Femtosecond laser surface texturing of 3D poly-ε-caprolactone matrices for bone tissue engineering applications

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Femtosecond laser surface texturing of 3D poly-ε-caprolactone matrices for bone tissue engineering applications

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Abstract. Fibrous 3D matrices were fabricated from poly-ε-caprolactone (PCL) by fused deposition modeling. Femtosecond laser irradiation was then used to demonstrate the possibility to affect the porosity of the 3D PCL fiber meshes. The surface characteristics were analyzed by scanning electron microscopy (SEM) and confocal microscopy. The interrelationship was examined between the laser processing parameters (number of pulses, pulse energy applied) and the response of the biomaterial. The formation was demonstrated of well-defined micropores, while the original fiber structure was retained. The study of cells cultivation on the laser-modified scaffolds showed good adhesion compared to a non-modified scaffold. The results obtained showed that femtosecond laser processing can be used as an alternative non-contact tool in enhancing the porosity of artificial constructs, thus influencing the cell adhesion into fibrous meshes.

1. Introduction

The deterioration of tissues or organs is a serious medical problem in trauma and orthopedic surgery, which is yet to be solved satisfactorily. Bone regeneration is proving invaluable in several applications and has given rise to hope in situations that had thus far been deemed beyond salvation, such as repair or replacement of irreversibly damaged and fully differentiated bone defects resulting from trauma, inflammation or tumor resection [1]. The limitations of the established techniques, such as distraction osteogenesis and implantation of autografts or allografts, involve problems with risk of infection, immune reaction and pain [1,2]. To address these challenges, diverse tissue engineering approaches have been attempted aimed to develop functional three-dimensional (3D) tissues matrices. In this respect, the scientific field of tissue engineering has emerged as an important technique for bone regeneration; it is based on forming a temporary porous matrix to guide the regeneration of the tissue

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into the desired three-dimensional shape. The extra-cellular matrix (ECM) is used as a micro-environment onto which cells can adhere and proliferate. In the field of regenerative medicine, a basic task is to design new biomaterials and methods for controlling their interface characteristics for guided tissue regeneration [3].

Synthetic matrices have evolved to become viable alternative materials for bone reconstruction. An ideal bone substitute should possess certain properties, including porosity, interconnectivity of the scaffold, osteoconductivity, biodegradability, as well as adequate mechanical properties; all this limits the particular material's ability to stimulate cell growth and generate new tissue [1,4]. Over the past years, a wide range of innovative synthetic materials have been developed to overcome the problems associated with autologous bone grafts. Newly developed implant materials are being studied as alternatives, including bioceramics, such as calcium phosphates (CaPs) and bioactive glasses, polymers (natural − collagen-I and synthetic − poly-caprolactone) and hybrid materials (a mixture of bioceramics and polymers), partly in combination with growth factors, bone marrow or mesenchymal stem cells [5,6]. A scaffold made of ceramic, such as calcium phosphate and calcium sulfate, is the most commonly used material for bone regeneration due to its bioactive properties. However, its brittleness and fast resorption rate pose clinical concerns. Biodegradable polymers are another promising type of potential bone graft substitutes. One such suitable candidate, namely poly-ε-caprolactone (PCL), is a biodegradable polymer, approved by the U.S. Food and Drug Administration, that has a low degradation rate compared with poly (lactic-co-glycolic acid) (PLGA) and polylactic acid (PLA) [7-9].

A current challenge in this field has to do with creation of 'smart' biomimetic, synthetic or natural materials (polymers, ceramics, and metals) by means of various chemical or physical techniques with the purpose of inducing enhanced surface functionalization by way of tailored topographic surface modification. Applying PCL scaffolds in bone regenerative medicine is mainly limited by problems related to volume vascularization of the artificial constructs. An additional laser-assisted treatment leading to surface modification by inducing surface roughness could provide enhanced cell infiltration [5,10]. Laser tailoring of the surface topography by modifying the respective material's surface offers advantages over both the chemical and physical methods. Moreover, the laser-assisted technique enables precise modification of certain surfaces that are difficult to treat with conventional chemical methods. Among its advantages is also the fact is that the modified surfaces are free from contaminants [11].

