

Calcium phosphate interactions with titanium oxide and alumina substrates: an XPS study

FLORENCE BARRÈRE^{1,2,3}, ALBERT LEBUGLE³, CLEMENS A. VAN BLITTERSWIJK^{1,2}, KLAAS DE GROOT^{1,4}, PIERRE LAYROLLE^{1*}, CHRISTIAN REY³

¹*IsoTis NV, PO Box 98, 3720 AB Bilthoven, The Netherlands*

²*iBME, Twente University, The Netherlands*

³*CIRIMAT, Laboratoire des Phosphates, Toulouse, France*

⁴*BRG, Leiden University, The Netherlands*

E-mail: pierre.layrolle@isotis.com

Besides the excellent mechanical properties of titanium and alumina (Al₂O₃) in the case of load bearing applications, their bone-bonding properties are very different. In osseous environment, Al₂O₃ ceramic is encapsulated by fibrous tissues, whereas bone can bind directly to titanium, via its natural titanium dioxide (TiO₂) passivation layer. So far, this calcification dissimilarity between TiO₂ and Al₂O₃ was attributed to respectively their negative and positive surface charge under physiological conditions. The present study aims at studying the chemical interactions between TiO₂ and Al₂O₃ (phase α) with the diverse ions contained in simulated body fluids (SBFs) buffered with trishydroxymethyl aminomethane (TRIS) at pH = 6.0 and pH = 7.4. After 1 h of immersion, TiO₂ and α -Al₂O₃ powders were analyzed by X-ray photoelectron spectroscopy (XPS). The results indicated that Ca and HPO₄ groups were present on TiO₂ surface. In addition, HPO₄ groups were found to be in a higher amount than Ca on TiO₂, which does not comply with the surface charge theory. With regard to Al₂O₃, little HPO₄ but no Ca was detected on its surface, and TRIS bound to Al₂O₃ substrate in all of the immersion experiments. The fact that both Ca and HPO₄ were present at the vicinity of TiO₂ might be at the origin of its calcification ability. On the other hand, Al₂O₃ did not show any affinity towards Ca and HPO₄ ions. This might explain the inability of Al₂O₃ substrate to calcify.

© 2003 Kluwer Academic Publishers

Introduction

In hip and joint surgery, titanium (Ti) and alloys, and alumina ceramics (Al₂O₃) are widely used because of their excellent mechanical properties, which are required for load-bearing applications. Both biomaterials have a successful clinical history in load-bearing applications, they are biocompatible, but they react differently in the body. In a bony environment, a rather thick fibrous tissue envelope usually covers Al₂O₃ implants. The presence of fibrous tissue layer affects bone fixation on the implants, leading at long term to decalcification [1, 2], and to the loosening of the implant [3, 4]. With regard to Ti implants, direct bone contact is often observed without intervention of fibrous tissues [5–8]. The superior osteointegration of titanium is attributed to the passive oxide (TiO₂) layer forming immediately on the surface in air or aqueous solutions, which could calcify.

It is clear that the physico-chemical properties of these two sorts of biomaterials affect their calcification ability. *In vitro* studies have shown that Al₂O₃ is not an efficient

calcium phosphate (Ca–P) nucleator when soaked in calcifying solutions, whereas titanium with its passive oxide layer (TiO₂) is [9, 10]. Li attributed this phenomenon to the difference of surface charge of these substrates immersed in simulated body fluids (SBFs) [10]. At physiological pH, Al₂O₃ is positively charged, whereas TiO₂ is slightly negatively charged [10, 11]. Additionally, TiO₂ surface has a gel-like structure in the presence of aqueous fluids [11–14]. With regard to TiO₂ surface charge, one should expect a higher amount of cationic species such as Ca²⁺ than anionic species such as HPO₄²⁻ on TiO₂ substrate. Experimentally, the initiation of Ca–P formation on Ti substrates from supersaturated calcifying solutions remains under discussion. Depth profile analyses of Ca–P coatings formed in SBF indicate that calcium and magnesium are detected deeper at the coating/substrate interface than HPO₄ groups [16, 17]. On the other hand, analyses performed on Ti materials soaked for various times in calcifying solutions indicate usually first the presence of HPO₄²⁻ on

* Author to whom all correspondence should be addressed.

the substrate, and second a lower amount of Ca^{2+} compared with HPO_4^{2-} [13, 18]. With regard to Al_2O_3 surface charge, one should expect interaction with HPO_4^{2-} due to its positively charged surface at physiological $\text{pH}=6-7$. If it is the case, why does Ca-P not form on Al_2O_3 substrate?

The goal of this study was to analyze the surface of TiO_2 and Al_2O_3 powder soaked in SBF solution at $\text{pH}=6.0$ and $\text{pH}=7.4$. Both substrates were analyzed by X-ray photoelectron spectroscopy (XPS) in order to quantify which elements are bound to the substrate, and to evaluate which chemical interactions favor the formation of Ca-P on inorganic substrates, and thus their calcification ability.

Materials and methods

Materials

Titanium oxide powder (TiO_2 , pure anatase, Prolabo) had an average particle size of 40 nm. Aluminum oxide (Al_2O_3 , pure α -phase) had an average particle size of 100 nm, and was a gift from Dr A.J.A. Winnubst (Twente University, The Netherlands).

