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

FLORENCE BARRÈRE[1,2,3], ALBERT LEBUGLE[3], CLEMENS A. VAN BLITTERSWIJK[1,2], KLAAS DE GROOT[1,4], PIERRE LAYROLLE[1]*, CHRISTIAN REY[3]
[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 (Al_2O_3) in the case of load bearing applications, their bone-bonding properties are very different. In osseous environment, Al_2O_3 ceramic is encapsulated by fibrous tissues, whereas bone can bind directly to titanium, via its natural titanium dioxide (TiO_2) passivation layer. So far, this calcification dissimilarity between TiO_2 and Al_2O_3 was attributed to respectively their negative and positive surface charge under physiological conditions. The present study aims at studying the chemical interactions between TiO_2 and Al_2O_3 (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, TiO_2 and α-Al_2O_3 powders were analyzed by X-ray photoelectron spectroscopy (XPS). The results indicated that Ca and HPO_4 groups were present on TiO_2 surface. In addition, HPO_4 groups were found to be in a higher amount than Ca on TiO_2, which does not comply with the surface charge theory. With regard to Al_2O_3, little HPO_4 but no Ca was detected on its surface, and TRIS bound to Al_2O_3 substrate in all of the immersion experiments. The fact that both Ca and HPO_4 were present at the vicinity of TiO_2 might be at the origin of its calcification ability. On the other hand, Al_2O_3 did not show any affinity towards Ca and HPO_4 ions. This might explain the inability of Al_2O_3 substrate to calcify.

Introduction

In hip and joint surgery, titanium (Ti) and alloys, and alumina ceramics (Al_2O_3) 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 Al_2O_3 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 (TiO_2) 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 Al_2O_3 is not an efficient calcium phosphate (Ca–P) nucleator when soaked in calcifying solutions, whereas titanium with its passive oxide layer (TiO_2) 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, Al_2O_3 is positively charged, whereas TiO_2 is slightly negatively charged [10, 11]. Additionally, TiO_2 surface has a gel-like structure in the presence of aqueous fluids [11–14]. With regard to TiO_2 surface charge, one should expect a higher amount of cationic species such as Ca^{2+} than anionic species such as HPO_4^{2-} on TiO_2 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 HPO_4 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 HPO_4^{2-} on

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* 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 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 pH = 6.0 and 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 $CaCl_2.2H_2O$, $MgCl_2.6H_2O$, $Na_2HPO_4.2H_2O$, $NaHCO_3$ and NaCl. These solutions were buffered with trishydroxymethyl aminomethane (TRIS, $(CH_2OH)_3CNH_2$) and HCl (1 M). SBF-7.4 was buffered at pH = 7.4 whereas SBF-6 was buffered at pH = 6.0. Second, Ca- and HPO_4-solutions were prepared by dissolving on one hand NaCl and $CaCl_2.2H_2O$, and on the other hand NaCl and $Na_2HPO_4.2H_2O$ in similar concentrations as SBF solution. These so-called Ca- and HPO_4-solutions were buffered respectively at pH = 6.0 and pH = 7.4. They were named Ca-6, Ca-7.4 for Ca-containing solution respectively buffered at pH = 6.0 and pH = 7.4, and HPO_4-6, HPO_4-7.4 for HPO_4-containing solutions respectively buffered at pH = 6.0 and 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 Al Kα: 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 $Ti2p_{3/2}$ at 458.3 eV and $Ti2p_{1/2}$ 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 $Ti2p_{3/2}$ 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 $Ca2p_{3/2}$ 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, $Ca2p_{3/2}$ and $Ca2p_{1/2}$ bands at 346.3 and 349.7 eV

TABLE I Composition of the various solutions in mM

	pH	NaCl	$MgCl_2 \cdot 6H_2O$	$CaCl_2 \cdot 2H_2O$	$Na_2HPO_4 \cdot 2H_2O$	$NaHCO_3$	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						

