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Electrically stimulated osteogenesis on Ti-PPy/PLGA constructs prepared by laser-assisted processes

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ABSTRACT

This work describes a versatile laser-based protocol for fabricating micro-patterned, electrically conductive titanium-polypyrrole/poly(lactic-co-glycolic)acid (Ti-PPy/PLGA) constructs for electrically stimulated (ES) osteogenesis. Ti supports were patterned using fs laser ablation in order to create high spatial resolution microstructures meant to provide mechanical resistance and physical cues for cell growth. Matrix Assisted Pulsed Laser Evaporation (MAPLE) was used to coat the patterned Ti supports with PPy/PLGA layers acting as biocompatible surfaces having chemical and electrical properties suitable for cell differentiation and mineralization. In vitro biological assays on osteoblast-like MG63 cells showed that the constructs maintained cell viability without cytotoxicity. At 24 h after cell seeding, electrical stimulation with currents of 200 μA was applied for 4 h. This treatment was shown to promote earlier onset of osteogenesis. More specifically, the alkaline phosphatase activity of the stimulated cultures reached the maximum before that of the non-stimulated ones, i.e. controls, indicating faster cell differentiation. Moreover, mineralization was found to occur at an earlier stage in the stimulated cultures, as compared to the controls, starting with Day 6 of cell culture. At later stages, calcium levels in the stimulated cultures were higher than those in control samples by about 70%, with Ca/P ratios similar to those of natural bone. In all, the laser-based protocol emerges as an efficient alternative to existing fabrication technologies.

1. Introduction

Electrical stimulation (ES) of cells has emerged as a powerful tool for tissue engineering, by stimulating cell adhesion, proliferation and differentiation within the nervous system, skin, bones and muscles [1–3]. ES was found to be particularly efficient for enhancing the proliferation, differentiation and mineralization of osteoblasts and, for this reason, much work has been devoted to bone regeneration and fracture healing using electrically conductive substrates such as metals, graphenes and certain polymers [2–5].

The most popular material for bone regeneration is Ti, because of its mechanical resistance that is able to support osteoblast adhesion and growth [3,6]. Apart from ES, cell behavior has also been controlled through the use of appropriate 3D architectures produced on Ti [1]. 3D micro and nanopatterning of Ti has been efficiently achieved by laser ablation with femtosecond (fs) pulses [7,8]. Fs laser ablation emerged as a clean and versatile method, having high spatial resolution. As compared to other processing techniques, fs laser ablation requires fewer steps and ensures better reproducibility and less contamination. Moreover, the use of short pulse durations limits the heat diffusion and minimizes the damage to adjacent areas. Depending on the irradiation conditions, conical micro/nanostructures, nanopores, nanoprotrusions, parallel grooved surface patterns and micro-roughnesses with various configurations were generated on Ti [7,8].

Despite their successful use for bone repair, improving the in vivo functionality of the Ti constructs remains challenging [6]. The low electrical resistance of Ti allows for much higher electric currents through the support than those encountered in normal tissues, causing adverse effects on the cells. Also, the mechanical mismatch between the rigidity of Ti and the softness of the surrounding tissues leads to the inflammation of the implantation site and activation of immunological responses. In addition, Ti corrosion is often accompanied by the release of cytotoxic products [3].

A viable solution to these problems is to coat the Ti supports with biocompatible surfaces, having chemical and electrical properties that are suitable for cell growth and proliferation. The most promising materials for this purpose are electrically conductive polymers, since they possess physical and chemical properties specific to organic polymers,

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as well as electrical characteristics of metals [9]. Owing to its good biocompatibility, environmental stability, low cost and lack of toxicity toward the surrounding tissues, the most used conductive polymer for bone tissue engineering is polypyrrole (PPy) [1–3]. However, its poor mechanical properties and its inability to degrade render PPy less suitable for practical use [10]. To improve its mechanical properties and biocompatibility, PPy has been blended with biocompatible and/or biodegradable polymers such as PU, PLA, PDLLA or chitosan [1–9].

The coating of Ti constructs with PPy-based layers is mainly achieved by electrodeposition [11,12], which has limited applicability when using blends of PPy with non-conductive polymers. Solvent-based coating techniques, such as spin coating and drop casting, are also unsuitable, since uneven wetting and evaporation of the solvent can lead to non-uniform layers containing solvent traces that are detrimental for the cells [13].

In all, the development of a versatile technology to fabricate Ti supports with controllable 3D micro-architectures, having surfaces that are both biocompatible and electrically conductive, would be a critical step forward toward achieving osteoblast growth, differentiation and mineralization.

In this work, we developed a simple and low cost laser-based protocol for producing Ti-PPy-based constructs for electrically stimulated osteogenesis. More specifically, we used a fs laser source for the patterning of Ti supports. The created microstructures provide mechanical resistance and physical cues for cell growth. Matrix Assisted Pulsed Laser Evaporation (MAPLE) was used to coat the micropatterned Ti with layers of polypyrrole/poly(lactic-co-glycolic) acid blends (PPy/PLGA) in order to achieve biocompatible, electrically conductive surfaces for cell cultures. The advantages of MAPLE are discussed in more detail elsewhere [13–15]. In brief, MAPLE is a low cost technique, which enables the obtaining of thin layers of polymers, including polymer blends [14], while preserving their chemical composition. Moreover, in a recent study, we deposited polymer blends on patterned polymeric substrates and we have shown that MAPLE also preserved the micro-patterns from the underlying layer [16].

