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Hydroxyapatite coating of titanium by biomimetic method

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The biomimetic method was used in order to deposit, on titanium substrates, an hydroxyapatite (HA) coating. The bioactive HA layer was obtained by using, in the first stage of the process, a glass having the composition $2.5CaO \cdot 2SiO_2$ different from the one proposed for the application of the biomimetic method. This glass can be obtained via sol–gel, a method that allows one to obtain, easily, very pure products. The growth of HA crystals was confirmed by Fourier transform infrared, SEM, EDS and X-ray photoelectron spectroscopy (XPS) results. The experimental results suggest that, as reported in the literature for other supports, the silicate ions released from the glass in the first stage bind themselves to the titanium support. In particular, from XPS analysis it is evident that the titanium substrate is well covered by a calcium phosphate layer of the type of HA.

Introduction

A new class of biomaterials was recently discovered [1, 2]; they are known as bioactive ceramics because of their ability to bind to living bone. The contact with blood plasma, in fact, has the surface covered with an hydroxyapatite (HA) layer, the osteoblasts can proliferate on. Recently, it was found [2–5] that a glass of composition $7CaO \cdot 5P_2O_5 \cdot MgO \cdot 0.056CaF_2$ can help to form the same kind of the apatite layer on the surface of other materials both organic (polyethyleneterephtalate, polymethylmethacrylate, polyethersulfone, nylon6, polytetrafluoroethylene, polyvinylalchol hydrogel) and inorganic (alumina, ceramic, carbon cloth, silicon single crystal). In the first stage of the biomimetic method, the substrate is soaked in a simulated body fluid (SBF) in contact with a glass of composition $7CaO \cdot 5P_2O_5 \cdot MgO \cdot 0.056CaF_2$. This stage is reported [2–5] to be essential to form HA nuclei that grow in the subsequent stage, when the support is soaked in a solution (1.5 SBF) with ions concentrations 1.5 times higher than SBF.

It is known that the pure titanium and some titanium alloys have been widely used to make dental and orthopedical implants by virtue of their excellent biocompatibility. Several studies demonstrated that the bone tissue could form on titanium surface with a very thin (order of magnitude 10 nm) cementum layer in between [6–8]. This phenomenon was named osseointegration [9]. Generally, it takes several months for titanium implants and bone tissue to reach integration. Hence, there is growing interest in shortening the process toward osseointegration. The formation of a HA layer on the titanium could improve the bonding to living bone and lead to higher stability of the interface by additional bioactive fixation. In this paper, it is shown that a HA layer can be formed on titanium by means of the biomimetic method using, in the first stage of the process, a glass of the binary $CaO–SiO_2$ system. As it is known [10–12] glasses of the system $CaO–SiO_2$ can be obtained via sol–gel, a method that allows to easily obtain very pure products.

Experimental

The glasses of composition $2.5CaO \cdot 2SiO_2$ was prepared by melting analytical grade reagents $CaCO_3$, and SiO_2 in a platinum crucible in an electric oven for 4 h at 1500 °C. The melt was quenched by plunging the bottom of the crucible into cold water.

A SBF with ion composition, reported in Table I, nearly equal to blood plasma and an aqueous solution (1.5 SBF) with ion concentration 1.5 times those of SBF were prepared by dissolving reagent grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 in distilled water as reported by other researchers [2–4]. They were buffered at pH 7.25 by using trishydroxymethylaminomethane (trizma base)

TABLE I Composition of the SBF

Ion	SBF (mM)
Na^+	142.0
K^+	5.0
Mg^{2+}	1.5
Ca^{2+}	2.5
Cl^-	147.8
HCO_3^-	4.2
HPO_4^{2-}	1.0
SO_4^{2-}	0.5

and trishydroxymethylaminomethane-clorid acid (trizma-HCl). In order to apply the biomimetic method, samples of titanium were first soaked, in contact with powdered (150–300 μm) $2.5CaO \cdot 2SiO_2$ glass in SBF for four days. The amount of titanium and SBF were such to have a ratio of exposed surface to volume of SBF equal to $S/V = 10\,mm^2/ml$ SBF, as the one reported in the original papers on this method [3, 4]. Then, the substrates were soaked for seven days in 1.5 SBF, whose volume was such to have the same S/V ratio. The compositional and structural changes occurring at the surface were followed by means of Fourier transform infrared (FTIR), SEM, EDS and X-ray photoelectron spectroscopy (XPS).

