Ultra-fast laser surface texturing of β-tricalcium phosphate (β-TCP) ceramics for bone-tissue engineering applications

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Abstract. β-tricalcium phosphate (β-TCP) has provoked particular attention as graft for bone regeneration applications due to its excellent biocompatibility and biodegradability. In this work, we investigated the interaction of femtosecond laser radiation with β-TCP pellets by varying the output laser parameters in order to estimate their influence on the surface roughness and on the morphological and topographical properties of the substrate before and after laser treatment. Femtosecond laser micro-processing for pulse duration of $\tau = 30$ fs and $130$ fs was performed by varying the number of laser pulses $N = 1 \sim 100$, at $\lambda = 800$ nm and a variable repetition rate. The microstructural changes induced were characterized by confocal microscopy. Comparing the experimental results, we concluded that the femtosecond laser method can be applied for biomaterials surface functionalization with a high-level of precision.

1. Introduction

Bone is a complex tissue with a variety of functions, such as providing mechanical stability for movement, protection of the inner organs, hematopoiesis and mineral homeostasis of the body. In order to respond adequately to all diverse challenges to the human body, bone consists of several different cell types and an extracellular matrix that is mechanically stable, yet flexible at the same time [1]. As we grow older, the human body is confronted more often with traumas, infections, fractures, tumors, surgery, etc., which could cause injury, deformations and even loss of bone tissue [2]. Reconstructing bone tissue defects has thus become a major challenge to modern medicine. Research in bone tissue engineering has aggressively moved into regenerative approaches in order to achieve improved clinical outcomes. A functional bone tissue can be developed to replace the damaged one

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through an intelligent application of “matrix, cells, and signals” components. Therefore, the basis of designing bone tissue depends on the level of understanding the interactions between molecules of the extracellular matrix (ECM) and the cells enclosed in it [3]. The main challenge in designing tissue substitutes is still the realization of a customized implant with good interface properties without any risk of subsequent inflammation. Creating novel temporary “platforms” have been extensively researched and innovated in order to obtain scaffolds fulfilling all requirements for seeding different types of cell cultures and improving cell adhesion, proliferation and differentiation. The perfect scaffolds should mimic the strength, stiffness and the mechanical properties of the natural bone, as these can significantly influence not only the cell behavior, but also the osteointegration of the implants and the surrounding “host” tissues. Human bone consists of about 70% of calcium phosphate (CaP) minerals, therefore CaPs are the materials of choice to repair damaged bone [1]. Special attention has been focused on β-tricalcium phosphate (β-TCP; Ca₃(PO₄)₂) as grafts for bone regeneration applications due to its excellent biocompatibility, biodegradability and direct bond formation with the connective tissue [4-6]. β-TCP has been shown to be resorbed relatively quickly in the human body through physico-chemical dissolution and cell-mediated resorption [7]. When studying the mechanism of β-TCP degradation in the body, it was found that after a few weeks of implantation, a new lamellar bone is formed at the contact surface between β-TCP implant and surrounding osteoblasts [8]. In other words, β-TCP is progressively absorbed in vivo by cell-mediated resorption and replaced by new bone [9, 10].

Designing the temporary scaffolds may include a variety of conventional techniques. The application of laser-assisted methods for development of improved quality constructs is an excellent strategy owing to the possibility of fabricating complex scaffolds with hybrid porosities. The natural hierarchical architecture of bone ranges from pores of around 1 μm (interaction with proteins), to 1-to-20 μm (cellular ingrowth), 100 μm, and up to more than 500 μm (for cellular growth and bone ingrowth) [11]. The micro/nano porosity has to be carefully considered when designing scaffold constructs, since it is an important condition for cell viability and tissue ingrowth. An interconnected pore structure will permit inward diffusion of oxygen and nutrients and outward diffusion of waste products from the scaffold [12]. The literature survey shows the existence of diverse methods [13, 14] altering the surface topography to improve the cell-surface attachment. Among them, sand-blasting and acid etching, argon or nitrogen plasma treatment, and ozone irradiation are very popular and have demonstrated increased topological modifications. However, the presence of residuals is the most significant problem facing these techniques due to the risks of toxicity and carcinogenicity. The advantages of the laser-matter interaction process reside in the non-thermal and non-destructive mode of modification leading to the production of a diversity of structures ranging from nano- to micro morphologies and dimensions; further, it is contactless and does not require any additional chemicals. It provides a possibility to treat the material’s surface and bulk with a high degree of precision in comparison to other techniques. Using ultra-fast laser processing allows on to control closely the hierarchical interconnected porosity, the chemical composition, and the external size and shape in order to further mimic the natural ECM structure. No special pre-treatment is required and the cells’ adhesion is improved, as the roughness of the substrate is increased. Control over the biomaterial’s characteristics might be achieved, as the interaction of ultra-short pulses with biomaterials results in reduced crack formation and heat diffusion, absence of molten zones, and reduced ablation thresholds.

In the research presented, we studied the interaction of femtosecond laser radiation with β-TCP pellets by varying the output laser parameters in order to estimate their influence on the surface roughness, and on the morphological and topographical properties of the substrate before and after laser treatment. Femtosecond laser micro-processing with a pulse duration τ = 30 fs and 130 fs was performed by varying the number of laser pulses \( N = 1 - 100 \), at \( \lambda = 800 \) nm and a variable repetition rate. The microstructural changes induced were characterized by confocal microscopy. Comparing the experimental results, we could conclude that the method of femtosecond laser texturing can be applied to surface functionalization of biomaterials with a high-level of precision.
2. Material and methods

2.1. Material preparation
The β-TCP powder was synthesized by aqueous precipitation of calcium nitrate and di-ammonium phosphate solutions. The as-obtained raw powder was heat-treated at 850 °C, ball-milled in water for three hours, and filtered and dried at 100 °C. Pellets (d = 12.5 mm) were produced by uniaxial pressing at 55 MPa followed by sintering at 1100 °C for a dwell time of two hours (5 °C min⁻¹). The β-TCP pellets were then polished by SiC paper discs and diamond pastes (down to 0.5 – 3.0 μm) to obtain a smooth surface.