This article discusses the laser induced post-modification of poly-ε-caprolactone (PCL) scaffolds surface as a potential bone graft substitute in bone defects. The scaffolds design and fabrication, its surface modification and the influence on the cellular activities were evaluated by scanning electron microscopy (SEM), confocal microscopy and fluorescence microscopy measurements.

2. Material and methods

2.1. Fabrication and processing of 3D – PCL scaffolds

Three-dimensional (3D) scaffold constructs (5×5 mm in width and 3 mm in height) from poly-ε-caprolactone (PCL), (Sigma-Aldrich, St. Louis, MO) with a molecular weight (Mn) of 45 kDa, melt index of 0.95 g/ 10 min at 95 °C (ISO 1133), melting temperature of 57 °C, and a glass-transition temperature of −60 °C were designed using Solid Works 3D CAD Design Software. The geometrical model was fabricated by a Bioscaffolder machine (SYSENG, Germany) as an STL file. The internal architecture of the tested scaffolds was then designed with lay-down patterns 0/90/180.

To create the wood-pile structures, the PCL granules were heated to $T = 70$ °C. Once the material melted, air at a pressure of 5 bar was applied to the reservoir in order to transfer the molten polymer for extrusion. The layer-by-layer plotted fibrous scaffolds were characterized by theoretical parameters, namely, spacing between fibers in the same layer (140 μm) and layer thickness (130 μm).

The femtosecond laser modification experiments were carried out by a Ti:Sapphire laser (Quantronix-Integra-C) delivering 30-fs pulses at a central wavelength $\lambda = 800$ nm and tunable

repetition rate. The number of laser pulses applied (N) was controlled by a computer-driven fast mechanical shutter synchronized by the controlling software. The experiments were performed in air with the laser beam focused to a focal spot with a diameter of 50 µm using a lens of focal length 20 cm at normal incidence, figure 1.

The focusing lens was placed on a translation stage equipped with a micrometer screw for fine adjustment of the focus position on the specimen's working surface. The sample was positioned perpendicular to the focusing beam on a high-precision XYZ translation stage. The experimental setup was controlled via specially written LabView software. The effect of the laser processing parameters on the created surface microstructures was followed by scanning electron microscopy (Microscope FEI, Quanta 200F) and confocal microscopy- µsurf explorer (Nanofocus). We thus examined the laser-induced modification effect on the adhesion of an MG63 osteoblast-like cell line. The cell culturing was initiated by seeding MG63 osteoblast-like cells at a density of 5×10^5 cells/cm^2. The cell viability was monitored after three days of culturing in Dulbecco's Modified Eagle Medium (DMEM). The imaging of the cells was achieved by adding Rhodamine Phalloidin conjugated staining to visualize the actin cytoskeleton, and DAPI (blue) to visualize the cell nuclei.

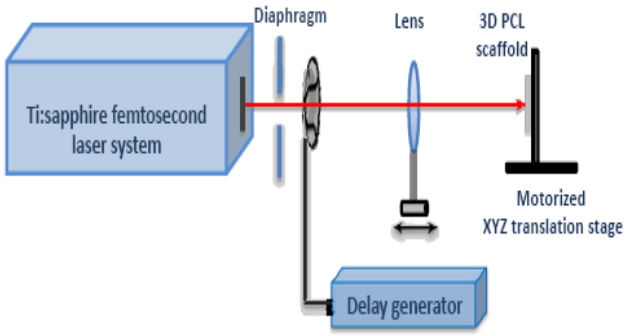

Figure 1. Experimental setup for femtosecond laser irradiation of PCL scaffold.