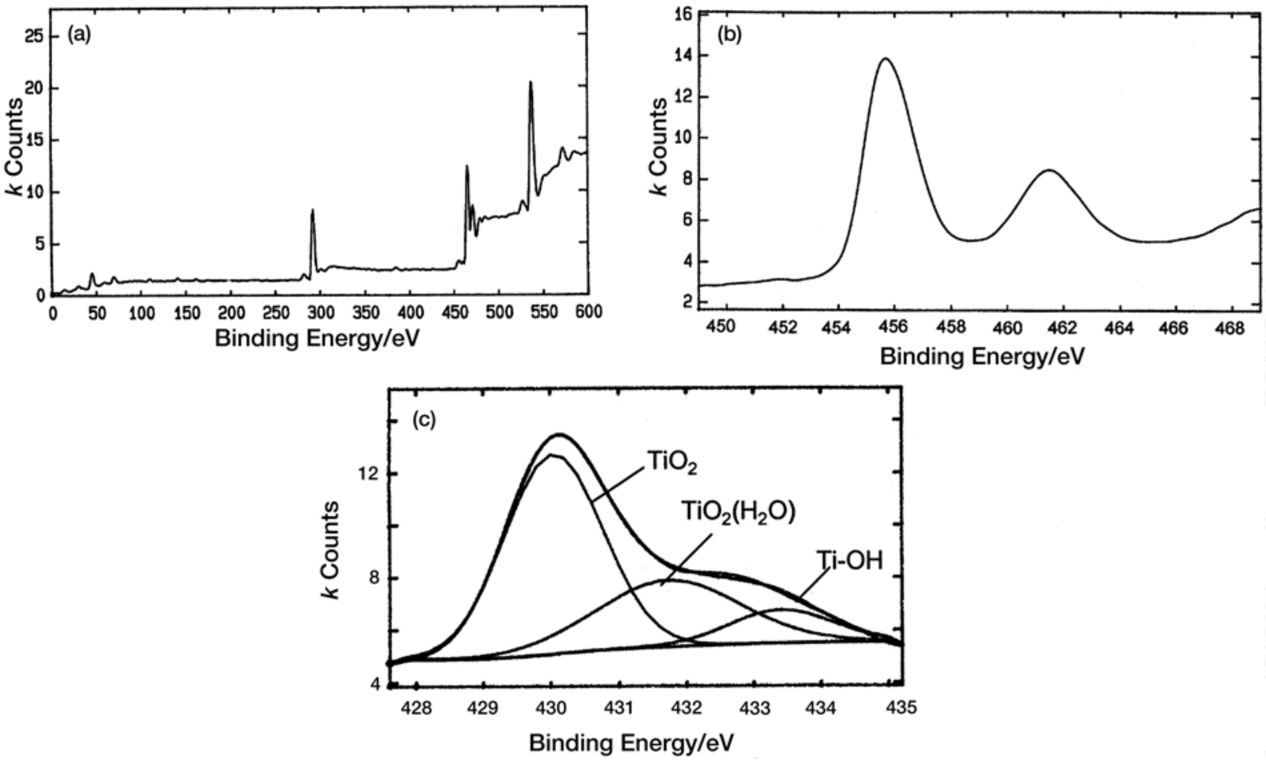

Figure 1 XPS spectrum of raw TiO_2: (a) general spectrum, (b) Ti2p bands, (c) O1s bands with deconvolution of TiO_2, Ti(OH) and $TiO_2(H_2O)$.

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 O1s, the position and the relative contribution (%) of three O1s 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
Ti2$p_{3/2}$	458.3	458.4	458.3	458.5	458.5	458.3	458.3
O1s	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%)
Ca2$p_{3/2}$	nd	346.7	346.7	nd	nd	346.7	346.3
P2p	nd	nd	nd	132.6	133	132.8	132.5
C1s	284.6	284.6	284.6	284.6	284.6	284.6	284.6

