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Surface structuring of β-TCP and transition to α-TCP induced by femtosecond laser processing

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Abstract. Tricalcium phosphate ($Ca_3(PO_4)_2$, TCP), is one of the most studied and used as material for bioresorbable implants. The β phase has a slower dissolution dynamic and ensures mechanical support for a longer time in biological environment, while a faster release of ions characterize the α phase that trigger a stronger biological response. In this work a femtosecond laser system was used to process β-TCP pellets surface. The femtosecond laser processing results in surface morphology modification, by turning the flat mirror polished surface into a rough and opaque one. The morphological and phisycochemical characteristics of material surface were studied by means of SEM, AFM, Raman, XRD and contact angle measurement. The processed surface showed the formation of micro and nano roughness alongside, furthermore a partial phase transformation from β-TCP to α-TCP was detected. A significant improvement in surface wettability for three different liquids (i.e.water, ethylene glycol and diiodo-methane) is reported. This implies an increase in surface free energy as well. The combination of α and β phase, together with the increased roughness obtained by laser processing, could positively affect the cell adhesion and metabolic activity.

1. Introduction

Due to its bone-like composition and its bioactivity (being able to show osteoconductive and even osteoinductive behaviour [1]), calcium phosphate (CP) finds applications in bone regeneration in a variety of forms (coating, cements and scaffold) [2]. The bone regeneration process is positively affected by the release of calcium (Ca) and phosphorus (P), which triggers the activity of osteoblasts and osteoclasts [3]. Different crystalline phases of CP provide different behaviors in terms of solubility, releasing of ions, mechanical properties, etc [4]. Tricalcium phosphate ($Ca_3(PO_4)_2$, TCP), is one of the most studied CP for applications in regenerative medicine; TCP has a C/P ratio of 1.5. The β phase, shows slower dissolution dynamics in biological environment providing mechanical support for a longer time, while the α phase ensures a faster release of ions, able to trigger the biological response. The physical properties of material surface play a fundamental role as well, the surface free energy and topography are of major importance in regulating initial cell adhesion and spreading. Their effect may even be more important than the surface chemistry as demonstrated by Dos Santos et al. [5].

A femtosecond laser was used to process β-TCP tablets modifying surface topography and wettability, and inducing crystalline phase transformation as well. The laser processing, providing such a combination of structural and physicochemical characteristics could positively affect the material performance for biomedical application improving its biocompatibility, favoring cell adhesion and stimulating the tissue regeneration. The effects of laser processing on surface morphology were studied by Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM), which gave us a better insight on the micro and nano surface features formations. X-Ray diffraction (XRD) has been exploited to detect phase transformation from β-TCP to α-TCP and contact angles measurement allowed to estimate the surface free energy.

2. Material and Methods

A Ti:Sapphire laser system (Quantronix-Integra-C, Hamden, CT, USA), generating pulses with duration $\tau < 130$ fs at the central wavelength $\lambda = 800$ nm and repetition rate $\omega = 1$ kHz with a peak power above 1 W, was used to process β-TCP tablets with a 1 cm diameter and a 3 mm thickness.

β-TCP tablets were produced according to Somers et al. [6] starting from the synthesis (by aqueous precipitation) of an apatitic tricalcium phosphate, $Ca_9(HPO_4)(PO_4)_5OH$ precursor of β-TCP. A diammonium phosphate solution $(NH_4)_2HPO_4$ was gradually added to a calcium nitrate solution $Ca(NO_3)_2.4H_2O$, keeping constant pH and temperature at a value of 6.7 and 31 °C respectively, in the double-wall reactor used for the synthesis of the powder. The apatitic tricalcium phosphate precipitate was filtered, dried and calcinated. The calcination is a necessary step to remove residual by-products of the syntesis and to transform the amorphous apatitic precursor into crystallized β-TCP. The powder, after being ball milled, was then dried and shaped to pellets by mold casting. Pellets were then sintered in an electric furnace.