3. Results and discussion

3.1. Femtosecond laser ablation and characterization of modified fibrous scaffolds

We varied the laser pulse energy and effective pulse number and followed the effect on the size and structure of the modified zone of the PCL scaffolds. To study the effect of laser fluence on the PCL fiber meshes, the struts were irradiated at a fluence of 0.105 J/cm^2 and 0.629 J/cm^2. The confocal microscopy observations showed that irradiation by a single laser pulse induced the formation of bump

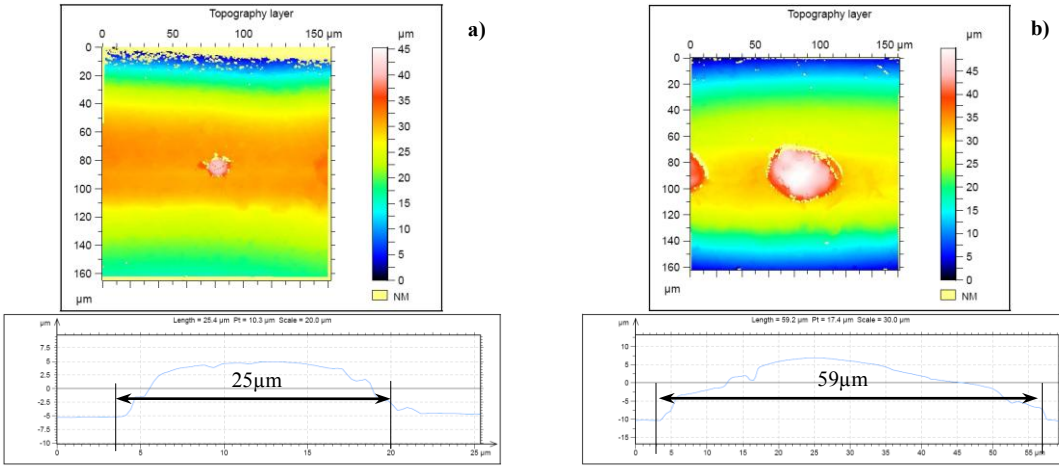

Figure 2. Confocal microscopy image of the laser induced modification; a) topography and cross-section image for $N = 1$, $F = 0.105$ J/cm^2, $\tau = 30$ fs, $\nu = 1$ kHz; b) topography and cross-section image for $N = 1$, $F = 0.629$ J/cm^2, $\tau = 30$ fs, $\nu = 1$ kHz.

like structures with a diameter of 25 μm and 59 μm, and a height over the surface level of 10 μm and 17 μm, for laser fluences of 0.105 J/cm^2 and 0.629 J/cm^2, respectively (figure 2).

We further monitored the changes in the surface of scaffold fibers initiated due to irradiation with increasing number of laser pulses. The porosity of the mesh was altered when the number of pulses (N) exceeded five. Figure 3 is an example of PCL fiber meshes irradiated by $N = 5$, 10 and 30.

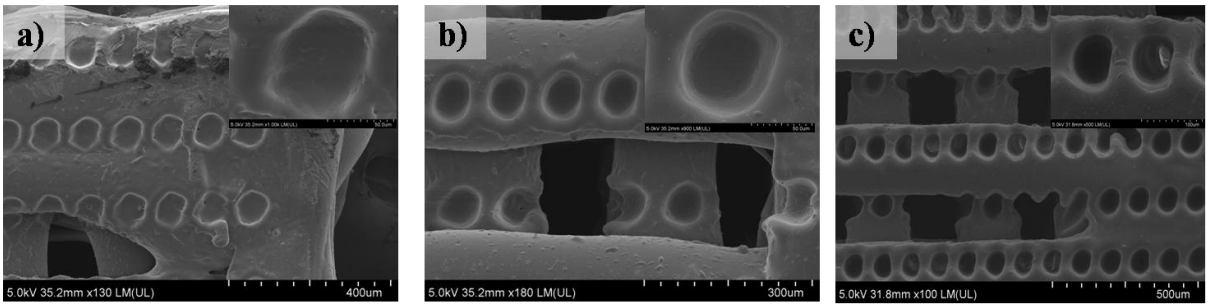

Figure 3. SEM micrographs of PCL fiber meshes (a) $N = 5$, $F = 0.105$ J/cm^2 (b) $N = 10$, $F = 0.105$ J/cm^2; (c) $N = 30$, $F = 0.105$ J/cm^2.