FTIR transmittance spectra were recorded in the 500–1400 cm^{-1} region using a Mattson 5020 system equipped with a single reflection attenuated total reflectance (ATR) accessory, with a resolution of 2 cm^{-1} and 20 scans. The FTIR spectra were elaborated by means of a Mattson software (first macros). FTIR was performed on small disks of 5.0 mm in diameter and 2.0 mm thick soaked as such in SBF and 1.5 SBF.

An electron microscope (Cambridge Stereoscan 240) equipped with an energy dispersive analytical system (EDS) LINK AN 10000 was used in order to verify the morphology of the coated sample and to make a qualitative elemental analysis.

XPS was used as analytical technique; it is appropriate for elemental analysis of surface, chemical state identification of surface species and relative quantification of elements [13].

Surface chemical composition was studied by XPS technique in a VG ESCALAB MKII spectrometer equipped with a 5-channeltron detection system. A standard Al-Kα excitation source (Hν = 1486.6 eV) was employed. The samples were positioned with respect to the analyzer at the electron takeoff angle normal to the surface, and the analyzer was set to constant pass energy $E_p = 50\,eV$. The binding energy (BE) scale was calibrated by measuring the reference peak of C 1s (BE = 285.0 eV) from the surface contamination. The accuracy of the measured BE was ± 0.2 eV. The relative concentration of chemical elements were calculated by standard quantification routine, including Wagner *et al.* energy dependence of attenuation length [14].

Results and discussion

In Fig. 1 the FTIR spectrum of a soaked sample is reported. The peaks at 1035 and 580 cm^{-1} correspond to P–O stretching and bending vibration modes [15], respectively. Therefore, FTIR data suggest that the method is effective in promoting the formation of a phosphate surface layer.

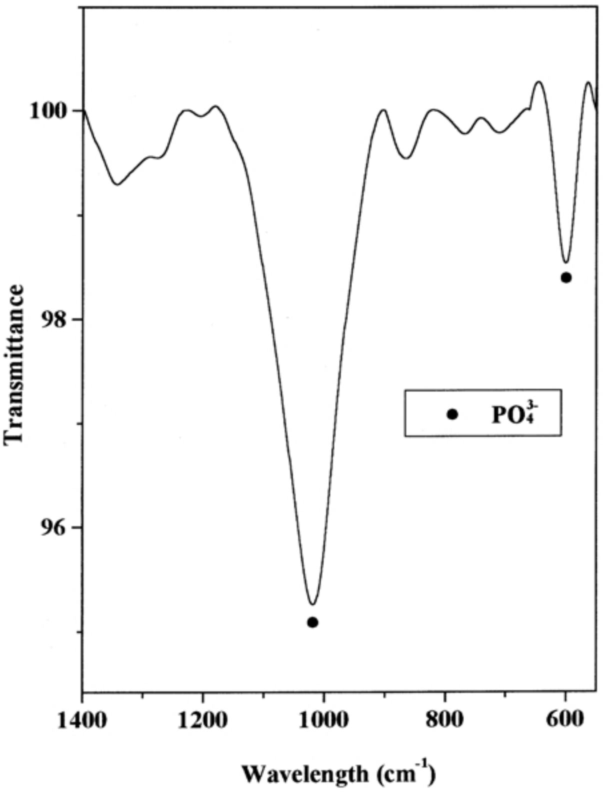

Figure 1 IR spectrum of a titanium substrate after biomimetic method.

The effectiveness of the method is confirmed by SEM, EDS and XPS results. In Figs 2(a), 3 and 4(a), the SEM micrographs of a sample soaked for four days in SBF, in contact with $2.5CaO \cdot 2SiO_2$ glass, and seven days in 1.5 SBF are shown. The results of SEM analysis at low magnifications are reported in Fig. 2(a). As it can be seen the whole surface is covered by a calcium phosphate layer. Fig. 3 shows a SEM micrograph of the same sample at higher magnifications. The characteristic apatite globular crystals are clearly visible. Fig. 4(a) shows a typical crystalline agglomerate of the apatite layer, which looks as it is made of a large number of fused together globular crystals. As it can be seen, the EDS results reported as Figs 2(b) and 4(b) confirm that the surface layer observed in the SEM micrograph is composed of calcium phosphate. In the EDS spectrum of Fig. 2(b) a peak, of very low intensity, of Si is also observed. The presence of Si is coherent with the mechanism suggested in the literature [16]. In fact, calcium and silicate ions are released from the surface of bioactive glasses owing to the reactions of cation exchange and hydrolysis occurring during soaking in SBF. The calcium ions increase the ionic activity product of the apatite in the narrow gap between the substrate and the glass particles. Moreover, it is believed [16] that the silicate ions bind themselves to the surface of the substrate and catalyze, there, the nucleation of apatite, in the same way as on the surface of the bioactive glasses [2].