The β-TCP tablet is placed on a software-controlled (LabView) two-axis motorized translation stage (Thorlabs, Newton, New Jersey, United States), and the laser beam was focused on the surface by an achromatic biconvex lens with a 100 mm focal distance. The tablet surface was processed by raster scanning the surface at a fixed speed $V = 8$ mm/s with hatch distance $h = 30$ μm using a laser power of $P = 50$ mW, corresponding to a fluence $F = 12.7$ J/cm^2. The whole process was carried out in air and at room temperature. The morphology and physicochemical characteristics of treated and untreated surface were studied and compared. Images of the samples surface were acquired with a SEM (Auriga Carl Zeiss Microscopy, Deutschland GmbH, Germany), using a 5 kV accelerating voltage and a secondary electron detector. The sample surfaces were scanned by AFM (Park System, NX20, Suwon, South Korea), acquiring 20 μm x 20 μm frames, to study the topography and evaluate the roughness. Three different areas were scanned on both treated and untreated samples and the values of roughness were averaged. XRD (Panalytical X'PERT PRO) was used to study the crystalline phase of the tablets and its change after laser processing. XRD data were processed using the Profex software to identify the different crystalline phases and quantify their contribution. The tablets were also analyzed by Raman spectroscopy (spectrometer Horiba MicroRaman, Kyoto, Japan), the spectra were obtained using a 532 nm laser and each reported spectra are the average of five acquired in different points on the same sample. The surface wetting properties and free energy were studied using a drop shape analyzer system (DSA100E, KRUSS GmbH, Germany). The reported contact angles are the average of three different measurements.

3. Results

The mirror polished pellets of β-TCP, turned to homogeneously opaque after the laser treatment. The observed change in appearance is due to the modified surface morphology. As shown by SEM pictures (see figure 1) the unprocessed surface appears smooth and flat, while, after laser exposure a drastic change occurs, a more complex morphology arises from the femtosecond laser induced ablation process.

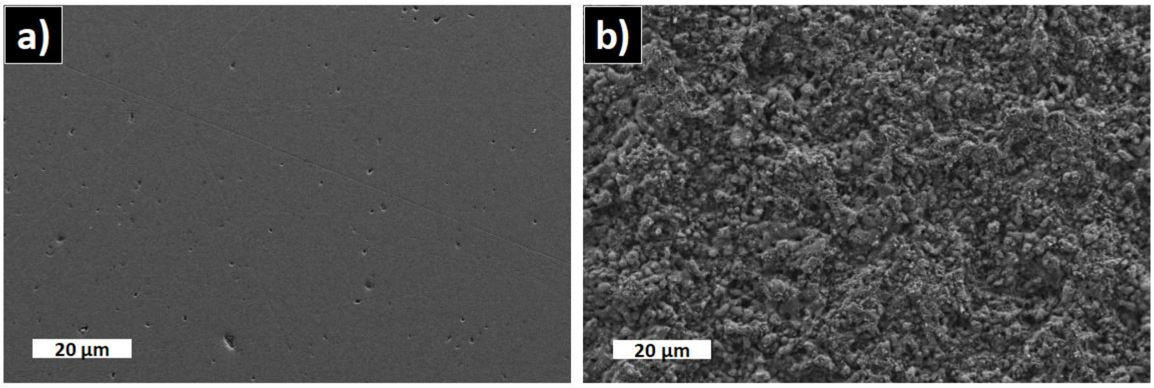

Figure 1. SEM pictures of β-TCP tablet surface, scalebar is 20 µm; a) polished surface, before laser processing; b) laser processed surface.

The surface topography analyzed by AFM (see figure 2) showed in more detail the change in roughness and morphology occurred. The polished surface showed only nano roughness (Sa = 4 ± 3 nm and Sq = 12 ± 15 nm) while the laser processed surface is characterized by the presence of micron and nano sized features (Sa = 0.56 ± 0.12 µm and Sq 0.70 ± 0.15 µm).

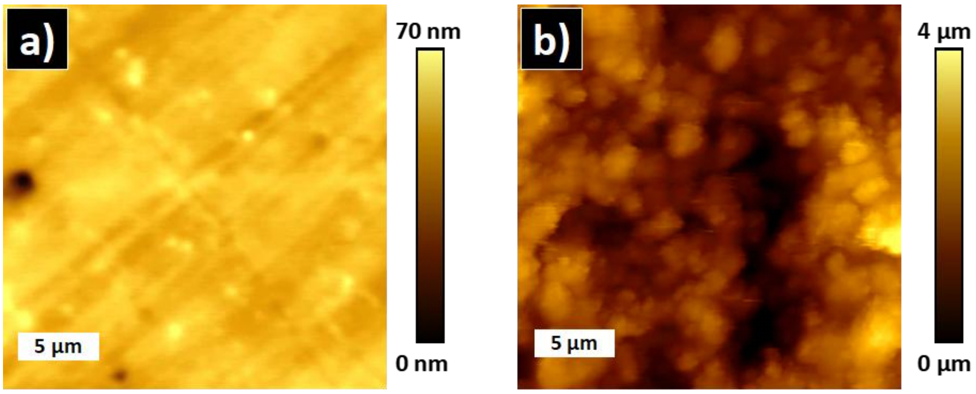

Figure 2. 2D AFM images of β-TCP tablet surface, scalebar is 5µm; a) polished surface, before laser processing; b) laser processed surface.