The results showed that pores with an average diameter size of 70 μm can be formed without additional side effects to the scaffold structure by optimizing the laser energy and number of laser pulses applied.

The SEM investigations confirmed that fs laser irradiation of printed scaffolds resulted in a minimal collateral damage and thermal side effects. Regarding the effect of the number of laser pulses applied (N), this was varied from 1 to 30 pulses. Differences were observed in the depths of the created craters when the number of applied laser pulses was increased while the laser fluence was kept constant. The most pronounced modification in the volume of the sample was achieved by irradiation by the highest number of pulses, figure 3(c).

3.2. Cellular adhesion on topographically structured PCL matrix

Previous studies [12,13] have demonstrated that topographical post-modification of scaffold surfaces with microscale features influences the cells' behavior. We observed by confocal laser microscopy the morphologies of MG63 osteoblasts adhering to PCL constructs, figure 4.

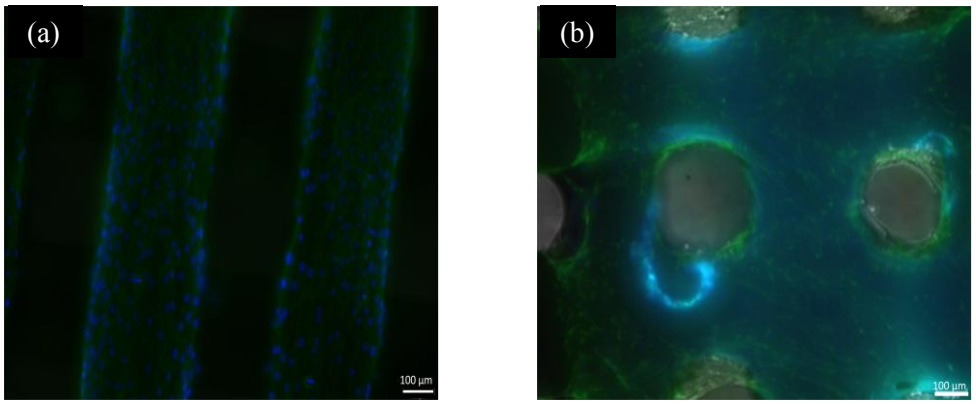

Figure 4. Confocal laser microscopy images of MG63 osteoblast cells cultured for three days on 3D PCL matrices, demonstrating the actin staining and visualizing the fibrillar network of actin cytoskeleton a) non-irradiated surfaces, scale bar 100 μm b) laser-modified fiber surface, scale bar 100 μm.

Fluorescence microscopy images showed that the MG63 osteoblast cells adhere homogeneously to untreated and treated scaffold fibers. These basic cellular studies demonstrated that cells tend to grow completely over the areas where a new structured zone is formed. These preliminary results need a further detailed study, which is out of the scope of this article.

4. Conclusions

The results reported here show that the laser-induced modification can be optimized by controlling the laser processing parameters. The physical integrity of the 3D PCL fiber meshes is thus preserved, as confirmed by SEM analysis. Additional sample modification can affect the mechanical properties of the produced matrices and provide a path to enhancing the cell's infiltration in the volume for improved vascularization. Therefore, femtosecond laser processing could be used as an alternative method for micropatterning of polymeric meshes with a high-level of precision, thus offering a way of enhancing the surface functionalization in tissue applications.

Acknowledgments

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