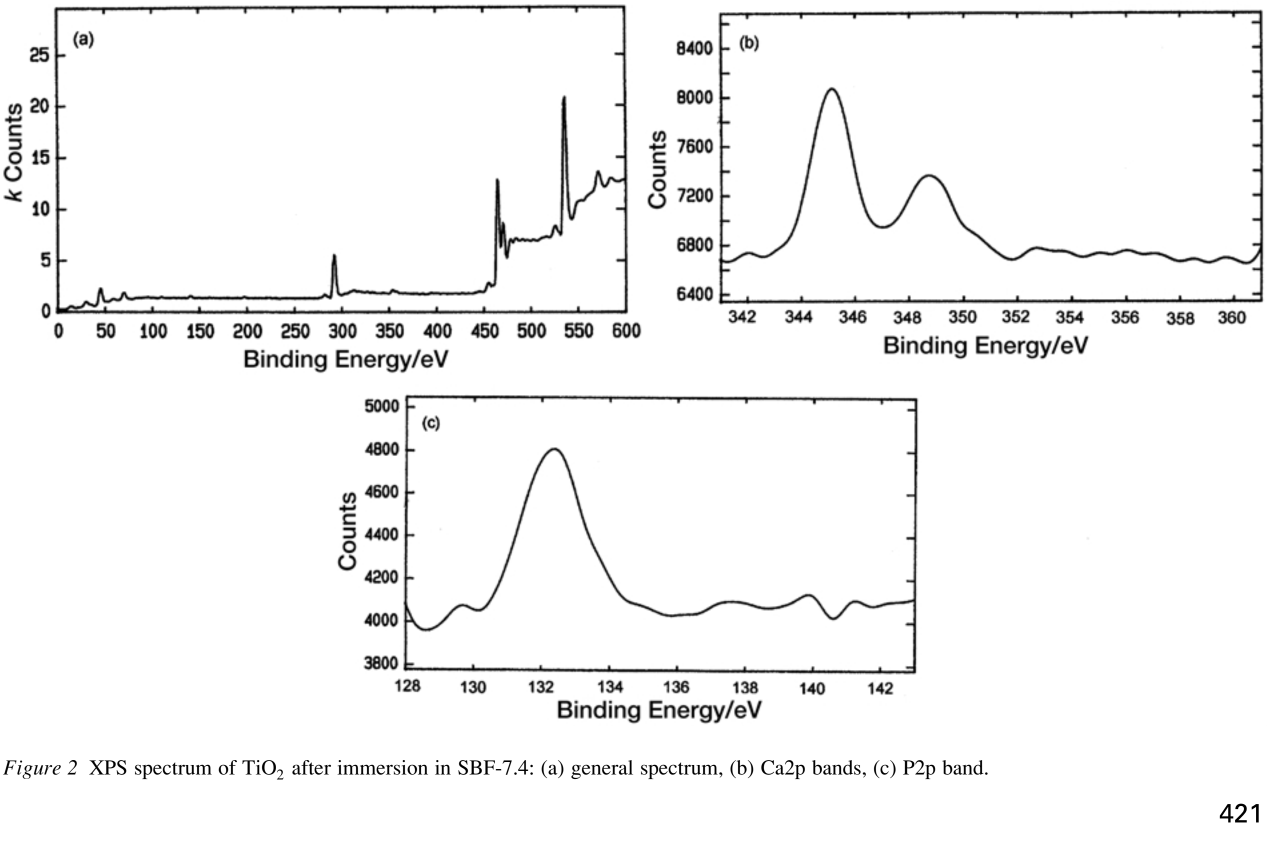

Figure 2 XPS spectrum of TiO_2 after immersion in SBF-7.4: (a) general spectrum, (b) Ca2p bands, (c) P2p band.

TABLE III Relative quantification of the elements detected by XPS on TiO_2 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 TiO_2 samples. Raw TiO_2 powder was P- and Ca-free. The O/Ti ratio of raw TiO_2 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 HPO_4-6 and HPO_4-7.4 solutions the Ti/P ratio was quite similar for both pH. When TiO_2 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 HPO_4-amount were relatively independent of pH. Ca-amount was comparable to Ca-7.4 experiment, and HPO_4-amount was comparable to HPO_4-6.0 and HPO_4-7.4 experiments. The quantity of orthophosphate groups was two times higher than the quantity of Ca^{2+} ions.

α-Al_2O_3

Fig. 3(a) exhibits the general XPS spectrum of α-Al_2O_3 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-Al_2O_3 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-Al_2O_3 materials [21, 22]. Hereby, the surface chemistry of Al_2O_3 was not affected by immersion in the various solutions. Despite the presence of Ca^{2+} ions in the working solutions, Ca2p peak was never detected onto the surface of α-Al_2O_3 powder. When α-Al_2O_3 powder was immersed in HPO_4-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 HPO_4^{2-} in SBF-6 and SBF-7.4 solution, P2p peak could

