J Mater Sci: Mater Med (2010) 21:2743–2749
DOI 10.1007/s10856-010-4137-y

Studies of early growth mechanisms of hydroxyapatite on single crystalline rutile: a model system for bioactive surfaces

Carl Lindahl · Per Borchardt · Jukka Lausmaa · Wei Xia · Håkan Engqvist

Received: 20 March 2010 / Accepted: 17 July 2010 / Published online: 1 August 2010

Abstract Previous studies have shown that crystalline titanium oxide is in vitro bioactive and that there are differences in the HA formation mechanism depending on the crystalline direction of the titanium oxide surface. In the present study, the early adsorption of calcium and phosphate ions on three different surface directions of the single-crystal rutile TiO_2 substrate has been investigated. A crucial step in the nucleation of HA is believed to be the adsorption of Ca^{2+} and PO_4^{3-} from phosphate buffer solutions. The (001), (100) and (110) single crystalline rutile surfaces were soaked in phosphate buffer saline solution for 10 min, 1 h and 24 h at 37°C. The surfaces were then analyzed using time-of-flight secondary ion mass spectrometry (TOF-SIMS) and X-ray photoelectron spectroscopy (XPS). The results show that the adsorption of Ca^{2+} and PO_4^{3-} is faster on the (001) and (100) surfaces than on the (110) surface. This study also shows that TOF-SIMS can be used as a tool to better understand the adsorption of calcium and phosphate ions and the growth mechanism of HA. This knowledge could be used to tailor new bioactive surfaces for better biological reaction.

C. Lindahl · W. Xia · H. Engqvist
The Angstrom Laboratory, Department of Engineering Sciences, Uppsala University, Uppsala, Sweden

C. Lindahl · P. Borchardt · J. Lausmaa · W. Xia · H. Engqvist (✉)
BIOMATCELL VINN Excellence Center of Biomaterials and Cell Therapy, P.O. Box 412, 405 30 Gothenburg, Sweden
e-mail: hakan.engqvist@angstrom.uu.se

P. Borchardt · J. Lausmaa
SP Technical Research Institute of Sweden, Box 857, 501 15 Borås, Sweden

1 Introduction

Recent studies have shown that crystalline titanium oxide is in vitro bioactive. This is interesting for several reasons; crystalline TiO_2 has a well documented chemistry, it is non-resorbable and is possible to produce as coatings [1–3]. The potential bioactivity of a surface is normally studied in simulated body fluid (SBF) or phosphate buffer saline (PBS) solution [4]. If HA forms on the surface within 4 weeks of soaking the surface is considered to be bioactive. This HA layer is anticipated to act as a bonding layer between bone and biomaterial in vivo resulting in an improved biological functionality of the implant.

A partially crystalline or amorphous titanium dioxide layer is in most cases present as the surface layer on titanium implants. Crystalline titanium dioxides are thought to be bioactive due to the presence of hydroxyl groups on the surface. The hydroxyl groups induce hydroxyapatite formation from body fluids [5, 6].

In the article by published previously by Lindberg et al. [7] it was observed that there were differences in the formation of hydroxyapatite on the different faces of rutile (001), (100) and (110) at soaking times up to 4 weeks in phosphate buffer saline solution (PBS). It was shown that the coverage of the (001) surface was faster, due to epitaxy between the initial hydroxyapatite crystals formed and the crystal directions of the substrate. On the (110) surface the hydroxyapatite crystals first formed will be oriented with the $(100)_{HA}$ surface growing outwards from the face. On the (100) surface the crystals seem to attach to the substrate after first being formed in solution. The authors have shown that the (100) surface has a low degree of HA formation and low surface area compared to the (001) surface, resulting in HA crystals that are lying flat on the surface. In the paper the authors note that speculations about the

growth mechanism of hydroxyapatite on the different crystal faces requires a better understanding of the interactions on an atomic level [7].

Whereas HA growth on surfaces from solutions such as SBF and PBS has been quite expensively studied, very few studies have addressed the early phenomena taking place before the surface has become coated by HA. Adsorption of calcium and phosphate is supposed to be an important early step in the nucleation of hydroxyapatite on bioactive surfaces [8]. Studies of the kinetics of Ca and phosphate adsorption and the dependence on the crystalline phases and directions of the substrate can therefore contribute to an increased understanding of the early stages of hydroxyapatite formation at surfaces. Such studies in turn require the use of surface sensitive techniques such as X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) in order to be able to detect the small amounts of substances involved.