2.2. Laser treatment
For femtosecond laser micro-processing of the β-TCP disks prepared we employed a Ti:sapphire laser (Femptopower-Compact pro) and (Quantronix- Integra-C) systems with a pulse duration τ of 30 fs and 130 fs at a central wavelength of λ = 800 nm and a variable repetition rate and number of laser pulses N = 1 – 100. The experiments were performed in air with the laser beam focused to a focal spot with a diameter of approximately 50 μm using a lens with a 20-cm focal length. The focusing lens was placed on a translation stage equipped with a micrometer screw for fine adjustment of the focus position on the sample’s surface. The sample was positioned normally to the focused beam on a high-precision XYZ translation stage (figure 1). The experimental setup was controlled by LabView software. The samples were processed by scanning the focused laser beam over the material surface at precisely-defined separation intervals dₓ and dᵧ in order to avoid a spatial overlap between the separate laser spots.

2.3. Surface analysis
To evaluate the surface topography of the ceramic pellets before and after the fs laser treatment, a confocal laser scanning microscope (CLSM) (Keyence VK-X250) with a standard resolution of 1024×768 and an optical 3D μsurf confocal microscope (Nanofocus) were used. A 100× objective lens was used to measure the field size of 150 μm×150 μm with a vertical resolution of 20 nm by a motorized positioning unit and with a fine positioning (1 nm, piezo-driven) unit. The resolution of the instrument was 0.31 μm (lateral resolution). We thus obtained 3D topography images and cross-sections of 3D reconstructed images of the laser-patterned surfaces.

3. Results and discussion
The femtosecond laser micro-processing with a pulse duration of 130 fs was performed at λ = 800 nm and a variable number of laser pulses. Figure 2 shows representative images of the confocal observations performed on the β-TCP surfaces patterned by fs pulses N = 1, 2, 4, 10 (a) and N = 10, 20, 50, 100 (b) at a fixed laser fluence F = 0.61 J/cm².

The spots presented in figure 2 were created in a series of irradiations with an increasing number of laser pulses (N = 1 – 100). For N = 1 – 4, the onset of material modification was clearly detectable (figure 3a and figure 3b) at the β-TCP surface. As N was increased further, ablation of the material’s
surface started. With increasing the number of laser pulses applied ($N > 10$), the structures’ porosity started to change, and removal of material was initiated, leading to a deeper crater formation (figure 2d).

A clear difference was observed in the morphological patterns of the β-TCP surfaces. These results demonstrated that microporous structures that could find numerous applications in bone regenerative medicine were fabricated by way of femtosecond laser irradiation at specifically selected exposition parameters.

**Figure 2.** General view of the irradiated area of the β-TCP samples with: (a) topography image of β-TCP surface irradiated by $F = 0.61$ J/cm$^2$ and $N = 1, 2, 4, 10$; (b) topography image of β-TCP surface irradiated by $F = 0.61$ J/cm$^2$ and $N = 10, 20, 50, 100$; (c)-(d) cross-sections of the reconstructed 3-D images of the irradiated craters as a function of the number of applied laser pulses from 1 – 100.

**Figure 3.** Fs-irradiation-formed craters on the β-TCP samples at (a) $F = 0.61$ J/cm$^2$ and $N = 1$; (b) $F = 0.61$ J/cm$^2$ and $N = 4$.

**Figure 4.** Optical, 3D and cross-section images of fs-irradiated areas of β-TCP samples processed by $F = 0.61$ J/cm$^2$, $\tau = 30$ fs and (a) $N = 1$; (b) $N = 5$. 
Furthermore, a series of experiments was performed where, apart from the number of pulses (N), the laser fluence (F) was also varied, as follows N = 1, 2, 4 and F = 0.03 J/cm², 0.06 J/cm², 0.17 J/cm², 0.35 J/cm². The general trend observed was that raising either parameter, F or N, the effect on the surface of the β-TCP disks was expressed in increasing the depth of the craters formed. To optimize further the micro-structuring of the sample surface, irradiation of β-TCP was performed at a reduced pulse duration τ = 30 fs and at F = 0.35 J/cm². A “gentle” modification was thus achieved for processing with N = 1-5, without formation of cracks and melted zones around the treated zones (figure 4 (a) and (b)).

4. Conclusions
The results obtained provide comprehensive evidence that the femtosecond laser processing of β-TCP disks improves the characteristics of bio-ceramic surfaces. Patterned surfaces with tunable surface roughness were experimentally demonstrated when a selected set of parameters of the femtosecond laser radiation was used. In the case of treatment by a higher number of fs pulses and laser fluence, formation of cavities through removal of material was observed, and destruction of the micro-porosity started to take place. It is further shown that a surface with a uniform roughness and creation of self-induced porosity due to the nature of the non-contact interaction between the fs laser radiation and the β-TCP surface could be achieved when the number of applied laser pulses and the laser energy are carefully chosen. We thus confirmed that fs laser processing is a technique suitable for bio-ceramic surface functionalization for further biomedical applications.

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