The binding energy values of the main XPS peaks (Ca_{2p}, P_{2p}, Ti_{2p}, O_{1s}) in the investigated samples are

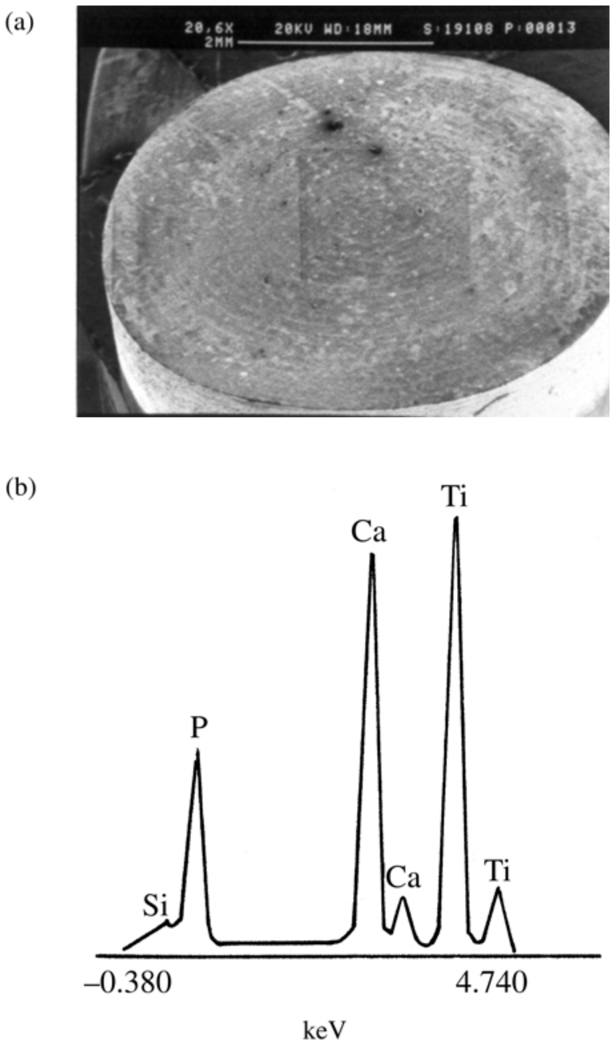

Figure 2 (a) SEM micrograph at low magnification (21 ×) of the surface of a titanium sample after biomimetic method; (b) EDS spectrum relative to whole surface.