XPS is a well established surface analysis technique in materials science, and has also been frequently used for surface characterization of biomaterials. The TOF-SIMS technique is a surface sensitive analysis technique that has been used in materials science [9, 10]. It can detect inorganic and organic compounds on solid surface at the same time [11, 12] and has recently been used to study the bone mineralization on implant surfaces and to evaluate the early stages of the osseointegration process on titanium surfaces [13].

This article describes experimental observations of the initial adsorption of ions from PBS on three different rutile TiO_2 single crystalline surfaces, (001), (100) and (110). The aim of the study was to investigate which changes that had occurred at the crystal surfaces as a result of soaking in the PBS for different time periods, and to which extent these changes were influenced by the crystal direction of the surfaces.

2 Materials and methods

2.1 Surface preparation of crystals

Single crystals (rutile) with dimensions 5 mm × 5 mm × 0.5mm, cut and polished in three different crystalline directions (001), (100) and (110), were purchased from MTI Corporation (Richmond California). Before and between the different experimental series the single crystals were pre-cleaned ultrasonically in 75 ml 0.5M HCl for 30 min and then rinsed 3 times in milliQ water. After that they were cleaned ultrasonically in 75 ml milliQ water for 30 min and finally dried in a flow of nitrogen gas. The crystals were polished with a 1 μm diamond paste on acetate cloth (Struers) for 2 × 2.5 min with light finger pressure. After polishing the crystals were ultrasonically cleaned for 10 min each in ethanol and milliQ water, respectively and then placed in a clean glass Petri dish. The crystals were treated in UV-ozone for 60 min and then immediately covered with milliQ water. The surfaces were at this point checked for cleanliness with TOF-SIMS. After the TOF-SIMS analysis the UV-ozone treatment was repeated followed by immersion in milliQ water before PBS immersion. This crystal surface preparation protocol efficiently removed all traces of HA from previous experiments, without introducing any unintentional structural or chemical changes, as judged by high resolution SEM and TOF-SIMS analyses.

2.2 Immersion in PBS

As soaking medium Dulbecco's phosphate-buffered saline (PBS) (Aldrich, USA) was used. The composition of this PBS was: Na^+ (145 mM), K^+ (4.3 mM), Mg^{2+} (0.49 mM), Ca^{2+} (0.49 mM), Cl^- (143 mM) and HPO_4^{2-} (9.6 mM).

After surface preparation as described above, the samples were transferred as quickly as possible and without any intervening air exposure to covered plastic petri dishes containing about 40 ml of 37°C PBS solution, in which they were kept for 10 min, 1 h, and 24 h. After immersion, the samples were transferred to a clean glass crystallization beaker with >100 ml milliQ water, dried with nitrogen gas and stored in PTFE containers prior to the analyses.

2.3 Surface analysis

Chemical characterization of the sample surfaces was done using time-of-flight secondary ion mass spectrometry (TOF-SIMS, TOF-SIMS IV, ION-TOF GmbH, Münster, Germany). Positive spectra and negative secondary ion mass spectra were measured from three areas of 500 μm × 500 μm on each crystal using 25 kV Bi_3^+ primary ions at a pulsed target current of 0.1 pA. Interesting peaks in the spectra were identified, and their relative intensitites were calculated by normalization either against the total ion of the spectrum, or against a well known substrate peak (the TiO^+ ion at $m/z = 64$). Since these normalization procedures do not yield absolute concentrations from the TOF-SIMS data, XPS analysis was done on selected samples to verify the amount of elements on the surfaces.

3 Results

3.1 X-ray photoelectron spectroscopy (XPS)

Because the quantitative analysis with TOF-SIMS is difficult, a selection of the single crystals was analyzed with