Methods

First, two SBF solutions were prepared by dissolving $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, NaHCO_3 and NaCl . These solutions were buffered with trishydroxymethyl aminomethane (TRIS, $(\text{CH}_2\text{OH})_3\text{CNH}_2$) and HCl (1 M). SBF-7.4 was buffered at $\text{pH}=7.4$ whereas SBF-6 was buffered at $\text{pH}=6.0$. Second, Ca- and HPO_4 -solutions were prepared by dissolving on one hand NaCl and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and on the other hand NaCl and $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in similar concentrations as SBF solution. These so-called Ca- and HPO_4 -solutions were buffered respectively at $\text{pH}=6.0$ and $\text{pH}=7.4$. They were named Ca-6, Ca-7.4 for Ca-containing solution respectively buffered at $\text{pH}=6.0$ and $\text{pH}=7.4$, and HPO_4 -6, HPO_4 -7.4 for HPO_4 -containing solutions respectively buffered at $\text{pH}=6.0$ and $\text{pH}=7.4$. Ca-6, Ca-7.4, HPO_4 -6, and HPO_4 -7.4 had a similar ionic strength as SBF solution. All of the chemicals were reagent grade (Prolabo) and they were precisely weighted. Table I summarizes the composition of the various solutions.

XPS analyses

TiO_2 and Al_2O_3 powders were individually soaked in Ca-6, Ca-7.4, HPO_4 -6, HPO_4 -7.4, SBF-6 and SBF-7.4 at room temperature (250 mg/500 ml). After 1 h of soaking,

the powder was filtrated through a Millipore filter (0.2 μm). Thereafter, the particles were gently washed with demineralized water and dried in a dessicator overnight. XPS allowed the determination of the elemental composition of the first surface atomic layers up to a depth of 30–40 Å and the identification of surface species. The analyses were performed using a spectrometer Escalab Mk II (VG Scientific, France). X-ray source was generated by the $\text{Al K}\alpha$: 1486.6 eV, with a power of 250 W. The resolution of the spectrometer was 1 eV. The aliphatic C1s peak (284.6 eV) was used as an internal standard to correct the peak shifts due to the accumulation of surface charge on insulating samples. The accuracy of the binding energy was ± 0.1 eV. The relative atomic concentrations were determined on C1s, O1s, Ti2p, Ca2p, Mg1p and Al2p peaks by integrating with Lorentzian and Gaussian functions and using the Scofield sensitivity coefficients [15]. The peaks were fitted using the VGS 5000 ESCA software. The atomic ratios calculated with the semi-quantitative analyses were given with an uncertainty of about 5%. Three determinations have been performed for each sample.

Results

TiO_2

The general spectrum of TiO_2 powder prior to immersion exhibited the Ti2p, O1s and C1s bands (Fig. 1(a)). The Ti2p band was composed of $\text{Ti}2\text{p}_{3/2}$ at 458.3 eV and $\text{Ti}2\text{p}_{1/2}$ at 464.1 eV (Fig. 1(b)). The O1s bands were asymmetrical, with shoulders tending to higher binding energies (Fig. 1(c)). After deconvolution, raw TiO_2 exhibited a three-component O1s band that can be attributed to a combination of the oxygen from the titanium dioxide, titanium hydroxide and associated water molecules onto the substrate [12].

Table II summarizes the band positions of the various elements detected for all the studied samples. The position of the O1s and $\text{Ti}2\text{p}_{3/2}$ peaks did not change with the diverse immersions, indicating that the TiO_2 surface had not changed. When Ca^{2+} ions were present in the solution, the $\text{Ca}2\text{p}_{3/2}$ peak was detected between 436.3 and 436.7 eV. When HPO_4^{2-} ions were present in the solution, the P2p peak was detected between 132.5 and 133 eV. The values were in the range of binding energies determined for orthophosphate groups [13]. Magnesium was never detected.

As an example, Fig. 2 displays the XPS spectrum of TiO_2 powder immersed into SBF-7.4 solution. In addition to the initial Ti2p, O1s and C1s bands, $\text{Ca}2\text{p}_{3/2}$ and $\text{Ca}2\text{p}_{1/2}$ bands at 346.3 and 349.7 eV

TABLE I Composition of the various solutions in mM

	pH	NaCl	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	NaHCO_3	TRIS
SBF-6	6.0	140.0	1.5	2.5	1.0	4.2	50.0
SBF-7.4	7.4						
Ca-6	6.0	140.0	—	2.5	—	—	50.0
Ca-7.4	7.4						
HPO_4 -7.4	6.0	140.0	—	—	1.0	—	50.0
HPO_4 -6	7.4						