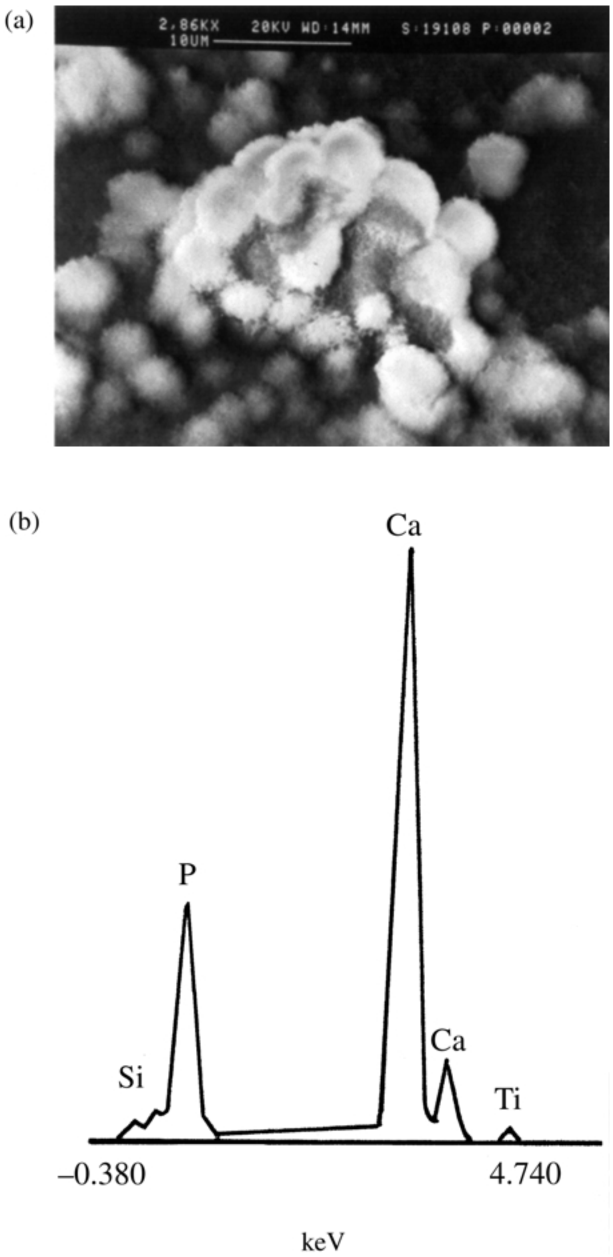

Figure 4 (a) SEM micrograph (2860 ×) of a typical agglomerate of apatite globular crystals; (b) corresponding EDS spectrum.

reported in Table II together with the surface chemical composition. The atomic ratio trend of the elements present on the surface of the samples is showed in Figs 5 and 6. In all the investigated samples (Ti substrate, two samples A and B of Ti–HA obtained by biomimetic method) the binding energy values for Ca_{2p}, P_{2p}, Ti_{2p} are the ones characteristic of the elements in their normal oxidation states: Ca^{2+} at $BE = 347.4$ eV, P^{5+} at $BE = 133.3$ eV, Ti^{4+} at $BE = 458.5$ eV, in good agreement with literature data reported for $Ca_3(PO_4)_2$ HA and TiO_2 (due to the grown of an oxide native layer on the metallic substrate) [17, 18]. As a result of XPS peak fitting, three components are present in the O_{1s} spectra of all the investigated coatings, at $BE = 529.8$ eV, $BE = 531.4$ eV, and $BE = 532.5$ eV, which can be assigned to oxide species (O^{2-}), hydroxide and phosphate groups (OH^-, PO_4^{3-}) and adsorbed water (H_2O) respectively. The XPS analysis of the coatings obtained by the biomimetic method shows that the substrate is completely covered by a calcium phosphate layer, because no Ti peaks are observed in photoemission spectra, as reported in Table II, and these coatings could be due to phosphate calcium compound similar to the HA. The stoichiometric HA $Ca_{10}(PO_4)_6(OH)_2$ must have

Figure 3 SEM micrograph at high (1780 ×) magnification of the surface of a titanium sample after biomimetic method.

TABLE II Atomic ratio and BE of main elements present on the surface of the investigated samples

Samples		O 1s			Ca 2p	P 2p	Ti 2p	Ca/P
		Oxide	PO_4^{3-}, OH	H_2O				
Ti substrate	At. Ratio	1.86	0.861	1.09	—	—	1	
	BE (eV)	530.0	531.5	533.0	347.5		458.5	
Ti + HA	At. %	—	2.50	0.304	1	0.624	—	1.60
Sample A	BE (eV)	—	531.5	533.0	347.4	133.3	—	
Ti + HA	At. %	—	3.13	0.410	1	0.613	—	1.63
Sample B	BE (eV)	—	531.5	533.0	347.4	133.2	—	

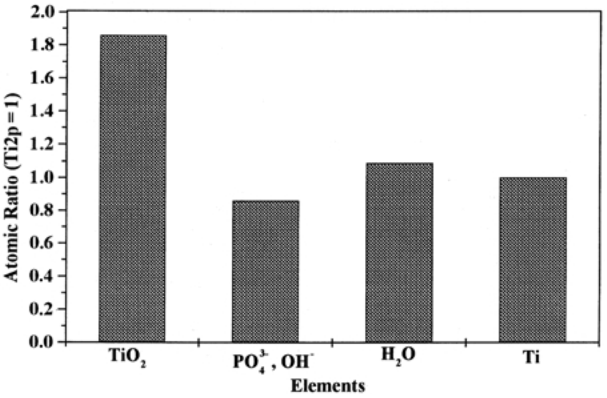

Figure 5 Atomic ratio of the main elements present on the surface of a titanium sample.

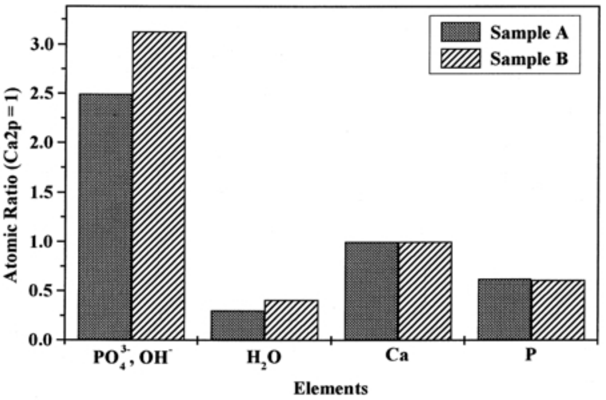

Figure 6 Atomic ratio of the main elements present on the surface of two titanium samples after biomimetic method.

a ratio of Ca/P $\cong$ 1.67. The values of this ratio for the analyzed samples reported in Table II (1.60 and 1.63 for the sample A and B, respectively) are very close to the stoichiometric value for the HA. Therefore, the BE value obtained for this compounds and the ratio Ca/P are indicative that the coatings on the Ti substrate obtained by the biomimetic preparation, is covered by a layer of phosphate calcium of the type of HA.