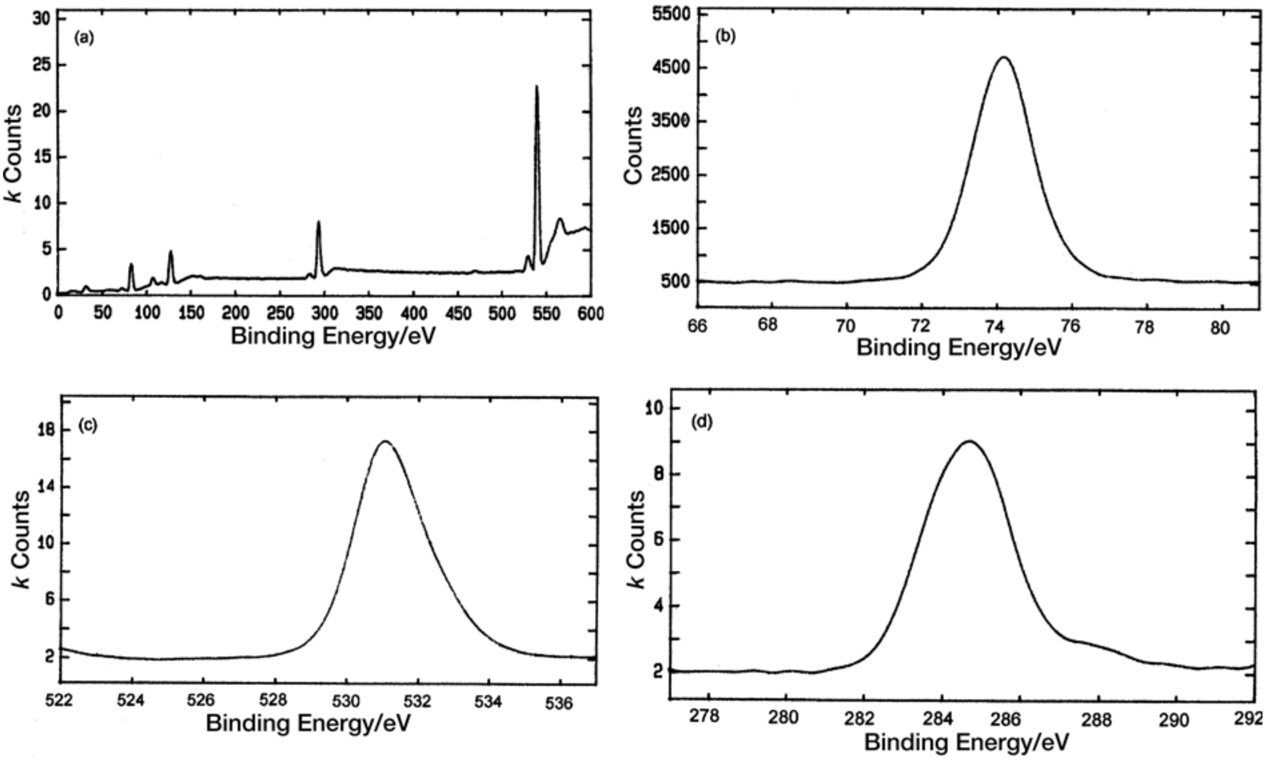

Figure 3 XPS spectrum of raw α-Al_2O_3: (a) general spectrum, (b) Al2p band, (c) O1s band, (d) C1s band.

TABLE IV XPS binding energies (eV) and the corresponding elements detected on Al_2O_3 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_2O_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