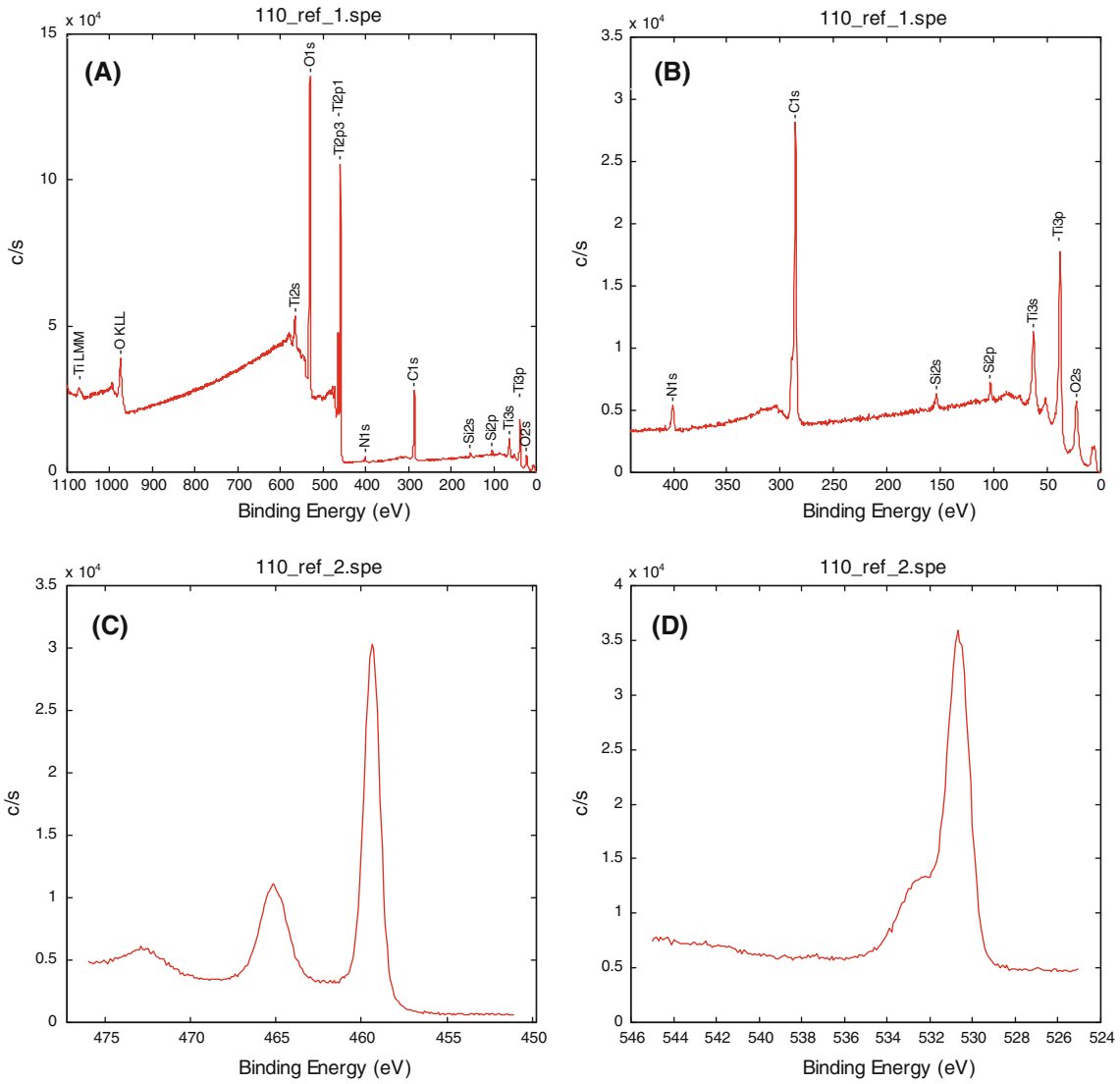

Fig. 1 XPS spectra from non-immersed TiO_2 (110) surface: Survey spectrum (**a**), detail of survey spectrum (**b**), high resolution Ti 2p (**c**), and high resolution O 1s (**d**)

X-ray photoelectron spectroscopy (XPS). Figure 1 shows an XPS survey spectrum, and high resolution spectra of the Ti 2p and O 1s peaks, for a non-immersed crystal surface. Spectra from the non-immersed samples were all similar, and dominated by Ti and O signals. A relatively strong signal from carbon was also detected in all cases, and also weak signals from nitrogen and silicon. The latter two are most likely due to contamination from air (adsorbed hydrocarbons), and possibly also from the polishing procedure. The carbon concentrations varied between 20 and 30 at.%, and the nitrogen and silicon levels were between 1 and 2 at.%. The Ti 2p 3/2 peak showed a single well defined contribution at 459 eV and a spin orbit splitting of 6 eV, both of which are consistent with TiO_2 stoichiometry. The O 1s peak is dominated by a peak around 531 eV due to TiO_2, and a weaker but pronounced peak around 533 eV which is most likely due to hydroxyl groups.

Figure 2 shows representative spectra from the immersed sample, in this case exemplified by a (110) surface immersed in PBS for 24 h. The spectra for the immersed samples all showed similar features. They were dominated by Ti and O, and relatively strong carbon signals and weak nitrogen signals were also observed. However, in no cases was Si detected. Instead the immersed samples all showed signals from Ca and P, at intensities depending on immersion time. The relative atomic concentrations are shown in Table 1.