Conclusions

The experimental results proved that a HA layer can be obtained by means of the biomimetic method, using, in the first stage, a glass of composition $2.5CaO \cdot 2SiO_2$. The results well agree with the mechanism reported in the literature. The coating of HA on titanium by the biomimetic process is expected to improve the interface titanium–bone and make this system very interesting for repairing hard tissue.

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References

1. L. L. HENCH, *J. Amer. Ceram. Soc.* **74** (1991) 1487.
2. T. KOKUBO, Bol. Soc. Esp. Ceram. Vid. (Proc. XVI Int. Cong. Glass. Madrid, vol. 1, 31-C1 (1992) 119.
3. T. KOKUBO, K. HATA, T. NAKAMURA and T. YAMAMURO, in "Bioceramics", Vol. 4, edited by W. Bonfield, G. W. Hasing and K. E. Tunner (Butterworth-Heinemann, Guildford, London, 1991) p. 113.
4. M. TANAHASHI, K. HATA, T. KOKUBO, M. MINODA, T. MIYAMOTO, T. NAKAMURO and T. YAMAMURO, in "Bioceramics", Vol. 5, edited by Kobunshi, Kankokai, Yamamoro, T. Kokubo and T. Nakamura (Kyoto, 1992), p. 57.
5. F. BRANDA, A. COSTANTINI, G. LAUDISIO, A. BURI, L. AMBROSIO, *J. Mat. Sci.* **34** (1999) 1319–1322.
6. T. ALBREKTSSON, P.-I. BRANEMARK, H.-A. HANSSON, B. IAVARSSON and U. JOHSSON, in "Clinical Applications of Biomaterials," edited by A. J. C. LEE, T. ALBREKTSSON and P.-I. BRANEMARK (Wiley, Chichester, 1982) pp. 167–177.
7. L. SENNERBY, P. THOMSEN and L. E. ERICSON, *Mater. Med.* **3** (1992) 262–271.
8. D. E. STEFLIK, A. L. SISK, G. R. PARR, L. K. GARDNER, P. J. HANES, F. T. LAKE, D. J. BERKERY and P. BREWER, *J. Biomed. Mater. Res.* **27** (1993) 791–800.
9. P.-I. BRANEMARK, *J. Prosth. Dent.* **50** (1983) 399–410.
10. N. P. BANSAL, *J. Mat. Sci.* **27** (1992) 2992.
11. A. BURI, A. COSTANTINI, G. LAUDISIO, M. CATAURO and F. BRANDA, *J. Therm. Anal.* **49** (1997) 863–868.
12. G. LAUDISIO, M. CATAURO, A. COSTANTINI, F. BRANDA, *Thermochim. Acta* **322** (1998) 17–23.
13. D. BRIGGS, M. P. SEAH, in "Practical Surface Analysis" (John Wiley & Sons, Chichester, England, 1990).
14. S. KACIULIS, G. MATTOGNO, A. NAPOLI, E. BEMPORAD, F. FERRARI, A. MONTENERO and G. GNAPPI, *Relat. Phenom.* **95** (1998) 61.
15. Y. KIM, A. E. CLARK and L. HENCH, *J. Non-Cryst. Solids* **113** (1989) 195.
16. M. TANAHASHI, T. YAO, T. KOKUBO, M. MINODA, T. MIYAMOTO, T. NAKAMURA and T. YAMAMURO, *J. Mater. Sci.: Mater. Med.* **6**(6) (1995) 319.
17. J. F. MOLDER, W. F. STICKLE, P. E. SOBOL, K. D. BOMBEN, in "Hand-book of X-Ray Photoemission Spectroscopy" (Phys. Electronics, Eden Prairie, USA, 1995).
18. S. KACIULIS, G. MATTOGNO, L. PANDOLFI, M. CAVALLI, G. GNAPPI, A. MONTENERO, *Appl. Surf. Sci.* **151** (1999) 1–5.

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