

Calcium Phosphate Formation on Alkaline-Treated Titanium by Biomimetic Synthesis

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Abstract- The hydroxyapatite coating on metal implants is a suitable method to create a bioactive surface and to increase the bone-implant bonding strength. In this research, at first the titanium surface was treated with NaOH solutions ; 5N and 10N at 60 °C for 24 h and 5N at 60 °C for 24 h followed by heating at 600 °C for 1 h. The samples were immersed in the simulated body fluid (SBF) for 28 days to generate a calcium phosphate coating on titanium substrates. The modified substrates and coatings were characterized using SEM and XRD. According to the results obtained in this work the concentration increase of the NaOH solution has influenced the grain boundaries, whereas the heat treatment at 600 °C has induced deeper and larger pores. Both the SEM images and the XRD spectra have illustrated the fact that the bone-like apatite had successfully been deposited on the titanium surface.

Key words: calcium phosphate, biomimetic, simulated body fluid

substrate [7]. It is a simple and cost-effective way to acquire a calcium phosphate coating at low temperature. In addition, coatings could be produced on to complex-shaped or/and microporous implants [3].

The bone formation around an implant is a complex process and it is not fully understood. The physicochemical and topographical surface characteristics of materials are some of the most influential factors in the improvement of osseointegration [9]. The surface energy also seems to be crucial for the cell attachment, even though the relationship between this parameter and the osseointegration event has only recently started to be discussed [10]. A simple method to estimate the bone-bonding potential of the materials is the immersion of these materials in the simulated body fluid. The bone-like apatite formed on biomaterials seems to activate the signaling proteins and cells that have an important role in the formation of bone [11, 12].

The aim of this study was to investigate the effect of alkaline and heat treatment of titanium substrates on the formation of calcium phosphate coating in simulated body fluid solution.

1- INTRODUCTION

TITANIUM has been widely used in orthopedic and dental materials because of their superior mechanical property and high corrosion resistance. However bioactivity of metal surface is not high enough to induce growth and fixation of the bone tissue [1]. The bioactive metal surfaces, prepared by chemical and heat treatments, led to the creation of a bonelike apatite layer in the SBF (simulated body fluid) [2]. The hydroxyapatite formation is an effective method to bioactivate the metal surface and enhance the implant – bone bonding strength [4]. The bone tissue direction is enhanced by the calcium phosphate coating on the metal implant surface [3]. Concerning the biomimetic method, it is one of the most promising techniques for the production of biomaterials in body environment conditions [5]. The biomimetic chemical process has been used to modify the cp-Ti surface with the formation of a deposit apatite layer (a calcium phosphate compound) [6].

In this method, a substrate was soaked in a solution that simulated the physiologic conditions, for a period of time enough to form a desirable calcium phosphate layer on the

II- MATERIALS AND METHODS

A. Treatment of cp-Ti with NaOH solution

The samples of cp- titanium were cut into sheets of (10 mm ×10 mm × 1 mm). The specimens were washed with distilled water and sequentially polished with abrasive papers (400-, 600-, 800-, 1000-, 1200-and 1500-grit). They were cleaned in acetone for 15 min, in 70% alcohol solution for 20 min and finally in distilled water for 20 min. Three samples were kept untreated and used as control. A total of three treated samples of each of the three types were used for a total of 9 treated samples. The detail of treated samples is summarized in Table 1.

TABLE I
THE TREATED SAMPLES

Designation of sample	Treatment
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S0	Untreatment
S1	5 N NaOH, 60 °C, 24 h
S2	10 N NaOH, 60 °C, 24 h
S3	5N NaOH, 60 °C, 24 h, 600 °C 1 h

B. SBF preparation and samples immersion in SBF

After the chemical treatment, the substrates were immersed in the simulated body fluid (SBF) solution for 28 days. The SBF solution had a pH value of 7.4 and an ion concentration nearly equal to those of human blood plasma. The SBF solution was prepared by dissolving the reagent grade chemicals of NaCl, NaHCO₃, KCL, K₂HPO₄.3H₂O, MgCL₂.6H₂O, CaCL₂ and Na₂SO₄ into distilled water and buffering at 7.4 with (TRIS) or tris-(hydroxymethyl)-amino methane and hydrochloric acid 1 M at 37 °C. The SBF solution was renewed every day. The ion concentrations of SBF are summarized in (Table 2.)