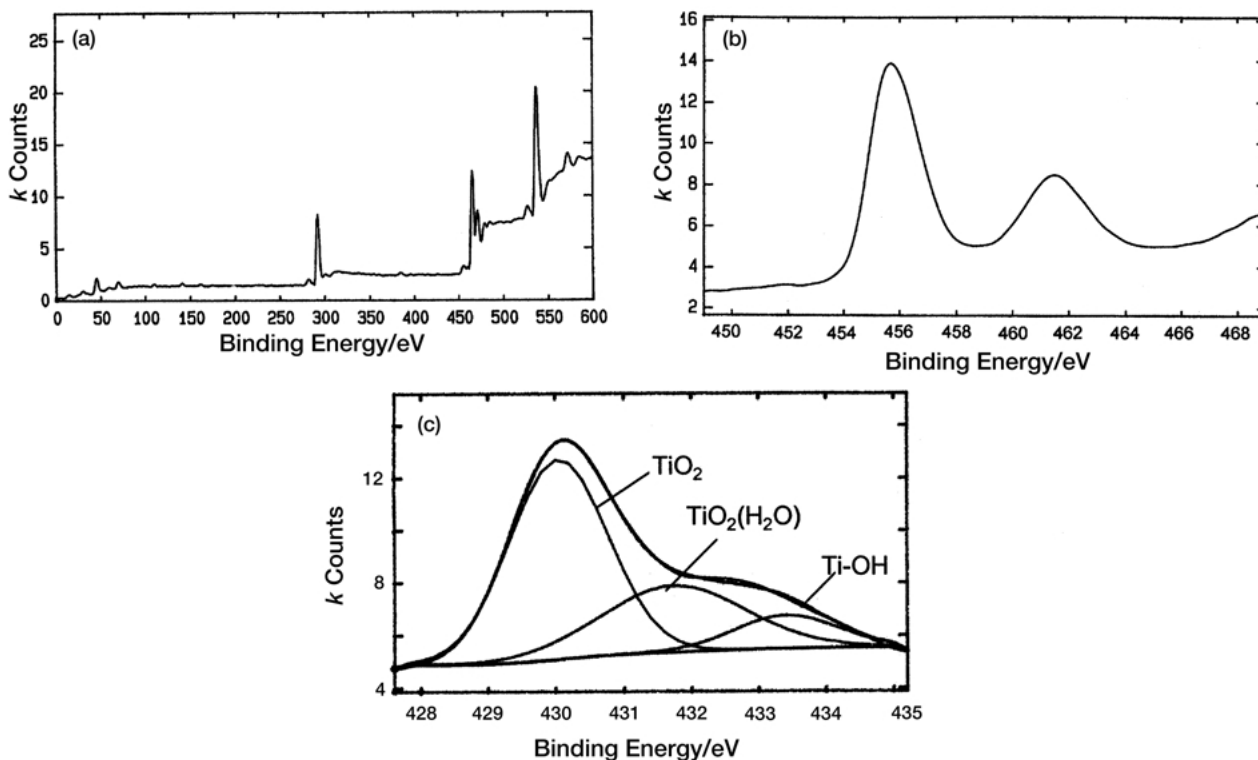


Figure 1 XPS spectrum of raw TiO_2 : (a) general spectrum, (b) $\text{Ti}2p$ bands, (c) $\text{O}1s$ bands with deconvolution of TiO_2 , $\text{Ti}(\text{OH})$ and $\text{TiO}_2(\text{H}_2\text{O})$.

TABLE II XPS binding energies (eV) and the corresponding elements detected on TiO_2 powder in the diverse studied conditions (nd: not detected). In the case of $\text{O}1s$, the position and the relative contribution (%) of three $\text{O}1s$ bands are indicated after deconvolution

TiO_2	Raw	Ca-6	Ca-7.4	HPO_4 -6	HPO_4 -7.4	SBF-6	SBF-7.4
$\text{Ti}2p_{3/2}$	458.3	458.4	458.3	458.5	458.5	458.3	458.3
$\text{O}1s$	529.7 (60%)	529.7 (70%)	529.6 (70%)	529.9 (73%)	529.7 (71%)	529.6 (64%)	529.9 (74%)
	531.3 (23%)	531.4 (19%)	531.2 (22%)	531.3 (19%)	531.1 (23%)	531.2 (32%)	531.5 (19%)
	533.0 (11%)	532.9 (11%)	532.8 (8%)	532.9 (8%)	532.8 (6%)	533.0 (5%)	533.0 (6%)
$\text{Ca}2p_{3/2}$	nd	346.7	346.7	nd	nd	346.7	346.3
$\text{P}2p$	nd	nd	nd	132.6	133	132.8	132.5
$\text{C}1s$	284.6	284.6	284.6	284.6	284.6	284.6	284.6