XRD spectra highlighted that the laser exposure induced not only morphological changes, the presence of α-phase of TCP could be spotted as well. A partial phase transformation, form β-TCP to α-TCP has been detected and studied. In figure 3 (a), the XRD spectra of processed (black line) and unprocessed (blue line) material, together with reference patterns of α-TCP and β-TCP (red and green line respectively) are reported. The unprocessed sample shows a XRD pattern perfectly matching with the β-TCP reference. The processed materials instead showed two characteristics peaks in the range 22 - 25 degrees, those are completely absent in the unprocessed sample spectrum. Such peaks can be attributed to the formation of α-TCP by comparison with reference spectrum (from Profex software database). Further analysis, performed by Profex software allowed to estimate the α phase contribution, calculated to be about 11% of the total signal. The phase transition, can be ascribable to the high temperature induced by the laser exposure. The Raman spectra (see figure 3 (b)) instead, showed no significant difference analyzing the material before and after laser processing. Both spectra perfectly resemble the signal of β-TCP, the presence of α-TCP was not evident, and its contribution cannot be distinguished [7,8]. Due to that, and considering a maximum X-ray penetration depth of about 18 µm (as estimated by software calculation), it can be assumed that only the first 1-2 µm from the surface were affected by the transformation into α-TCP, leaving the bulk material unaltered.

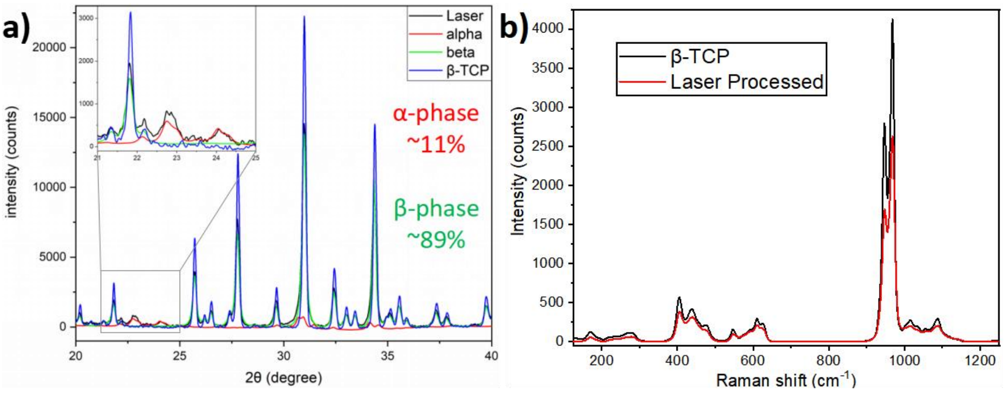

Figure 3 a) XRD spectra of β-TCP tablet before (blue line) and after (black line) laser treatment. Reference pattern of α-TCP (red line) and β-TCP (green line) for comparison, the inset shows a zoom of the signal between 21 and 25 degree; b) Raman spectra of β-TCP tablet before (black line) and after (red line) laser treatment.

The increased surface roughness, obtained after the laser treatment, provides a significant improvement in surface wettability and free energy of the sample [9]. Table 1 reports the contact angles, measured with three different liquids (water, ethylene glycol and diiodo-methane) and the surface free energy values resulting from such measurements.

Table 1. Contact angles values measured for water, ethylene glycol, diiodo-methane and surface free energy of β-TCP tablet surface before and after laser processing.

	water [°]	ethylene glycol [°]	diiodo-methane [°]	surface free energy [mN/m]
Polished	**59 ± 2**	**48 ± 8**	**46 ± 2**	**41 ± 2**
Laser Processed	**10 ± 2**	**10,5 ± 0.6**	**5,2 ± 0.8**	**57 ± 11**

The increased roughness offers a three-dimensional topography, which provides an increased surface area that could improve the cell adhesion and proliferation, due to the enhanced wettability as well. Moreover, the presence of α-TCP, could boost the activity of osteoblasts and osteoclasts, inasmuch the released ions play a role in regulating their activity, thus facilitating the growth of new bone tissue.

4. Conclusion

Femtosecond laser processing appears to be a promising tool for β-TCP surface modification improving material performances for biomedical applications. The surface morphology obtained by laser processing showed a complex topography and an increased roughness. The wettability of the surface results improved and the surface free energy increases as well. Moreover, the laser exposure induced the partial phase transformation of sample surface from β-TCP to α-TCP. The improved ions release, provided by the higher solubility of α-phase, together with the enhanced wettability could positively impact on cell adhesion and proliferation triggering their metabolic activity, facilitating bone regeneration after material implantation.

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