

PATTERNED SURFACES FOR *IN VITRO* HYDROXYAPATITE GROWTH

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During the past several decades, hydroxyapatite (HA) has been used in many medical, orthopaedic, and dental applications. In this study, patterning by sequential implantation of Ca and P ion through a mask was used for selective surface modification of three classes of materials (stainless steel, silicon and silica glass). Some of the patterned samples were immersed and simply soaked in a solution resembling human blood plasma (simulated body fluid, SBF). Another solution-based approach, laser-liquid-solid interaction (LLSI), was also employed. The method included a deposition system and a process which allowed simultaneous interaction between a scanning laser beam and the SBF, and also irradiation of the patterned substrates during the laser-liquid interaction, thereby stimulating HA nucleation. The study showed that immediately after the end of the LLSI process in SBF, NaCl dendrite formations and CaP particles were observed, and could be considered as nuclei formed due to the applied LLSI, which further facilitated the growth of the layer.

(Received December 9, 2004; accepted January 26, 2005)

Keywords: Patterning, Ion implantation, Hydroxyapatite, Laser irradiation

1. Introduction

HA [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] is the basic material for the preparation of calcium phosphate (CaP) ceramics that are considered the most bioactive and biocompatible synthetic hard tissue material available, and the most likely candidates for use as bone and teeth substitutes [1]. CaPs are also constituents of kidney stones [2]. Thus, it is important to understand the mechanisms responsible for CaP formation, in order to heal damaged bones or to prevent diseases like kidney stones.

Ion beam surface modification is widely used to create surfaces with tailored biological activity. The formation of surface design by ion-beam micro-patterning gives the opportunity to control the chemistry and topography of the substrates on which HA is deposited or cells are attached, and could be exploited in biosensor technology or to model various *in vivo* situations.

There has been an increasing interest in exploring the potential of the simple immersion *in vitro* method from aqueous solution, in order to mimic the process of biological apatite formation [3]. Its disadvantage is the long time (usually weeks or months) required to produce HA coatings. Methods such as ultrasound, ultraviolet and microwave irradiations have been used to stimulate HA formation *in vitro*, by application of external fields [4,5]. Recently, the idea of a synergistic effect of the simultaneous exposure of the surfaces to radiation and water has been discussed [6].

In the present study, the influence of laser irradiation of the solid substrates immersed in SBF, and their consequent soaking in irradiated SBF for a short time, was examined. A laser was chosen due to its possibility for precise and flexible irradiation of small and complex shapes and for its high power density. Stainless steel, silicon and silica glass were used as representatives of the three classes of

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materials (i.e. metal, semiconductor and insulator), to create an experimental model for studying the formation of minerals like HA from aqueous solutions.

2. Experimental details

Substrates (8×8 mm) were prepared by standard mechanical treatment of n-type silicon (S) with (100) orientation, silica glass (SG) Herasil and stainless steel (SS) AISI 316. The substrate patterning included sequential implantation of Ca and P ions through a molybdenum mask. Its geometry and sizes, as well as the applied ion energies and doses, are discussed in [7]. The experimental set-up included an open deposition system, in which the supersaturated solution (400 ml SBF, pH 7.4, 37 °C) was placed in a reaction container and the temperature was controlled by a thermostat. Reagent-grade chemicals NaCl, NaHCO₃, KCl, K₂HPO₄-3H₂O, MgCl₂-6H₂O, Na₂SO₄-10H₂O, and CaCl₂-6H₂O were dissolved in distilled water and buffered at pH 7.4. The SBF was prepared with 1.5 times higher Ca and P concentrations than those used by Kokubo [3]. To evaluate the ability of the patterned samples to induce HA growth, a kinetic study by two methods was performed. In the first, a simple soaking process in SBF was applied for 4 or 24 h, with the samples immersed horizontally or vertically in the SBF. In the second, a LLSI process that allowed simultaneous interaction between a scanning pulsed laser (CuBr, $\lambda = 578.2$ nm, 330 mW, 50 MW/cm²) and the modified substrate immersed in SBF, with subsequent horizontal sample soaking in the irradiated SBF for 4 or 24 h was applied (samples SS/CaP_LLSI_4h and SS/CaP_LLSI_24h). To study the influence of the laser energy on the HA nucleation and growth, some samples (SS/CaP_LLSI_0h) were taken immediately out of the SBF after the laser irradiation. The laser beam was focused onto the surface of the sample and, by a scanning system, a design of seven squares at a distance of 200 μ m was created at the end of each sample. In this way, the centre of the substrate (about 6x6 mm) was not irradiated. The design was repeated 500 times, and the interaction time was within 5 min. The kinetic study was performed by FTIR spectroscopy (IRScope II, Bruker Optics IFS 66/S).