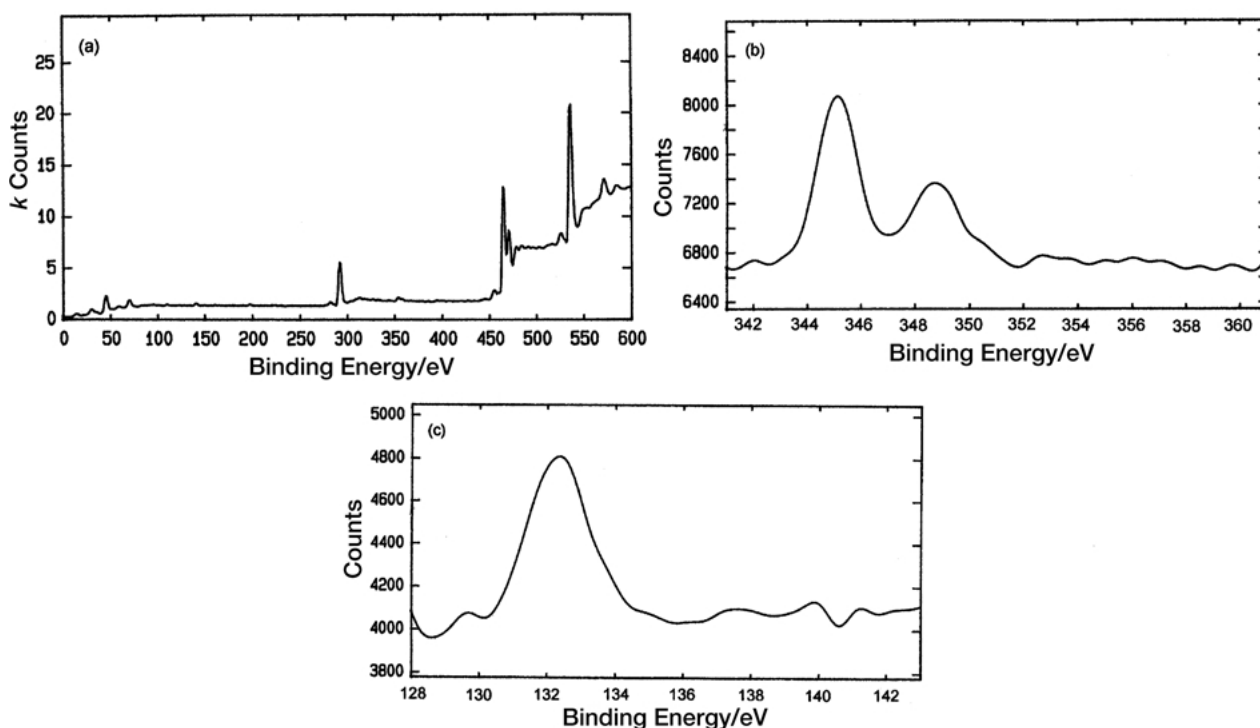


Figure 2 XPS spectrum of TiO_2 after immersion in SBF-7.4: (a) general spectrum, (b) $\text{Ca}2p$ bands, (c) $\text{P}2p$ band.

TABLE III Relative quantification of the elements detected by XPS on TiO₂ powder in the diverse studied conditions

	Ti	P	Ca	O	Ti/Ca	Ti/P	O/Ti
TiO ₂	1	—	—	3.225	—	—	3.225
HPO ₄ -7.4	1	0.077	—	2.571	—	13.0	2.571
HPO ₄ -6	1	0.088	—	2.73	—	11.4	2.73
Ca-7.4	1	—	0.049	2.897	20.4	—	2.897
Ca-6	1	—	0.023	2.842	43.5	—	2.842
SBF-7.4	1	0.084	0.057	2.909	17.5	11.9	2.909
SBF-6	1	0.093	0.046	2.977	21.7	10.8	2.977

respectively (Fig. 2(b)), and P2p band at 132.5 eV (Fig. 2(c)) were detected.

Table III summarizes the quantification of various elements detected on TiO₂ samples. Raw TiO₂ powder was P- and Ca-free. The O/Ti ratio of raw TiO₂ appeared much higher than that of the theoretical ratio at 2. This excess of oxygen decreased significantly for all samples treated in aqueous media but the O/Ti ratio still remained well above 2. With regard to HPO₄-6 and HPO₄-7.4 solutions the Ti/P ratio was quite similar for both pH. When TiO₂ was soaked into Ca-containing solutions at pH = 6.0 and pH = 7.4, the Ti/Ca ratio depended on the pH. In these solutions, the Ca-amount was two times higher at pH = 7.4 than at pH = 6.0. In SBF-6 and SBF-7.4 experiments the Ca- and HPO₄-amount were relatively independent of pH. Ca-amount was comparable to Ca-7.4 experiment, and HPO₄-amount was comparable to HPO₄-6.0 and HPO₄-7.4 experiments. The quantity of orthophosphate groups was two times higher than the quantity of Ca²⁺ ions.

α -Al₂O₃

Fig. 3(a) exhibits the general XPS spectrum of α -Al₂O₃ powder prior to immersion. The Al2p band at 74.1 eV, the O1s bands at 531.0 eV, and the C1s band at 284.6 eV were detected in respectively Fig. 3(b)–(d). All of the bands were symmetrical, and the O1s band corresponded to aluminum oxides and hydroxide compounds [19, 20].

Table IV summarizes the peak positions for the α -Al₂O₃ samples. The peak position of Al2p was found between 73.9 and 74.1 eV. The range of these values is in agreement for α -Al₂O₃ materials [21, 22]. Hereby, the surface chemistry of Al₂O₃ was not affected by immersion in the various solutions. Despite the presence of Ca²⁺ ions in the working solutions, Ca2p peak was never detected onto the surface of α -Al₂O₃ powder. When α -Al₂O₃ powder was immersed in HPO₄-containing solutions, P2p transition band was detected (Fig. 4(b)). The P2p peak position could be attributed to orthophosphate group [13]. Despite the presence of HPO₄²⁻ in SBF-6 and SBF-7.4 solution, P2p peak could