TABLE2
ION CONCENTRATIONS OF SBF IN COMPARISON WITH THOSE IN HUMAN BLOOD PLASMA

Ion concentrations (mM)	Blood plasma	SBF
Na ⁺	142.0	142.0
K ⁺	5.0	5.0
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	2.5
Cl ⁻	103.0	147.8
HCO ₃ ⁻	27.0	4.2
HPO ₄ ²⁻	1.0	1.0
SO ₄ ²⁻	0.5	0.5
pH	7.2-7.4	7.40

C. surface observation and analysis

The surface of the samples before and after soaking in the SBF was examined via a scanning electron microscope (LEO-4401). The Ca/P ratio of the calcium phosphate layer on the surface of samples was determined by EDAX analysis. Phase characterization of the coatings was carried out using X-ray diffraction analysis (XRD, PW1700, Philips).

II. RESULTS AND DISCUSSION

Titanium is a highly reactive metal, so that whenever it is exposed to air or other environments containing available oxygen, a thin layer of oxide is formed on the surface [6]. Therefore, the creation of a negatively charged microporous metallic surface is an effective way to initiate apatite nucleation from an SBF solution. The titanium oxide surface has been generally accepted to be negatively charged by OH⁻ in aqueous solution. The sub micrometer sized pores play a significant role in the Ca-P nucleation [3]. Fig. 1 illustrates the scanning electron micrographs of all titanium samples, treated in the different conditions before soaking in

the SBF. For the S1 specimen, homogeneously distributed nan oporous of 100-300 nm were produced (Fig. 1a).

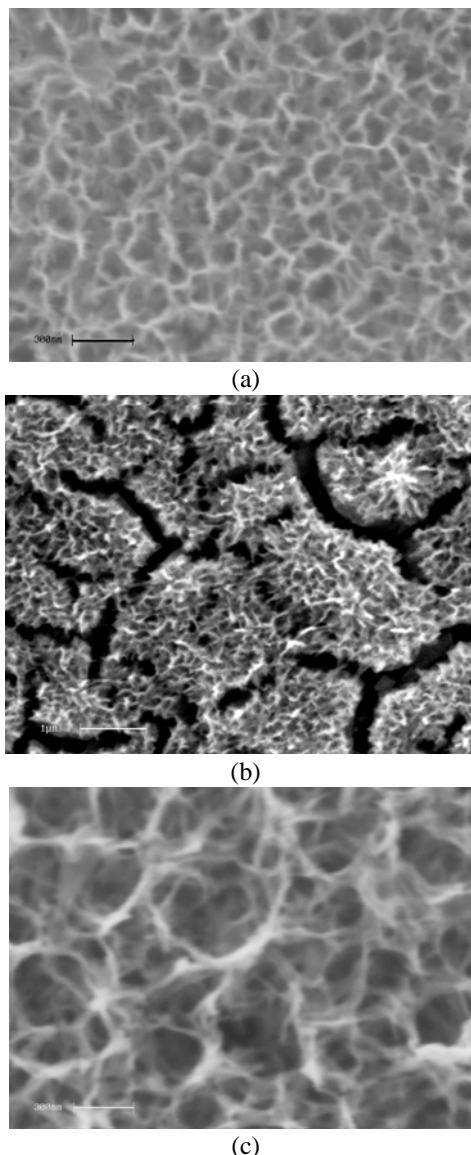
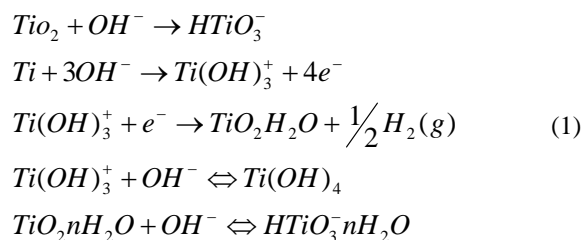


Fig1. SEM micrographs of the surface of Ti substrate which were subjected to(a) 5 N NaOH (b)to 10 N NaOH (c) 5 N NaOH and heated in 600 °C

