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Biomimetic calcium phosphates-based coatings deposited on binary Ti-Mo alloys modified by laser beam irradiation for biomaterial/clinical applications

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ABSTRACT:

Biomimetic Method has been widely used to prepare calcium phosphate coatings on Ti and its alloys. This modification is based on a Synthetic/simulated Body Fluid (BSF) which facilitates the mimicking of the biological process in order to provide hard tissue repairs. The formation of HA and other calcium phosphates under biological medium and SBF occurs in the presence of Ca^{2+} and PO_4^{3-} ions, as well as essential ions such as: Mg^{2+}, HCO_3^-, K^+ and Na^+. Ti-15Mo alloy samples were irradiated by pulsed Yb: YAG pulsed laser beam under air and atmospheric pressure. Sequentially, calcium phosphate coatings were deposited on the irradiated surfaces by the biomimetic method. The biomimetic calcium phosphates-based surfaces were submitted to heat treatment conditions at 350°C and 600°C. The present study correlates two conditions of fluency (1,91 and 5,54 J.cm^{-2}) as established have a sufficient energy to promote ablation on the laser beam irradiated surfaces. Likewise, it has been demonstrated the processes of fusion and fast solidification from the laser beam irradiation, under ambient atmosphere, inducing the formation of stoichiometric TiO_2 and non-stoichiometric titanium oxides, including Ti_3O_5, TiO, Ti_3O and Ti_6O with different oxide percentages depending on the fluency applied. Besides that, laser modification has allowed a clean and reproducible process, providing no traces of contamination, an important feature for clinical applications. The physico-chemical and morphological analysis indicated the formation of a multiphase coatings depending on the heat treatment temperature performed to 350 °C (ACP1 and 2, HA_D, HA phases) and 600 °C (HA_D, HA and β-TCP phases). It is worth noting that multiphasic bioceramic systems has been gaining attention for biomedical applications. Thus, the laser beam irradiation associated to bioactive coatings of calcium phosphates of biological interest have shown to be promising and economically feasible for use in dental and orthopedic implants.

INTRODUCTION

Currently, the demand for artificial implants in humans is increasing due to the population increase in countries such as Japan, the United States, Germany, Brazil, India, China and the loss of body functions due to aging and accidents. The materials, used as Biomaterials, must have properties such as: biocompatibility, biofunctionality, bioadhesion, adequate and compatible mechanical properties with bone, processability and resistance to corrosion, and especially market values consistent with Brazilian reality [1]. Since the sixteenth century, metals have been the materials most used for the application as biomaterials for bone repair in both dental and orthopedic applications. The metallic materials most used as implants cover three groups: stainless steels, cobalt-based alloys (Co) and titanium-based alloys (Ti) [2] Titanium and its alloys are the most commonly used materials in dental and orthopedic implants due to the combination of high corrosion resistance, low modulus of elasticity compared to other metals used as biomaterials and excellent biocompatibility [3]Currently, research on metal implants is focused on the development of a new generation of type Ti alloys composed of low toxicity elements such as Nb, Mo and Sn, which combine biocompatibility, low modulus of elasticity and better processability. Although the development of Ti-Mo alloys allows the production of materials with mechanical properties, resistance to corrosion, modulus of elasticity and biocompatibility suitable for application as biomaterial, these materials still need a study of the biological response from the surface interaction of the biomaterial / biological environment. Thus, it is necessary to use surface modification methods to improve the biological activity of these materials and to promote osseointegration [3]

The surface modification of cp Ti and its alloys can be obtained using laser beam irradiation. However, it is necessary to establish the correct relation between the parameters of the beam, such as frequency, time of application, energy and intensity, with the obtained composition and morphology of the irradiated surface. In the process of cell adhesion to the surface of biomaterials, topography and surface energy play a fundamental role in the osteoblastic adhesion that occurs in osseointegration [4]

In the last years, work has been published correlating the parameters of the laser beam, the atmosphere used and the phases formed, as well as the surface morphology. Gyorgy *et al*. studied the formation of oxides during pulsed laser irradiation and observed there is a higher concentration of oxygen in the center-edge and surface-to-depth direction, associating the observed at high temperatures, oxygen diffusion is avoided at that location, thus migrating to the edges, these also present a very high degree of oxidation, this is due to the fact that in the center and the surface there is the formation of non-stoichiometric oxides Ti_xO_{2x-1} due to deficiency of oxygen atoms. These oxides are of great interest and importance for medicinal use due to their biocompatibility and their bio-enrichment [5]