3. Results and discussion

The morphology of the grown layers was observed by SEM, coupled with EDX (Fig. 1). Irrespectively of the sample position in the SBF or the duration of soaking, the simply soaked samples induced a sponge-like layer on the non-implanted areas, and a flat dense layer with white piles on the implanted areas (Fig. 1, upper image). The measured elemental composition showed that the layer and the white piles consisted of Ca and P (inset). We consider that the grown layers simply repeated the morphology of the surface observed before the immersion in SBF by atomic force microscopy (data not presented). When the LLSI process was applied on the patterned surfaces in SBF, some samples were taken out immediately after the irradiation. Predominant dendrite formations, as well as randomly distributed white particles, were observed (Fig. 1, lower left and right). EDX analyses showed that the dendrites were composed of NaCl (inset in the lower left image). The white particles consisted of Ca and P (inset in the lower right image). The NaCl dendrites and the CaP white particles could be considered as nuclei formed due to the applied LLSI, which further facilitated the growth of the layer. Such a fast formation of nuclei was not observed when the samples were simply soaked in SBF. With 4 or 24 h soaking after the LLSI, a white inhomogeneous layer covered the whole surface of the three materials.

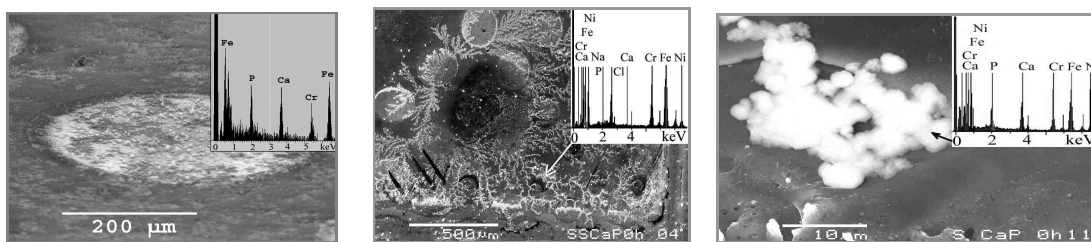


Fig. 1. SEM images of the layers grown after a simple soaking process (upper left) and immediately after the LLSI process (lower images). The insets show the corresponding elemental composition of the layers, measured by EDX.

The FTIR reflection spectra (Figs. 2a, b), recorded at different time intervals, demonstrate the kinetics of layer growth on the ion implanted SS in the region 800 - 4000 cm^{-1} (samples SS/CaP). The spectra in Fig 2a were recorded after the soaking process in horizontal and vertical positions of the samples in SBF, for periods of 4 or 24 h. The ν_1 and ν_3 P-O stretching vibrations characteristic of HA were registered in the region of 963 - 1113 cm^{-1} . Incorporation of carbonate (CO_3^{2-}) after 4 h of soaking was deduced from a shoulder at 874 cm^{-1} , characteristic of ν_2 C-O bending in CO_3 .

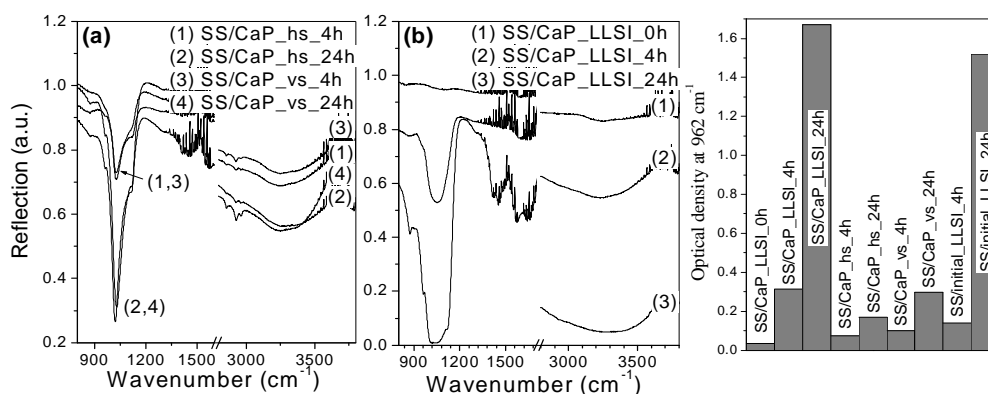


Fig. 2. FTIR spectra of the HA grown on SS/CaP by (a) a simple soaking in SBF; (b) a LLSI in SBF and subsequent soaking; (c) the histogram shows the kinetics of layer growth by the two applied methods.

