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Micro-structuring of titanium collectors by laser ablation technique: a promising approach to produce micro-patterned scaffolds for tissue engineering applications

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Abstract

Multi-scale micro-structured scaffolds can sustain attachment and orientation of different cells phenotypes. An innovative use of laser ablation technique to build micro-structured titanium surfaces to be used as collectors in both electrophoretic deposition and electrospinning processes was investigated. To produce micro-patterned scaffolds, a negative replica patterning was exploited by designing specific patterns to be laser ablated on titanium plates. This method allows the deposition of the scaffolds on the mold, thus reproducing the micro-features on the scaffold surface. The titanium surface morphology depending on ablation parameters was studied and the capability of the process in replicating the micro-pattern was characterized.

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Keywords: Patterning, laser ablation, electrospinning, electrophoresis, polymers.

1. Introduction

In medical device industry surfaces are the primary place of contact between biomaterial and organism. In this environment the need to design and manufacture materials to make them highly compatible with living tissue (including bio fluids such as blood) has become a major priority [1,2]. Direct fabrication of a scaffold mimicking the structure and functions of heart and skeletal muscle tissues from raw synthetic or biological materials is an approach distinct from decellularization of biological scaffolds [3,4]. In addition, morphology configuration and mechanical properties of these scaffolds can be controlled. The disadvantages are that their structure may not fully mimic the complexity of the native tissue with varied mechanical properties [5].

Regarding the scaffold, material selection and scaffold fabrication techniques are key points. The material utilized in producing cellular supports must be biocompatible with cell attachment sites [6]. Further, scaffold morphology can affect cellular adhesion and differentiation in a particular phenotype [7]. In particular, an interconnected porous structure allows nutrients and waste cellular product exchanges and a two-dimensional micro-pattern shape may play a role in controlling cellular morphology of embryonic stem cells [8].

In electrospinning and electrophoretic deposition tests a metal collector is used for the deposition of fibers and hydrogels [9,10,11]. Several approaches have been used to produce scaffolds with a controlled structure by the application of an external electrical field and by replacing the traditional two-dimensional (2D) plates collectors with rotating metallic mandrels or metal rings [12,13]. Recently, scaffolds with precise architectures and patterns have been produced by using wire meshes and drilling structures on metallic plates that were accurately mimicked in the resulting collected scaffolds [14]. Despite these advances, these new approaches are relatively slow and not fully reproducible experimental techniques. Moreover, the identification of a flexible and scalable method to design and fabricate collector plates with accurately controlled structures remains a challenge. Furthermore, the possibilities to scale-up manufacture of patterned scaffolds using these methods

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could be limited. For these reasons, techniques that allow the design of collector plates with precise structures according to the specific requirements of the end-user, have to be combined with electrospinning and electrophoretic deposition to collect scaffolds with well defined micro-topography at the surface. Novel collector geometries with complex 3D structures have been designed and produced by additive manufacturing techniques and combined with electrospinning to obtain scaffolds consisting of both random fibres and a defined 3D micro-topography at the surface [15,16].

The manufacturing of periodic surface topography on the collector may be executed using lasers with medium power density (from W/cm^2 to MW/cm^2) [17]. This method allows to form micro- and sub- micrometer structures with well defined long range ordering [18].

In this paper, laser ablated titanium collectors are used as molds during electrospinning and electrophoretic deposition tests to produce scaffolds with a surface topography that replicates the ablated micro-pattern geometry. The influence of the patterns on cellular attachment and orientation was evaluated.

2. Pattern design and laser process

2.1. Definition of the geometry

A total of five pattern configurations were considered: three patterns for the electrospinning tests (ES), namely A, B and C (Fig.1) and two patterns for the electrophoretic deposition tests (EPD), namely D and E (Fig.2).

Configurations A, B and C are characterized by grooves having three different pitches (d_0) equal to 25 μm, 50 μm and 75 μm respectively.

The grooves of the configurations D and E are characterized by dimensions of 125 μm x 250 (l x L) defining rectangular structures. The whole pattern of each configuration has a 5x5 mm^2 area.

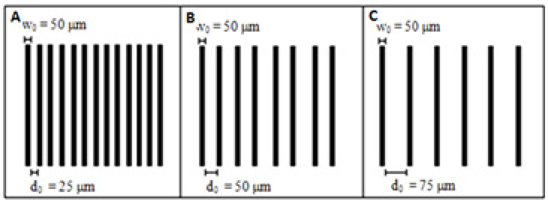

Fig. 1. Imposed pattern configurations A, B and C.

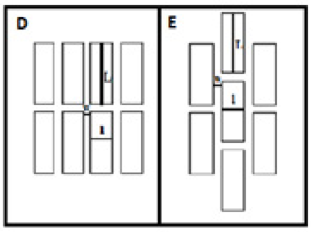

Fig. 2. Imposed pattern configurations D and E.

2.2. Laser ablation

The Titanium patterns (Ti CP Gr2) for ES and EDP application were realized by a laser micro-structuring process, using a LEP Lee Laser (Nd:YVO4, 8 W q-switched, λ=532 nm) for the laser ablation of flat collectors 0.5 mm thick. The laser machine is controlled by a software where it is possible to set the q-switch frequency, the duty cycle, the laser path strategy and the loop number of cycles of ablation. The high quality beam (M^2=1) can work at pulse frequency of 200 kHz and pulse minimum duration of 12 ns.

Pure Argon was chosen as assist gas to reduce piled material through a homogeneous flux oriented at 180° with respect to the specimen [19]. The ablation parameters set to obtain the selected configurations are reported in Table 1.

Each titanium collector was fixed on a frame at a distance equal to the focal distance (160 mm) from the galvo head. Five repetitions for the same working parameters were carried out to study the laser cycle effects on channels configuration homogeneity in terms of width and depth distributions. The number of ablation loop was set to 30 to investigate the effect of the refund material on the grooves geometry.

Table 1. Designed laser-ablation cycles.

Parameter	Description	Equation	Set value
Area [mm^2]	Sample area	-	25
f [kHz]	q-switch frequency	-	30
P_{avg} [W]	Average power	-	2.64
v_s [mm/s]	Scan speed	-	304.8
d [mm]	Spot diameter	-	0.1
abs [%]	Absorption coefficient [18]	-	50
dc [%]	Duty cycle	-	30
dt [ns]	Pulse width	$dt = \frac{dc}{f}$	10^4
P_{peak} [W]	Peak power	$P_{peak} = \frac{P_{avg}}{f \cdot dt}$	8.80
t_{loop} [s]	Time per loop	$t_{loop} = 3\frac{Area}{d} \cdot \frac{1}{v_s}$	2.46
E_{pulse} [J]	Energy per pulse	$E_{pulse} = P_{peak} \cdot dt$	1.33
F_{pulse} [J/mm^2]	Fluence per pulse	$F_{pp} = abs \cdot \frac{4}{\pi d^2} \cdot E_{pulse}$	$5.6 \cdot 10^{-3}$
# loop	Number of loops	#	30

3. Pattern characterization

3.1. Optical microscope

The titanium samples surface was observed using the Mitutoyo Quick Scope QS-200z optical microscope system and the Zeiss LEO EVO 40 scanning electron microscope.

The optical images were used to analyze the details of the micro-patterns while the laser modification performed on an area of 25 mm^2 were evaluated by means of the SEM images. The samples showed a morphology sketched by the ablated configurations as shown in Fig. 3 and Fig. 4.