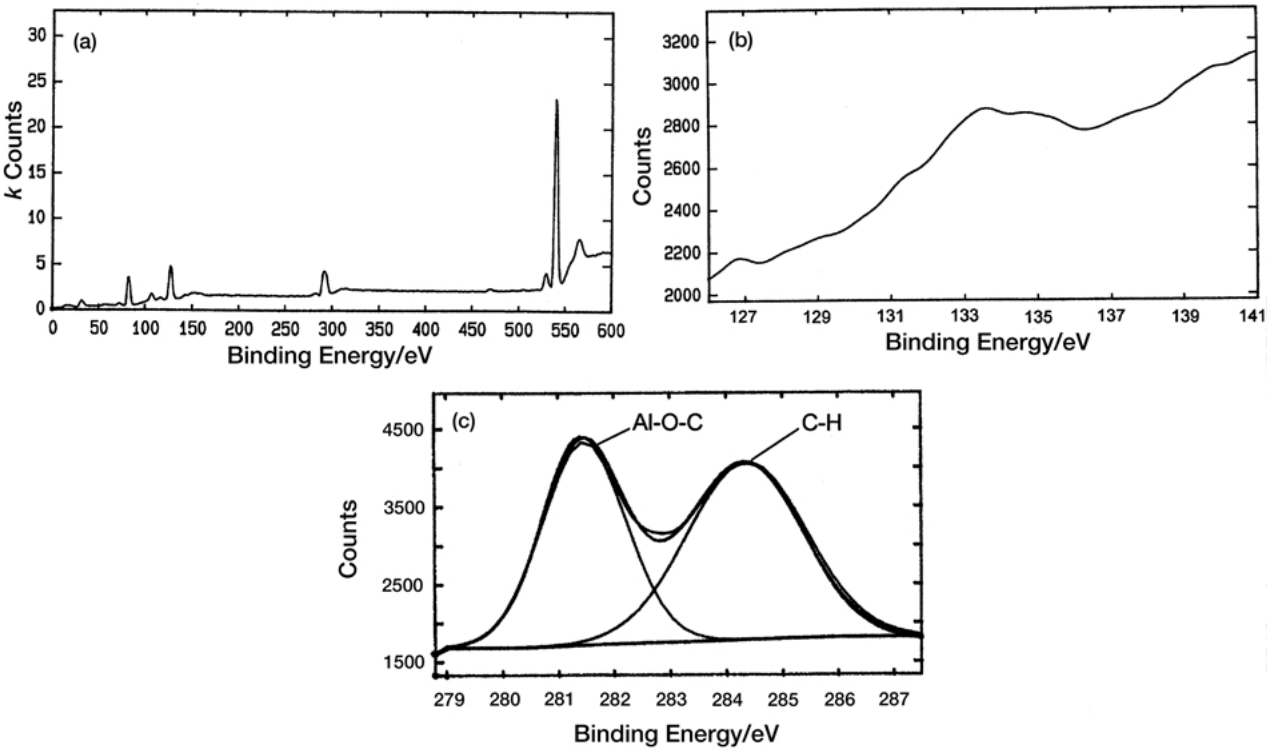

Figure 4 XPS spectrum of α-Al_2O_3 after immersion in SBF-7.4: (a) general spectrum, (b) P2p band, (c) C1s band after deconvolution exhibiting the aliphatic C band (C–H) and the Al–O–C band.

not be detected on α-Al_2O_3 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-Al_2O_3, indicating that solely the immersion of α-Al_2O_3 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 Al_2O_3 samples. The O/Al ratio of raw Al_2O_3 substrate appeared close to that expected for the theoretical O/Al ratio at 1.5. This ratio remained similar for the immersion preformed 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 HPO_4-6 and HPO_4-7.4 experiments; they were respectively 47.6 and 37.0.

Discussion

TiO_2 and α-Al_2O_3 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 Al_2O_3 powder in the diverse studied conditions

	Al	P	Ca	O	Al/Ca	Al/P	O/AI
Al_2O_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):

$$\text{Me–O} + H_2O \leftrightarrow \text{Me–(OH)}_2 \qquad (1)$$

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):

$$\text{Me–OH} + H_2O \leftrightarrow \text{Me–O}^- + H_3O^+ \qquad (2)$$

$$\text{Me–OH} \leftrightarrow \text{Me}^+ + OH^- \qquad (3)$$

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 TiO_2, the XPS analysis of the raw TiO_2 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 TiO_2 surface may be therefore represented as $TiO_{2-x}(OH)_{2x}.nH_2O$. 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-Al_2O_3 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-Al_2O_3 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-Al_2O_3 surface according to equilibrium (1).

The various atomic ratios calculated for TiO_2 and α-Al_2O_3 revealed significant differences. With regard to TiO_2, 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 TiO_2 surface had a double affinity for HPO_4^{2-} and Ca^{2+} according to the reactions (4) and (5):

$$\text{Ti–OH} + HPO_4^{2-} \leftrightarrow \text{Ti–O–HPO}_3^- + OH^- \qquad (4)$$

$$\text{Ti–OH} + Ca^{2+} \leftrightarrow \text{Ti–OH–Ca}^+ + H^+ \qquad (5)$$

The HPO_4-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 HPO_4 at the Ca–P/Ti or TiO_2 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, TiO_2 substrate should therefore attract cations rather than anions, which is in disagreement with the present results. The bonding of HPO_4^{2-} might be kinetically and chemically favored despite the negative surface charge of the TiO_2 substrate. The small and easily solvated Ca_{2+} might be more labile than the large and hydrogen bonded HPO_4^{2-} 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 Ca_{2+} from the TiO_2 surface is an extremely rapid process. It might therefore be difficult to observe the chemical bonding between TiO_2 substrate and the solvated Ca_{2+} by using XPS spectroscopy. Further, the adsorption of calcium ions onto the TiO_2 substrate might occur in a later stage of the Ca–P precipitation requiring an increase of the saturation in Ca_{2+} and HPO_4^{2-} at the vicinity of the surface.