Lavisse *et al*. studied the formation of various titanium oxides during the laser irradiation process on the titanium surface, where the following parameters were set: 5 kHz frequency, 500 $mm.s^{-1}$ scanning speed and matrix which was 20 μm. After the irradiation the samples were characterized by XPS and EDS and with the help of the phase diagram of Ti-O, the formation of Ti_6O, Ti_3O and Ti_2O for these laser parameters was observed [5]

Bioactive-based coatings deposited on the titanium alloys has been widely used in dental and orthopedic implants due to combined mechanical properties of the metals to suitable bioactive behavior for the replacement and bone regeneration [1, 2, 3, 4]. The

calcium phosphates are used in different clinical applications for the treatment of the bone system, including partial and total replacements, or coatings on implant surfaces [4, 5, 6, 7]. One of the most important properties of calcium phosphates is water solubility. In general, the higher the Ca/P ratio, the lower the solubility [6, 7]. The hydroxyapatite (HA) phase is used as biomaterial due to its chemical and structural similarity to the bone and teeth mineral part, excellent biocompatibility and osteoconductivity. However, clinical use is limited because of its slow in vivo biodegradation. Due to the limitations of the use of the HA phase, other phases of calcium phosphates, such as ACP, OCP, TCP and low crystallinity carbonated HA, have aroused interest in the use as biomaterials for tissue engineering [7, 8, 9]. Amorphous calcium phosphate (ACP-$Ca_3(PO_4)_2.nH_2O$ phase, Ca/P ratio: 1,5) occurs as an intermediate phase during the formation of the HA phase in the biological environment and in aqueous systems. The higher solubility of the ACP phase compared to the HA phase becomes an important property for its use as biomaterial, due to a higher rate of degradation in the biological environment. In biological systems, the rate of degradation of a material is strongly related to osteoconductivity, as well as plays an important role in the initial fixation of implants with the bone tissue. A soluble material enables the exchange of Ca^{2+} and PO_4^{3-} ions with the biological medium, improving bone growth [6, 10]. Several methods have been considered on preparation of bioceramic materials. The Biomimetic method was reported by Abe *et al.* (1990) [11], known as Synthetic Body Fluid - SBF, with ionic composition similar to blood plasma. The basic principle of this system was maintained a glass G substrate in SBF condition for 7 days at 37°C, where a continuous and homogeneous layer of crystallites of the hydroxyapatite phase similar to the biological was formed on the glass substrate. The characterization of the coating showed it was the carbonated hydroxyapatite of low crystallinity, very similar to of the biological hydroxyapatite present in the natural bone tissue. Likewise, modified biomimetic solution and method were proposed and tested, in order to accelerating the deposition process and altering the crystallinity of the coating, including acid, alkaline and thermal pre-treatments, and the use of solutions with varied composition and concentration [7, 12, 13]. Aparecida (2007) [14] has performed a modified Biomimetic method represented a great importance throughout the area of biomaterials. With the increasing interest in the use of other calcium phosphates with more promising properties than the HA phase, the author developed 6 different SBF solutions, which were called modified SBF, allowing the different phases of calcium phosphates of biological importance and the planning of the coating composition according to the solution used. The proposed modifications to the solution and the Biomimetic method allowed the control of the bioceramic coating composition.

Therefore, the aim of this work was to modify the surface of the Ti-15Mo alloy by laser beam Yb: YAG, and deposition of calcium phosphate bioactive ceramics by the biomimetic method using the modified SBF solution denoted by SBFm. The influence of the thermal treatment in the formation of calcium phosphate phases present in the laser beam irradiated surface coatings was evaluated.