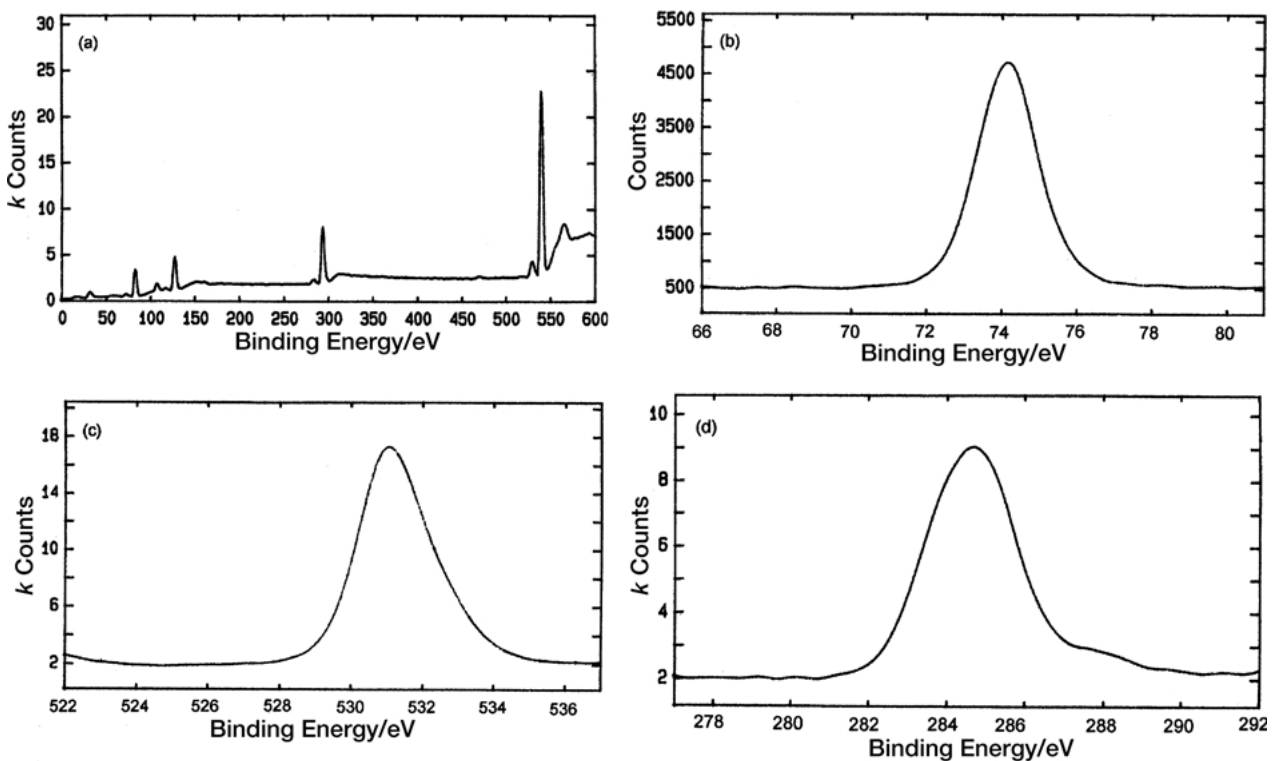


Figure 3 XPS spectrum of raw α -Al₂O₃: (a) general spectrum, (b) Al2p band, (c) O1s band, (d) C1s band.

TABLE IV XPS binding energies (eV) and the corresponding elements detected on Al₂O₃ powder in the diverse conditions (nd: not detected). In the case of C1s, the position and the relative contribution (%) of the two C1s bands are indicated after deconvolution

Al ₂ O ₃	Raw	Ca-6	Ca-7.4	HPO ₄ -6	HPO ₄ -7.4	SBF-6	SBF-7.4
Al2p	74.1	73.9	73.9	74	73.9	74.2	73.9
O1s	531.0	530.7	530.8	530.9	530.8	531.1	530.7
Ca2p _{3/2}	nd	nd	nd	nd	nd	nd	nd
P2p	nd	nd	nd	133.9	133.6	nd	nd
C1s	284.6	284.6	284.6	284.6	284.6	284.6	284.6
	—	282.5	283.3	281.9	281.7	282.0	282.2