It was observed that the size of the pores did not change significantly with the concentration of alkaline solution. Nevertheless, it seemed that the pores became deeper. The effects of grain boundary corrosion can be clearly observed on the surface of sample S2 because the high energy grain boundaries are more sensitive than the grains (Fig. 1b). After the alkaline treatment, the specimens were submitted to heat treatment at 600 °C, resulting in denser and larger sub micrometer scale pore sizes. The heat treatment at 600 °C caused the increasing of size and depth of pores. A pore size of about 300 nm was obtained in these samples (Fig. 1c) . Titanium metal is treated with NaOH aqueous solution, a titanate hydrogel layer can be formed on its surface in which negatively charged HTiO₃⁻ + nH₂O incorporating with

sodium ions can be found. During the alkaline treatment, the passive TiO₂ surface reacted in accordance with the equation (1):



After the heating treatment, the sodium titanate hydrogel layer will become amorphous [1]. In an *in vitro* study, Kim et. al exhibited that the surface layer of the alkali-treated titanium was mechanically weak. As a consequence, they recommended that this layer should be heated in order to form a more stable alkaline titanate layer [7]. With the immersion in the SBF solution, the sodium ions on the surface layer could replace the H₃O⁺ ions in the SBF, producing Ti-OH groups on the titanium surface to enhance the apatite nucleation. The Ti-OH groups would absorb Ca²⁺ by electrostatic force at first and, then, HPO₄²⁻ could reach the surfaces to form the apatite crystals. The Ca²⁺ and HPO₄²⁻ ions were consumed and diffused from the solution to the surface [4].

With this technique, it took 28 days to form the apatite deposition on titanium with a thickness of 60 μm, which is necessary for the bone tissue bonding. The SEM images of the samples, soaked in SBF for 28 days, revealed the formation of a nonporous calcium phosphate coating (Fig. 2). Increasing in the concentration of NaOH led to form coatings containing spherical grain morphology. As shown in Figure 2a and 2b the size of grains decreased with increasing in the concentration. The surface cracks of the coating could be attributed to an increase in the coating thickness due to an important reaction at grain boundaries. It was obvious that the S1 surface displayed a lower apatite nucleation level compared to S2 sample (Fig.2).

The heat treatment at 600 °C, following the alkaline treatment, could convert the sodium titanate hydrogel to an amorphous sodium titanate, which was stable and reduced the release rate of Na⁺ ions from the surface. The alkaline and heat treated samples (S3) offered a more uniform apatite nucleation than the alkali-treated ones. This phenomenon was caused by the slow Na⁺ ion release rate, which diminished the growing rate of the apatite crystals on the surface (Fig. 2c). When the pore size on the surface was big (Fig. 1c), the solute reached the bottom of the pores easily. Therefore, the apatite nuclei could grow, due to the continuous Ca²⁺ and HPO₄²⁻ absorbance. As a consequence, not only wide porous structures were created but also dense and uniform coatings were obtained on the whole surface (Fig. 2c). The EDAX resulting data displayed that, for the S₃ specimen, Ca and P could be detected when the samples