It is possible that an acid calcium phosphate phase (i.e. HPO_4 -containing) was also present in small amounts in the HA layers. Since the wavelength of the P-OH mode coincides with the ν_2 C-O mode, this could not easily be proven. With increasing time of soaking (i.e. after 24 h), a weak peak splitting at 1422/1454 cm^{-1} , characteristic of ν_3 C-O stretching in biological apatite, was detected. A band at 1650 cm^{-1} and a broad band from 2500 to 3700 cm^{-1} in all spectra contributed to the H-O-H vibrations in adsorbed H_2O . It is known that biological HA contains CO_3 , and that the OH sites are mostly replaced by CO_3 ions. The samples which were vertically oriented in the SBF also induced a thin HA layer on their surfaces, as seen from the spectra. The FTIR spectra recorded from layers grown on ion implanted S and SG (S/CaP and SG/CaP, spectra not shown) were found to be strongly influenced by reflection from the underlying substrate. Nevertheless, ν_3 P-O at 1022 cm^{-1} (for S/CaP), at 1021-1055 cm^{-1} (for SG/CaP), and a shoulder at 1118 cm^{-1} , as well as a ν_1 P-O mode at 960 cm^{-1} were observed after 24 h of soaking of SG/CaP. After applying the LLSI process (Fig. 2b), the HA bands in the FTIR spectra of the samples taken out immediately after the laser irradiation (SS/CaP_LLSI_0h) were hardly discernible, and only a weak band was observed at 858 cm^{-1} . This was assigned to acid phosphates. HPO_4 -containing phosphates are known to cause imperfections of the HA structure, and in this experiment they were regarded as a precursor phase in the formation of HA. A formless peak at 1048 cm^{-1} and a shoulder at 870 cm^{-1} were detected after 4 h in SBF (SS/CaP_LLSI_4h) and were assigned to HA (ν_3 P-O stretching in PO_4) and to CO_3 (C-O bending in CO_3) respectively. Further soaking for 24 h (SS/CaP_LLSI_24h) increased the crystallinity and thickness of the layer, and revealed additional vibrations due to the HA and CO_3 (ν_1 , ν_3 P-O modes at 963 cm^{-1} , 1120 cm^{-1} and a well resolved peak splitting at 1418/1454 cm^{-1} , ascribed to ν_3 C-O stretching). Vibrations due to the H-O-H bending in H_2O were detected at 1645 and 3300 cm^{-1} . The reflection of the S and SG substrates also influenced significantly the FTIR spectra recorded from the layers grown after the LLSI (spectra not shown), but the shoulder at 1020 cm^{-1} (ν_1 , ν_3 phosphate region) was present.

An estimate of the relative layer thickness (in a. u.) for the optical density at 962 cm^{-1} was performed, based on the FTIR spectra of SS/CaP (Fig. 2c). The calculated optical density showed that the samples soaked for 4 h in the vertical position (SS/CaP_vs_4h) induced a HA layer on their surfaces with a thickness of 0.10. This was comparable to that of the samples soaked for the same period in the horizontal position (0.08, SS/CaP_hs_4h), which confirmed that the layer deposition was not simply a question of gravity. When horizontal or vertical soaking for 24 h was applied, the

optical densities increased to 0.17 and 0.30, respectively. In comparison to simple soaking, the employment of the LLSI process yielded a higher optical density: 0.32 for 4 h of subsequent soaking (SS/CaP_LLSI_4h) and 1.67 for the samples soaked for 24 h (SS/CaP_LLSI_24h). This was assigned to a synergetic effect of the simultaneous use of the laser irradiation, the solid substrate and the SBF. In addition, Ca and P ion implantation also contributed to a higher thickness of the HA layers compared to non-implanted laser-irradiated samples of SS, where the optical density was 0.14 for 4h-soaked samples and 1.52 for 24h-soaked. We suggest that the Ca and P, stored on the surface by ion implantation through the mask, enhanced the observed synergetic effect.

4. Conclusions

Ca and P ion-beam patterning was carried out on SS, S and SG materials. SEM, coupled with EDX, and FTIR spectroscopy showed that the simple soaking of the samples in SBF yielded the growth of a sponge-like CO₃-containing HA layer on the non-ion implanted areas, and a flat dense layer with white CaP particles on the implanted areas. This occurred irrespectively of the sample position in the SBF (horizontal or vertical) or the duration of soaking. When the LLSI process was applied on the patterned surfaces in SBF, NaCl dendrite formations and CaP particles were observed on the samples taken out immediately after irradiation. They both could be considered as nuclei formed due to the applied LLSI, which further facilitated the growth of the layer. With increasing time of soaking after the LLSI, a white inhomogeneous layer covered the whole surfaces of the three materials. The formless peaks seen in the FTIR spectra after 4 h soaking were assigned to acid phosphates, and were regarded as a precursor phase in the formation of more crystalline HA. In comparison to simple soaking, the LLSI process yielded a higher optical density of the layers, as calculated from the FTIR spectra of the SS/CaP samples. This was assigned to a synergetic effect of the simultaneous use of the laser irradiation, the Ca- and P-implanted solid substrate, and the SBF.

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