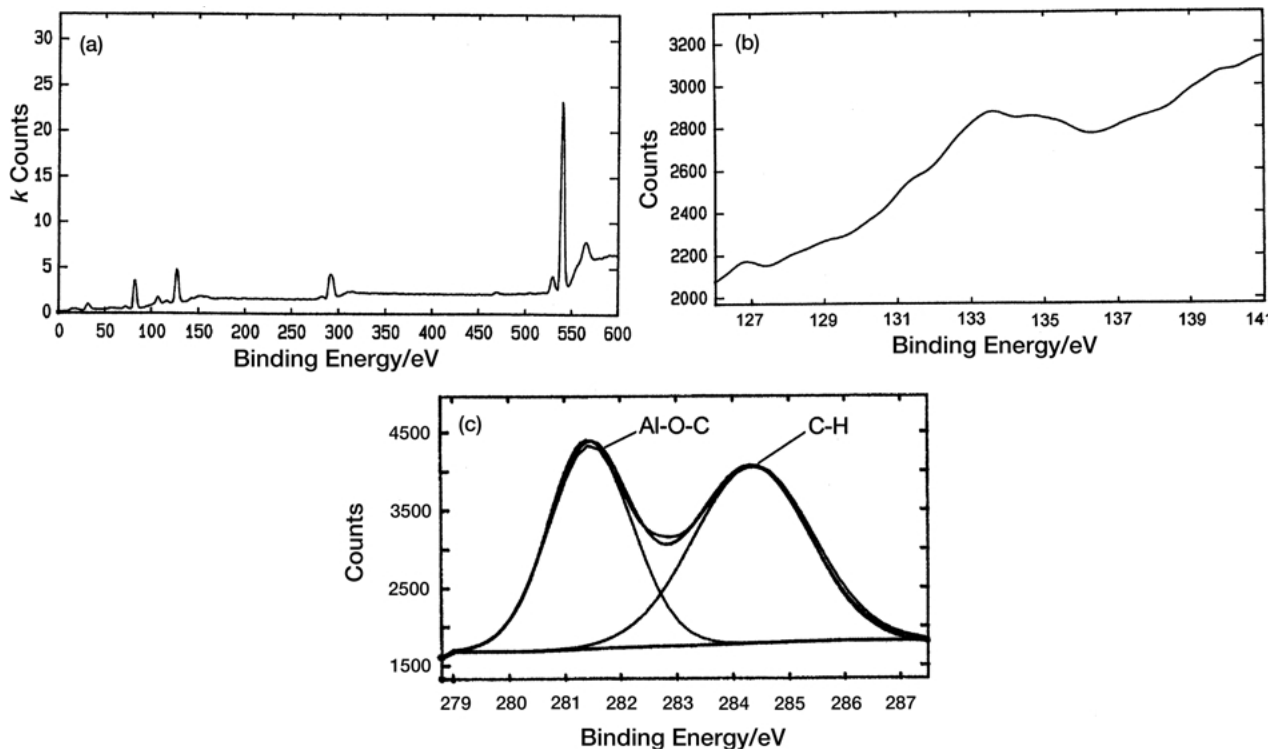


Figure 4 XPS spectrum of α -Al₂O₃ after immersion in SBF-7.4: (a) general spectrum, (b) P2p band, (c) C1s band after deconvolution exhibiting the aliphatic C band (C-H) and the Al-O-C band.

not be detected on α -Al₂O₃ surface. Additionally to the C1s peak attributed to aliphatic C-H at 284.6 eV, a second C1s band was detected between 281.7 eV and 283.3 eV, and contributed for approximately 50% of the total C band (Fig. 4(c)). This band was detected for all of the samples, except for raw α -Al₂O₃, indicating that solely the immersion of α -Al₂O₃ in the various solutions affected the C1s band. According to literature, this binding energy could correspond to C1s transition for carbures (Al-C) or Al-O-C. In the later case though, this C1s value was found for polyethylene containing Al [21].

Table V summarizes the quantification of various elements detected on Al₂O₃ samples. The O/Al ratio of raw Al₂O₃ substrate appeared close to that expected for

the theoretical O/Al ratio at 1.5. This ratio remained similar for the immersion performed at pH = 6.0 whereas the amount of oxygen slightly increased for the immersions in solutions at pH = 7.4. The sole Al/P ratios that could be calculated were for HPO₄-6 and HPO₄-7.4 experiments; they were respectively 47.6 and 37.0.

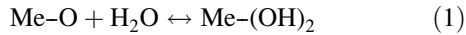
Discussion

TiO₂ and α -Al₂O₃ are oxides, which the isoelectric point (iep) is respectively 6.2 and 8.5 [11]. Below iep, their surfaces are positively charged, exhibiting more positively charged sites than negative ones. Vice versa, above

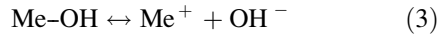
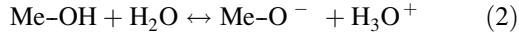
TABLE V Relative quantification of the elements detected by XPS on Al₂O₃ powder in the diverse studied conditions

	Al	P	Ca	O	Al/Ca	Al/P	O/Al
Al ₂ O ₃	1	0	0	1.511	—	—	1.511
HPO ₄ -7.4	1	0.027	0	1.59	0	37.0	1.59
HPO ₄ -6	1	0.021	0	1.525	0	47.6	1.525
Ca-7.4	1	0	0	1.44	—	—	1.44
Ca-6	1	0	0	1.525	—	—	1.525
SBF-7.4	1	0	0	1.565	—	—	1.565
SBF-6	1	0	0	1.52	—	—	1.52

the iep, their surfaces are negatively charged, exhibiting more negatively charged sites than positive ones. In water, their surface can hydrolyze according to equilibrium (1):



Depending on the iep, and on the nature of the Me-OH bonds, these hydroxylated surfaces can then show acidic or alkaline properties, according to equilibria (2) and (3):

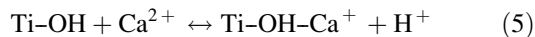
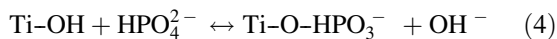


In both cases, an increase of the pH shall lead to a more negative surface either due to a decrease of the positive charges and/or to an increase of the negative charges.