EXPERIMENTAL SECTION

Laser-activated surface modification

Samples of titanium alloy (8x8x2mm) were subjected to Yb:YAG multipulse laser irradiation with a Laser OmniMark 20 F (λ = 1090 nm). The topography is related to surface morphology and roughness and surface energy will depend on the phases present [15]. The surfaces were modified under ambient pressure and air, using the

parameters (power, frequency and scan speed) with two fluency (ablation) of 1,91and 5,54 J/cm^2, Table S1. Two fluences were chosen in order to compare the oxides formed in both according to their respective values and the results obtained in their respective coatings. The laser parameters were set according to the procedure proposed by Braga [15]. After irradiation, the samples were treated ultrasonically and separately in solutions of ethyl alcohol, acetone and distilled water, followed by oven-drying, followed by oven-drying and characterization.

Coating of samples by the biomimetic method

The irradiated samples were immersed in modified SBF solution, identified as SBFm. This solution contained different ions in order to force the formation of the phases of interest. All the samples were coated at pH 7,25 and oven-dried at 37ºC for 4 days. The solutions were changed every 24 hours during the coating period. The reagents used were: NaCl (ACS), K_2HPO_4 (ACS) $CaCl_2$.$2H_2O$ (ACS) and HCl (ACS) - J. T. BAKER; Tris (hydroxymethyl) aminomethane (P.A) Mallinckrodt. Table S2 lists the ionic concentrations of the SBF solution employed here [3].

Preparation of the modified SBF solution and biomimetic coating of the substrates

The methodology of preparation of the modified SBFm solution, in order of addition of the salts used, was modified by the Biomaterials Group, in order to minimize the possibility of solution loss caused by its precipitation. The addition sequence of the salts initially proposed by Abe *et al.* (1990) and the one adopted in this work (Biomaterials Group) [6, 14]. Table S2 indicates the ionic concentrations of the SBFm solution used to obtain the calcium phosphate coatings on the surfaces irradiated by laser beam.

To obtain the calcium phosphate coatings using the modified SBF solution, the substrates were washed sequentially with alcohol, acetone and deionized water. All the samples were immersed in a volume of 50mL of modified SBF solution (pH 7.4), and remained in the oven for 4 days at 37ºC [6, 14]. The solution was exchanged every 24 hours for the purpose of promoting the super-saturation conditions of the solution and, consequently, inducing the formation of the calcium phosphate coating. After the period to obtain the coatings, the samples were air dried and submitted to thermal treatment at 350 and 600ºC for 2 hours, without atmospheric control. The heating and cooling rate used was 5°C/minute. The heat treatment used aims to increase the crystallinity of the calcium phosphates phases. All the coated and uncoated samples were characterized by scanning electron microscopy (SEM), X-ray diffraction and quantification by Rietveld refinement. The chemical bonds of the calcium phosphates coatings were characterized by vibrational infrared spectroscopy with a diffuse reflection DRIFT Collector^{TM}.

RESULTS AND DISCUSSIONS

The micrographies of the surfaces of the samples Ti-15Mo alloy submitted to laser beam using fluences (1,91 and 5,54 J/cm^2) are presented in Figure S1. It can be observed the increased fluency, due to longer exposure time of the laser beam to the alloy surface, producing typical morphologies with different surface energies. This can be explained through the formation of new structures (metal oxides) produced during the fast melt and solidification process [15, 16].

Figure S2 shows the diffractograms of samples 1 and 2 (1,91 and 5,54 J/cm^2). It can be verified a modified topography in the laser beam-treated surfaces, according to the ablation process. Therefore, it was possible to produce the formation of stoichiometric and non-stoichiometric oxides as predicted by the fluency equation [15]. X-ray diffraction spectra revealed, in addition to β-Ti peaks (#: 89-4913), the presence of TiO (#: 89-5010), Ti_3O (#: 76-1644), Ti_6O (#: 72-1471), TiO_2 (#: 77-441) [17].

Table S3 shows the oxide phases percentage obtained by Rietveld refinement, corresponding to laser beam-irradiated surfaces [18]. It is verified the higher the creep the greater the percentage of formation of oxides TiO_2 and Ti_3O_5 in the surface.