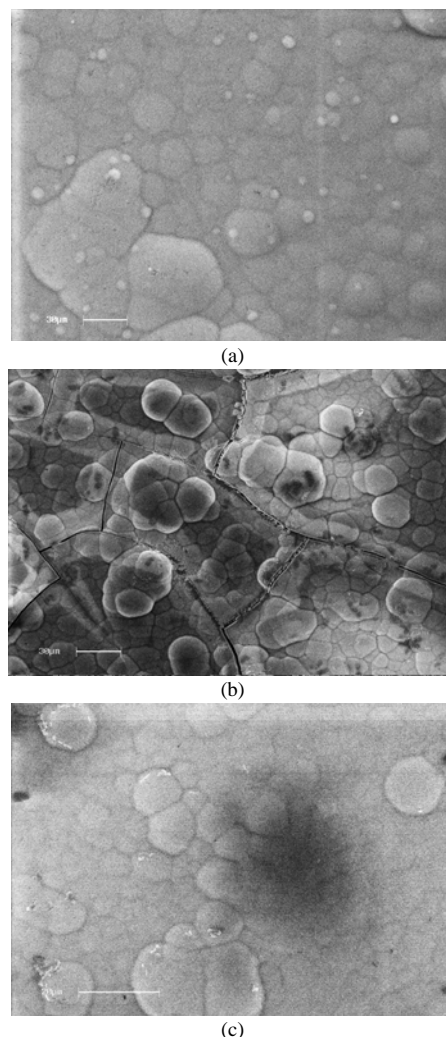


Fig. 2. SEM images of titanium treated after immersing in SBF for 28 days,

(a) S₁, (b) S₂, (c) S₃

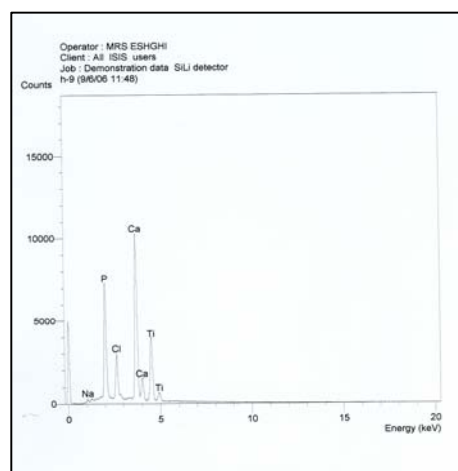


Fig. 3. EDAX analysis of specimen S₃

were soaked in the SBF for 28 days. As it is evident from Fig. 3, the Ca and P peaks are intensive. Table 3 lists the Ca/P atomic ratio of the above mentioned samples. Clearly, each one of those ratios is similar to that of a human bone. Fig. 4 demonstrates the XRD patterns of the separated coating from the S3 specimen surface after a period of 28 days in the SBF. The peaks of hydroxyapatite and calcium phosphate hydrate (hydrate TCP) ($Ca_3(PO_4)_2 \cdot xH_2O$) are observed.

TABLE 3
THE EDAX ANALYSIS OF SAMPLES SOAKED IN SBF FOR 28 DAYS

samples	Na	Mg	P	Ca	Ti	Total (%)	Ca/P
S1	0.53	1.28	34.43	63.69	0.08	100	1.84
S2	0.57	1.25	34.96	62.79	0.16	100	1.79
S3	0.71	1.58	35.42	62.26	0.03	100	1.75

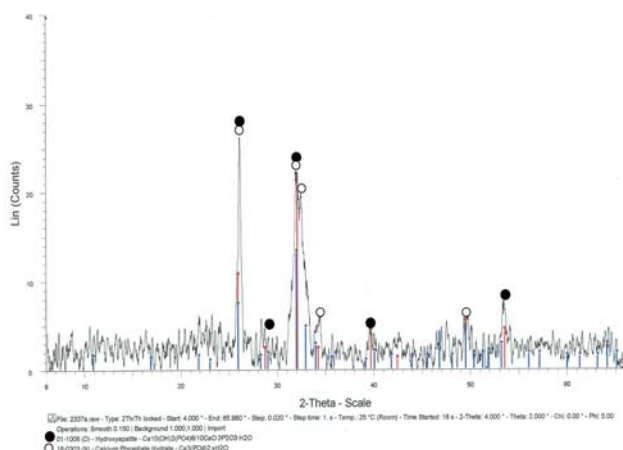


Fig. 4. XRD pattern of coating after 28 days in SBF. ●:Hydroxyapatite. ○: Calcium phosphate hydrate.

IV- CONCLUSIONS

This research described the studies that were conducted on the nanostructure bone formation. In detail, the apatite layer preparation on the Ti substrate surface was developed via a biomimetic method. A coating with uniform, homogeneous and sub micro crystallites was obtained, using a slow nucleation process. The thickness of the apatite layer was found to increase as the soaking period increased. The calcium phosphate microstructure was affected by two factors: firstly, the chemical compounds of the surface and, secondly, the porous size. A bioactive microporous titanium surface was formed by alkaline treatment. The concentration increase of the NaOH solution influenced the grain boundary, whereas the heat treatment at 600 °C induced deeper and larger pores in comparison with those of the alkali treated specimens.

Both the SEM images and the XRD spectra illustrated the fact that the bone-like apatite had successfully been deposited on the titanium surface.

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