With regard to TiO₂, the XPS analysis of the raw TiO₂ substrate exhibited solely oxygen element (O) and, respectively, titanium (Ti). Its surface composition exhibited an O/Ti ratio greater than the theoretical ratio of 2 indicating the presence of hydroxyl groups as exposed in equilibrium (1). The position of the 3-components O1s band was in agreement with the titanium dioxide hydrolysis [12, 13]. The TiO₂ surface may be therefore represented as TiO_{2-x}(OH)_{2x}.nH₂O. In aqueous solutions, the O/Ti ratio partially decreased. The water content of the surface layer seems to depend on the solution composition, related probably to faint surface alteration due to the fixation of mineral ions.

With regard to α-Al₂O₃ the O/Al ratio remained close to the theoretical ratio of 1.50. When the powder was immersed in solutions at pH=6.0, the O/Al ratio remained similar to the raw powder. The amount of oxygen slightly increased for α-Al₂O₃ powder immersed in all of the solutions at pH=7.4. This systematic increase for higher pH is consistent with the partial hydrolysis of α-Al₂O₃ surface according to equilibrium (1).

The various atomic ratios calculated for TiO₂ and α-Al₂O₃ revealed significant differences. With regard to TiO₂, Ca and P, under orthophosphate form, were detected on the surface, whereas Mg was never detected. The Ca- and P-amounts were identical for the overall immersion experiments, except for Ca-6 solution. This indicates that the TiO₂ surface had a double affinity for HPO₄²⁻ and Ca²⁺ according to the reactions (4) and (5):

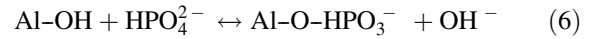


The HPO₄-amount was higher than Ca-amount as already observed by similar analysis technique [12, 13, 18]. However, this is in disagreement with surface charge considerations and previous depth profile analyses, whereby Ca was present in a higher amount than HPO₄ at the Ca-P/Ti or TiO₂ interface [16, 17]. Anatase shall be slightly negatively charged under our experimental condition since its iep has been determined at iep = 6.2 [11]. Above this value, the surface exhibit a higher density of negative charges compared to positive charges.

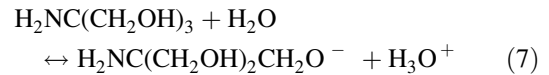
Electrostatically, TiO₂ substrate should therefore attract cations rather than anions, which is in disagree-

ment with the present results. The bonding of HPO₄²⁻ might be kinetically and chemically favored despite the negative surface charge of the TiO₂ substrate. The small and easily solvated Ca₂₊ might be more labile than the large and hydrogen bonded HPO₄²⁻ and thereby the rinsing step after immersion in SBF might have washed away most of the adsorbed calcium ions. This hypothesis is, however, extremely difficult to verify experimentally because desorption of Ca₂₊ from the TiO₂ surface is an extremely rapid process. It might therefore be difficult to observe the chemical bonding between TiO₂ substrate and the solvated Ca₂₊ by using XPS spectroscopy. Further, the adsorption of calcium ions onto the TiO₂ substrate might occur in a later stage of the Ca-P precipitation requiring an increase of the saturation in Ca₂₊ and HPO₄²⁻ at the vicinity of the surface.

With regard to α-Al₂O₃ substrate, HPO₄ groups were detected on the surface after immersion in HPO₄-6 and HPO₄-7.4 solutions. The Al/P ratio depended on pH, suggesting that HPO₄²⁻ interacted with the hydrolyzed sites of α-Al₂O₃ substrate according to reaction (6):

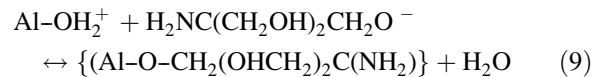
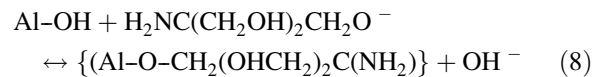


In the case of the SBF-6 and SBF-7.4 immersion experiments, HPO₄ groups were not detected on the α-Al₂O₃ substrate. On the other hand, the marked presence of non-aliphatic C1s suggested a Al-O-C binding [21], as a probable result of the grafting of TRIS on Al₂O₃ substrate. TRIS is a weak monoacidic base, which follows equilibrium (7) in water [22]:



pKa = 7.82 at 37 °C.

TRIS anions may interact with Al₂O₃ substrate according to equilibrium (8) or (9):



The interaction with anionic species complies with the positively charged surface of α-Al₂O₃ (iep ~ 9 [11]). However, despite the positive surface charge of α-Al₂O₃, the HPO₄-amount was significantly lower on α-Al₂O₃ than on the slightly negatively charged TiO₂ surface. This lower HPO₄-amount might result from a competition in favor to the bonding with TRIS. In addition, α-Al₂O₃ did not display any affinity for Ca²⁺ ions under our experimental conditions since Ca was never detected on its surface. A competition might take place between the solvated Ca²⁺, HPO₄²⁻ and the acidic α-Al₂O₃ substrate, resulting in a stronger affinity in the solution between Ca²⁺ and HPO₄²⁻ rather than HPO₄²⁻ with α-Al₂O₃ substrate. Therefore the supersaturation at the vicinity of the substrate cannot be reached enabling the further formation of Ca-P.