The fusion and solidification process by laser beam irradiation under ambient air, inducing the formation of titanium oxides with different degrees of oxidation. According Sorensen (1981), it can be an indication the laser energy favors the diffusion of O atoms or N, depending on the atmospheric condition used, as the rapid solidification to form these phases in a condition of non-equilibrium [19]. The presence of the Ti_3O, Ti_6O and Ti_3O_5, substoichiometric phases can be explained by interstitial oxygen diffusion in the Ti lattice [19]. Due to the high solubility of oxygen in titanium, this property leads to the formation of a large quantity of oxides, with an O/Ti ratio within the interval of 0-2. According Sorensen (1981), nonstoichiometric phases are found in several oxide systems at high temperatures, particularly, for cations may have various states (valences) of oxidation [19]. According György (2004), the β-Ti cubic phase at the surface can be understood in terms of the low oxygen content in the central area of the laser beam incidence angle, due to the dispersion of molecules [20]. The formation of TiO phase may be related to β-Ti phase [20]. The crystal lattice of titanium can absorb about 40% of atomic oxygen (18% w/w) in interstitial solid solution. Before the region of formation of TiO_2 (40% w/w), a phase is formed with lower oxygen content, the titanium oxide series Ti_nO_{2n-1}.

Coatings using SBF mod

Sbfm: 350°C

Figure S3 shows the X-ray diffraction patterns of the bioceramic coatings, obtained using the SBFm solution on the surfaces of the samples (1: 1,91 J/cm^2 and 2: 5,54 J/cm^2). In all samples the peaks corresponding to the phases of the Ti-15Mo alloy (#: 89-4913), a mixture of ACP phases, calcium deficient hydroxyapatite-HA_D (#: 46-905) and hydroxyapatite (#: 89-4405) were identified [17].

The ACP phase (amorphous calcium phosphate) occurs as a metastable phase in the early stages of the formation of calcium phosphates from aqueous solutions supersaturated with neutral or alkaline pH and during mineralization of living tissues. It may be converted directly to the HA phase or having as an intermediary phase the calcium-deficient HA (HA_D), as described [21]:

$$ACP \rightarrow HA$$

$$ACP \rightarrow HAD \rightarrow HA$$

The transformation of the ACP phase to the HA phase can occur directly from ACP1, whereas its transformation through the formation of intermediates occurs with ACP2 as another intermediate as described below [22]:

$$ACP1 \rightarrow HA$$

$$ACP1 \rightarrow ACP2 \rightarrow HA_D \rightarrow HA$$

The higher solubility of the ACP phase compared to HA becomes an important feature for its use as biomaterial, as it confers a higher degradation rate in the biological environment. A soluble material enables the exchange of Ca^{2+} and PO_4^{3-} ions with the biological medium, facilitating bone growth [22]. The spectra in the medium infrared region of the bioceramic coatings using the SBFm solution on the surfaces of samples 1 and 2 are shown in Figure S4. It can be observed all the spectra present bands in the regions between 1140-950 and 730 cm^{-1} indicating the asymmetric stretching of the P-O-P bond, and a band in the 1245 cm^{-1} region relative to the stretching of P = O [23, 24]. For the samples (1 and 2) the presence of a triplet in the region of 630, 570 and 490 cm^{-1} was identified. These bands may be associated with the stretching of the OH-group, the vibration of the PO_4^{3-} group, and unfolding of the PO_4^{3-} group [4, 24]. The band at 1650 cm^{-1} is due to the incorporation of water molecules. The bands at 1350 and 1464 cm^{-1} may be associated with the vibration of the CO_3^{2-} group, from the CO_2 of the atmosphere during the processes of dissolution, agitation, reaction and calcination, or to the formation of carbonated hydroxyapatite due to the possibility of substitutions occurring of the ions PO_4^{3-} or hydroxyl of the hydroxyapatite by the ion CO_3^{2-}, reaction below [21, 24, 25, 26].

$$2OH^-(\text{hidroxyapatite}) + CO_2(ar) \rightarrow CO_3^{2-}(\text{carbonated hidroxyapatite}) + H_2O$$

Figure S5 shows the morphologies of the coatings obtained in samples 1 and 2 are presented, using the SBFm solution and heat treated at 350ºC. The morphology of the ACP (amorphous calcium phosphate) phase is formed by spherical particles with diameters of 20 to 120 nm without structure, which are agglomerated of small particles and being identified as amorphous. Some authors describe the occurrence of two types of the ACP phase, called ACP1 and ACP2, which have the same composition but with characteristic morphology. The morphologies of the phases are, respectively, spherical grains and flocculent morphology [10, 27]. It was possible to identify the formation of a coating composed of different phases of calcium phosphates, evidenced by the presence of particles with different morphologies, characteristics of the ACP 1 and 2, HA_D and HA phases [21, 22].