The inset in Fig. 1 displays a schematic representation of the Ti-PPy/PLGA constructs. These were obtained using a three-step procedure: i) fs laser ablation of flat Ti surfaces, coupled with a computer-controlled patterning system, allowed the achievement of periodic microstructures having high spatial accuracy; ii) we used MAPLE to deposit an intermediate polyurethane (PU) layer acting as electrical insulator between the patterned Ti support and the top PPy/PLGA layer; iii) finally, MAPLE was once again used to cover the PU coated Ti supports with a layer of PPy/PLGA, which provided a biocompatible interface having chemical and electrical properties suitable for cell differentiation and mineralization. The incorporation of the conductive PPy within the insulating PLGA matrix aimed at improving the biocompatibility of the top polymer layer and to facilitate its processing.

The viability of the Ti-PPy/PLGA constructs was tested against osteoblast-like cultures subjected to electrical stimulation (ES), by monitoring the degree of cell differentiation and mineralization in electrically stimulated cultures, as compared to non-stimulated ones.

2. Materials and methods

2.1. Fabrication and characterization of the Ti-PPy/PLGA constructs

2.1.1. Laser ablation with femtosecond (fs) pulses

The patterning of the Ti supports was achieved using a pulsed Ti:sapphire laser source (Clark-MRX 2101) working at a wavelength of 775 nm, having 200 fs pulse duration and 2 kHz pulse repetition rate. The laser beam was focused and scanned using an f-theta lens, having a focal distance of 10 cm, on the Ti supports with a diameter of 1 cm, thickness of 0.1 cm, and surface roughness of approximately 5 nm (acquired from Goodfellow, purity >99.99%). The supports were placed in a slightly defocused position with respect to the focal point of the 10 cm f-theta lens, resulting in a laser spot size of few tens of microns across. This was done in order to create morphological irregularities about the same size as the cells to be tested in subsequent cultures. The pulse energy of the incident beam was set at 0.3 mJ.

2.1.2. MAPLE deposition of the polymer layers

MAPLE coating of the Ti supports with polymer layers was carried out as described in [13]. The PPy/PLGA blends were prepared by mixing PPy and PLGA (Sigma Aldrich) solutions (each having 1 wt.% in acetone) in 1/1 blending ratios, under continuous stirring. The target obtained by freezing the solution in liquid nitrogen was placed in a vacuum chamber and irradiated with a Nd:YAG laser emitting at 266 nm and having 10 Hz pulse repetition rate. The target was irradiated with 150,000 pulses at a laser fluence of 0.55 J/cm^2. The size of the laser spot reaching the target

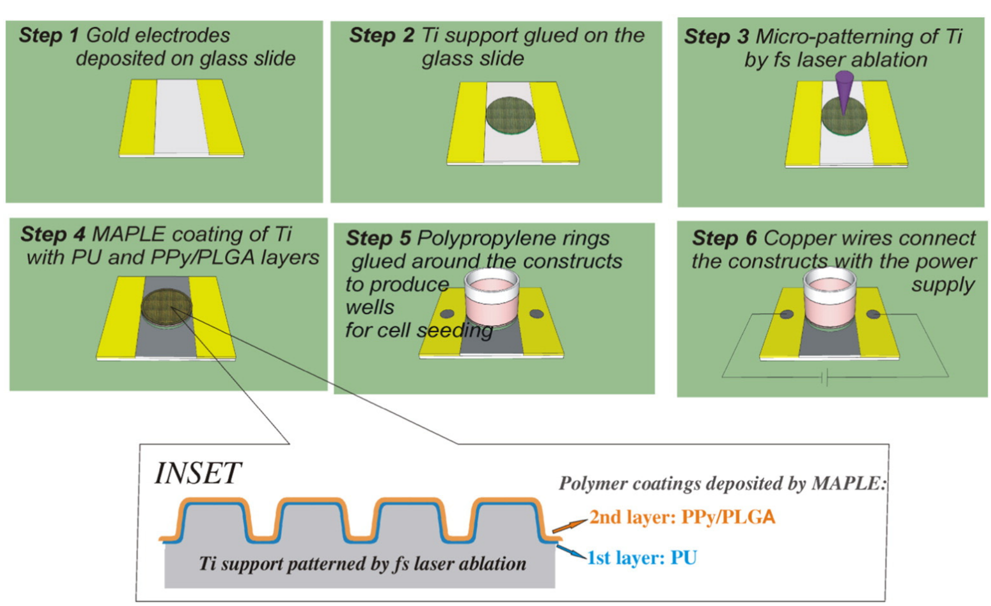

Fig. 1. Home-made cell culture device for electrical stimulation of the cultured cells: (inset) schematic representation of Ti-PPy/PLGA construct. Ti supports were first patterned by fs laser ablation and subsequently coated with 2 polymeric layers by MAPLE: a first layer of PU for electrical isolation of the Ti support and a second layer of PPy/PLGA acting as biocompatible, electrically conductive substrate for cell culture.