This inability of α-Al₂O₃ to calcify seems to be inherent to the material. Besides the surface charge characteristics, the dielectric constant values (ε) of the studied materials are markedly different: Al₂O₃ ε = 5 to

10, TiO₂ (anatase) $\epsilon = 48$ [11]. TiO₂ has a rather equal value with dielectric constant of water ($\epsilon = 78$), whereas Al₂O₃ has a dielectric constant significantly lower than water and TiO₂. The close dielectric constant values between water and TiO₂ suggests a water-like behavior of the surface, whereas the difference in dielectric constant values between α -Al₂O₃ substrate and water leads to the polarization of the surface, which is beneficial for interaction with hydrated macromolecules [11]. This may be consistent with the strong affinity between TRIS and Al₂O₃. In relation with this low dielectric constant, Al₂O₃ ceramics are relatively less hydrophilic than other bioceramics [23], exhibiting a lower wettability angle than TiO₂ and Ti substrates [24]. The approach of solvated cations might be therefore inhibited, enabling the further Ca–P nucleation on Al₂O₃ substrates.

Conclusions

The overview of the present study enlightens that TiO₂ and Al₂O₃ have a marked diverse reactivity towards SBF solution at pH = 6.0 and pH = 7.4. TiO₂ has a double affinity towards cations and anions. Ca and in a greater amount HPO₄ bound to TiO₂ substrate, resulting locally in a relatively high supersaturation, and in the further Ca–P nucleation. On the other hand, Al₂O₃ exhibits a low density of OH groups precluding interactions with the mineral ions present in SBF solutions. The local supersaturation is therefore too low at the vicinity of Al₂O₃ substrate to stimulate the Ca–P formation.

References

1. H. S. TALWAR, A. H. REDDI, J. MENCZEL JR., W. C. THOMAS and J. L. MEYER, *Kidney Int.* **29** (1986) 1038.
2. S. STEA, L. SAVARINO, A. TONI, A. SUDANESE, A. GIUNTI and A. PIZZOFERRATO, *Biomaterials* **13** (1992) 664.
3. M. BOEHLER, K. KNAHR, H. PLENK, A. WALTER, M.

- SALZER and V. SCHREIBER, *J. Bone Joint Surg. Br.* **76** (1994) 53.
4. B. W. DARVELL, N. SAMMAN, W. K. LUK, R. K. F. CLARK and H. TIDEMAN, *J. Dent.* **23** (1995) 319.
5. J. W. MCCUTCHEN, J. P. COLLIER and M. B. MAYOR, *Clin. Orthop.* **261** (1990) 114.
6. C. M. SERRE, G. BOIVIN, K. J. OBRANT and L. LINDER, *Acta Orthop. Scand.* **56** (1994) 323.
7. L. CARLSSON, L. REGNER, C. JOHANSSON, M. GOTTLANDER and P. HEBERTS, *J. Orthop. Res.* **12** (1994) 274.
8. T. ALBREKTSSON, P. I. BRANEMARK, H. A. HANSSON and J. LINDSTROM, *Acta Orthop. Scand.* **52** (1981) 155.
9. P. ROYER, S. AMRAH-BOUALI, M. FRECHE, C. REY, N. ROUQUET and G. BONEL, *Bioceramics* **5** (1992) 95.
10. P. LI, C. OHTSUKI, T. KOKUBO, K. NAKANISHI, N. SOGA and K. DE GROOT, *J. Biomed. Mater. Res.* **28** (1994) 7.
11. P. TENGVALL and I. LUNDSTROM, *Clin. Mater.* **9** (1992) 115.
12. K. E. HEALY and P. DUCHEYNE, *J. Biomed. Mater. Res.* **26** (1992) 319.
13. A. LEBUGLE, C. COMBES, C. REY and M. FRECHE, *Colloids Surf. B: Biointerfaces* **11** (1998) 15.
14. D. S. SUTHERLAND, P. D. FORSHAW, G. C. ALLEN, I. T. BROWN and K. R. WILLIAMS, *Biomaterials* **14** (1993) 893.
15. J. H. SCOFIELD, *J. Electron Spectroscop.* **8** (1976) 129.
16. F. BARRÈRE, C. A. VAN BLITTERSWIJK, K. DE GROOT and P. LAYROLLE, *Biomaterials* **23** (2002) 2211.
17. P. LI, in "In vitro and in vivo Calcium Phosphate Induction on Gel Oxides", PhD Thesis, Leiden University, The Netherlands (1993).
18. K. E. HEALY and P. DUCHEYNE, *Biomaterials* **13** (1992) 553.
19. P. M. A. SHERWOOD, *Surf. Sci. Spectra* **5** (1998) 1.
20. S. THOMAS and P. M. A. SHERWOOD, *Anal. Chem.* **64** (1992) 2488.
21. J. L. DROULAS, Y. JUGNET and T. M. DUC, *Le vide, les couches minces* **258** (suppl.) (1991) 39.
22. In "The Merck Index", edited by S. Budavari, 12th edn (Merck & Co Inc., Whitehouse Station, USA, 1996) p. 9899.
23. P. MILLEDING, S. GERDES, K. HOLMBERG and S. KARLSSON, *Euro. J. Oral. Sci.* **107** (1999) 384.
24. D. C. SMITH, R. M. PILLIAR and R. CHERNECKY, *J. Biomed. Mater. Res.* **25** (1991) 1045.

Received 11 March
and accepted 20 August 2002