Sbfm: 600°C

Figure S6 shows the X-ray diffraction patterns of the bioceramic coatings, obtained using the SBFm solution on the surfaces of the samples (1: 1,91 J/cm^2 and 2: 5,54 J/cm^2). In all samples the peaks corresponding to the phases of the Ti-15Mo alloy (#: 89-4913), a mixture of calcium deficient hydroxyapatite-HA_D (#: 46-905), hydroxyapatite (#: 89-4405) and tricalcium phosphate (β-TCP) (#:70-2065) were identified [17]. The formation of the β-TCP and HA phases may be related to the decomposition of the carbonated HA after the heat treatment, reaction below.

$$Ca_{10}(PO_4, CO_3)_6(OH)_2 \xrightarrow{\geq 600^\circ C} \beta - Ca_3(PO_4)_2 + Ca_{10}(PO_4)_6(OH)_2 + CO_2 \uparrow$$

A study by Kanazawa (1989) indicated the formation process of the β -TCP phase may be related to the decomposition of the non-stoichiometric hydroxyapatite phase, between 600 and 800 ° C, reaction below.

$$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}.nH_2O \rightarrow (1-x)Ca_{10}(PO_4)_6(OH)_2 + 3xCa_3(PO_4)_2 + H_2O_{(g)}$$

In Figure S7, the spectra in the medium infrared region of the bioceramic coatings can be observed using the SBFm solution on the surfaces of samples 1 and 2. It can be observed all the spectra present bands in the regions between 1200-940 e 760-730 cm^{-1} indicating the asymmetric stretching of the P-O-P bond, and a band in the 1250 cm^{-1} region relative to the stretching of P = O [23, 24]. For the samples (1 and 2), the bands were present at 3570 cm^{-1}, and at the regions 630, 570 and 495 cm^{-1}. These bands are associated with the stretching of the OH group, the vibration of the PO_4^{3-} group and the unfolding of the PO_4^{3-} group and refer to the probable formation of the hydroxyapatite phase [4, 28]. Bands in the 1765-1630 cm^{-1} region are attributed to the incorporation of water molecules. The bands in the regions of 1390-1500 cm^{-1} may be associated with the vibration of CO_3^{2-}, from the CO_2 of the atmosphere during the processes of dissolution, agitation, reaction and calcination, or the formation of carbonated hydroxyapatite due to the possibility of substitutions occurring of the PO_4^{3-} or hydroxyapatite ions of the hydroxyapatite by the CO_3^{2-} ion [1, 21, 24, 26].

Figure S8 shows the morphologies of the coatings using the SBFm solution and heat treated at 600ºC in samples 1 and 2. It was possible to identify the formation of a coating composed of different phases of calcium phosphates of biological interest, evidenced by the presence of particles with different morphologies and size characteristic of HA_D, HA and β-TCP phases.

CONCLUSIONS

The pre-modification of the surface by laser beam irradiation has influenced the physical-chemical interaction of the alloy / coating surfaces, improving the interaction with the calcium and phosphate ions. The physico-chemical characterization showed a multiphase coating with SBFm solution that can be obtained according to the heat treatment temperature used at 350°C (phases ACP1 and 2, HA_D, HA) and 600°C (HA_D, HA and β-TCP). The different morphologies and particle sizes obtained for the specific formed phases, in the thermal treatments at 350°C and 600 ° C, suggesting the different oxides formed by the laser beam obtaining influence the morphology of the phases of calcium phosphates. The best result was obtained for sample by fluency 5,54 J/cm^2, probably due to the higher amount of stoichiometric and non-stoichiometric oxides and the obtaining of phases of calcium phosphates that influence the process of formation and bone resorption, because the physico-chemical properties are fundamental to favor the phenomenon of osseointegration. The investigations carried out using the SBFm solution made it possible to obtain calcium phosphate coatings of biological importance according to the application and expected activity, promoting the optimization and the planning of the physico-chemical and biological reactions of the biomaterial surfaces.

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