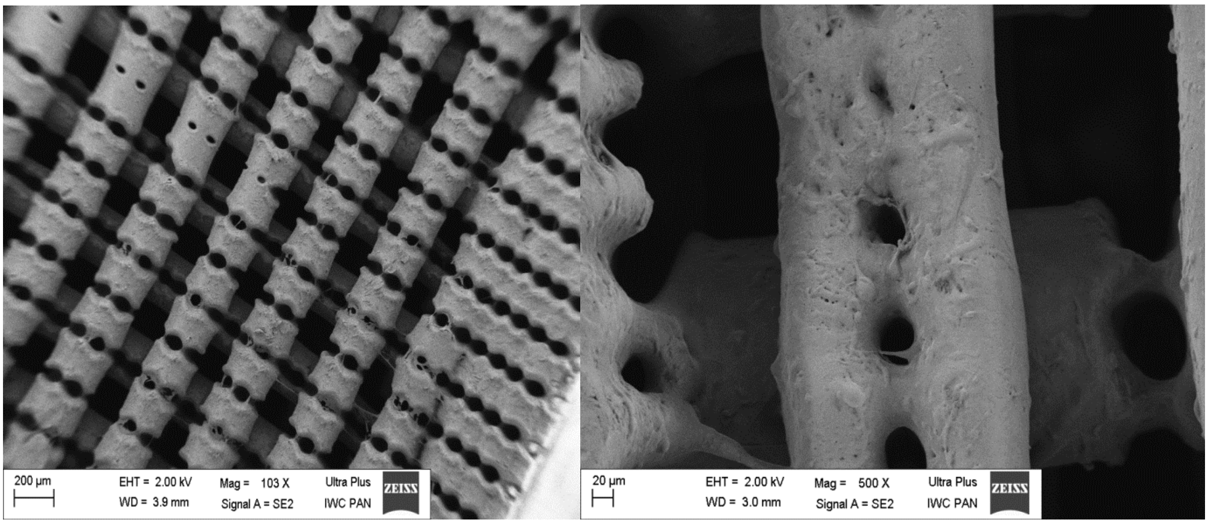
Fig. 3. Chitosan sample treated with different number of pulses, 150 fs, output power 0,1mJ, repetition rate 50 Hz: a) 1 pulse and b) 2 pulses

5. Cultivation of MG63 cell line for monitoring cell proliferation, adhesion, and differentiation on laser processed.

MG63 cell's proliferation was monitored after 7 days of cultivation period using scanning electron microscope. Creation of surface micro, nano-modifications demonstrated the possibility of the fs laser assisted method to induce additional surface scaffold treatment to improve implant adhesion characteristics. We found that cells tend to orient, attached, and grow along the laser modified surface. Fs laser induced modification of biocompatible materials exhibit direct control over MG63 cell's behaviour.

6. Morphological characterization by SEM of cell seeded laser modified PCL matrices

The topographical properties affect the cells interaction process. The main factors of cell-behaviour which are mainly influenced by the nanostructured surface are: cell's orientation, morphology and disposition. The MG63 cytoskeletons can be seen aligning between the modified PCL samples, Fig. 4.



a) b)

Fig. 4. SEM pictures of MG63 cells cultured for 7 days on the laser processed PCL sample, a) general view, b) single line from the sample

It is observed cells stretching between separate spots and in to them, also see Fig. 4b. The cell cultivation experiments indicate that cell orientation is significantly influenced by the surface topography of the samples, where cells were attached. It was observed cell selective orientation and elongation between the laser spots due to initial contact guidance.

References:

[1] Guimaraes A, Martins A, D Pinho E, Faria S, L Reis R, M Neves N 2010 *Nanomed.* **5(4)** 539–554

[2] Rebollar E, Cordero D, Martins A, Chiussia S, L. Reis R, M. Neves N and Leóna B 2011 *Appl. Surf. Sci.* **257** 4091–4095