With regard to α-Al_2O_3 substrate, HPO_4 groups were detected on the surface after immersion in HPO_4-6 and HPO_4-7.4 solutions. The Al/P ratio depended on pH, suggesting that HPO_4^{2-} interacted with the hydrolyzed sites of α-Al_2O_3 substrate according to reaction (6):

$$\text{Al–OH} + HPO_4^{2-} \leftrightarrow \text{Al–O–HPO}_3^- + OH^- \qquad (6)$$

In the case of the SBF-6 and SBF-7.4 immersion experiments, HPO_4 groups were not detected on the α-Al_2O_3 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 Al_2O_3 substrate. TRIS is a weak monoacidic base, which follows equilibrium (7) in water [22]:

$$H_2NC(CH_2OH)_3 + H_2O \leftrightarrow H_2NC(CH_2OH)_2CH_2O^- + H_3O^+ \qquad (7)$$

pKa = 7.82 at 37 °C.

TRIS anions may interact with Al_2O_3 substrate according to equilibrium (8) or (9):

$$\text{Al–OH} + H_2NC(CH_2OH)_2CH_2O^- \leftrightarrow \{(\text{Al–O–}CH_2(OHCH_2)_2C(NH_2))\} + OH^- \qquad (8)$$

$$\text{Al–OH}_2^+ + H_2NC(CH_2OH)_2CH_2O^- \leftrightarrow \{(\text{Al–O–}CH_2(OHCH_2)_2C(NH_2))\} + H_2O \qquad (9)$$

The interaction with anionic species complies with the positively charged surface of α-Al_2O_3 (iep ~ 9 [11]). However, despite the positive surface charge of α-Al_2O_3, the HPO_4-amount was significantly lower on α-Al_2O_3 than on the slightly negatively charged TiO_2 surface. This lower HPO_4-amount might result from a competition in favor to the bonding with TRIS. In addition, α-Al_2O_3 did not display any affinity for Ca^{2+} ions under our experimental conditions since Ca was never detected on its surface. A competition might take place between the solvated Ca^{2+}, HPO_4^{2-} and the acidic α-Al_2O_3 substrate, resulting in a stronger affinity in the solution between Ca^{2+} and HPO_4^{2-} rather than HPO_4^{2-} with α-Al_2O_3 substrate. Therefore the supersaturation at the vicinity of the substrate cannot be reached enabling the further formation of Ca–P.

This inability of α-Al_2O_3 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: Al_2O_3 $\varepsilon = 5$ to

10, TiO_2 (anatase) $\varepsilon = 48$ [11]. TiO_2 has a rather equal value with dielectric constant of water ($\varepsilon = 78$), whereas Al_2O_3 has a dielectric constant significantly lower than water and TiO_2. The close dielectric constant values between water and TiO_2 suggests a water-like behavior of the surface, whereas the difference in dielectric constant values between α-Al_2O_3 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 Al_2O_3. In relation with this low dielectric constant, Al_2O_3 ceramics are relatively less hydrophilic than other bioceramics [23], exhibiting a lower wettability angle than TiO_2 and Ti substrates [24]. The approach of solvated cations might be therefore inhibited, enabling the further Ca–P nucleation on Al_2O_3 substrates.

Conclusions

The overview of the present study enlightens that TiO_2 and Al_2O_3 have a marked diverse reactivity towards SBF solution at $pH = 6.0$ and $pH = 7.4$. TiO_2 has a double affinity towards cations and anions. Ca and in a greater amount HPO_4 bound to TiO_2 substrate, resulting locally in a relatively high supersaturation, and in the further Ca–P nucleation. On the other hand, Al_2O_3 exhibits a low density of OH groups precluding interactions with the mineral ions present in SBF solutions. The local supersaturation is therefore to low at the vicinity of Al_2O_3 substrate to stimulate the Ca–P formation.

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