High resolution spectra showed no clear changes in the Ti 2p peak after immersion. Interestingly, the O 1s signal from the immersed samples show a less distinct hydroxyl

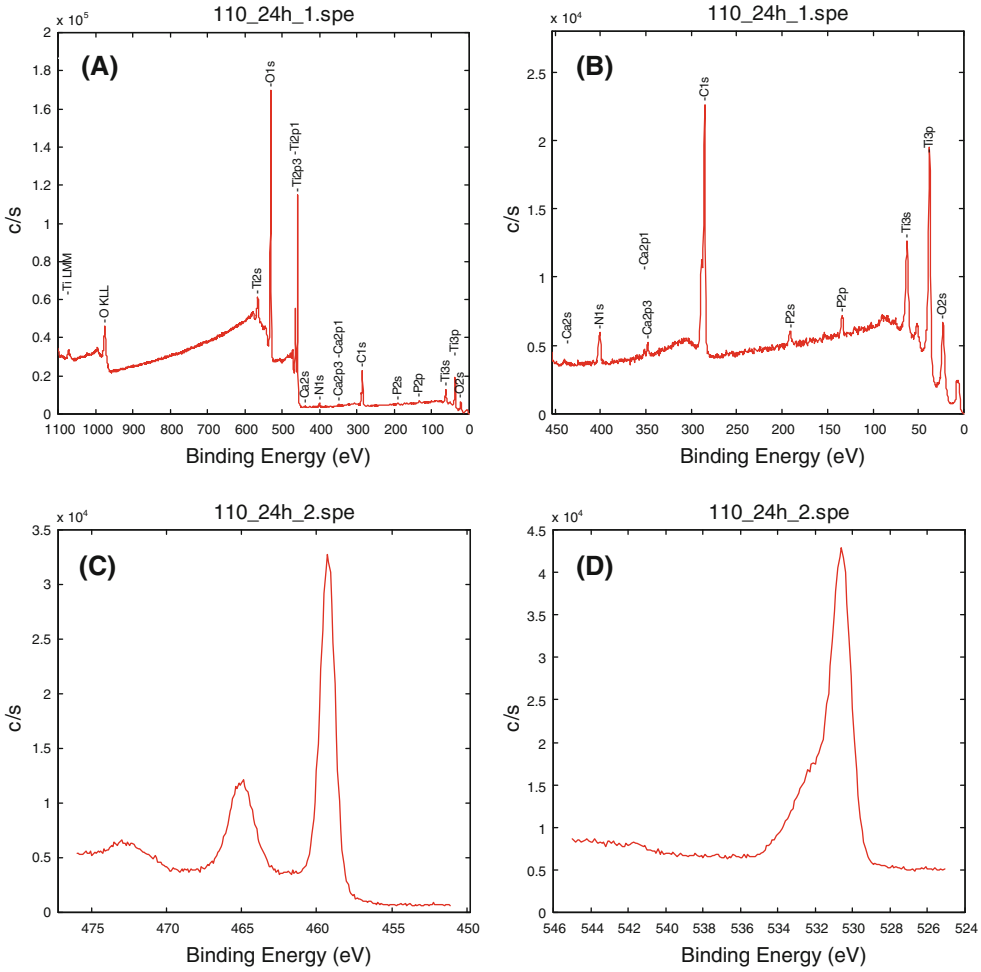

Fig. 2 XPS spectra from TiO_2 (110) surface immersed in PBS for 24 h: Survey spectrum (**a**), detail of survey spectrum (**b**), high resolution Ti 2p (**c**), and high resolution O 1s (**d**)

Table 1 Relative concentrations (at.%) of elements detected in XPS analyses. Also shown are the Ca/P ratios

	(110) ref	(110) 10 min	(110) 1 h	(110) 24 h	(001) 24 h
Ti	20.70	22.00	20.10	19.41	18.67
O	51.26	56.39	56.83	56.00	55.28
C	25.36	20.23	21.39	21.99	23.40
N	1.21	0.97	1.07	1.50	1.38
Si	1.04	0.00	0.00	0.00	0.00
Ca	0.00	0.03	0.08	0.20	0.29
P	0.00	0.41	0.53	0.91	1.01
Ca/P	n.a.	0.06	0.14	0.22	0.28

Values are mean of two measurement areas on each sample

peak, with a decreased intensity and a slight down-shift in binding energy. We interprete these changes as an indication that the hydroxyl groups are involved in the binding of ions from the PBS.

Table 1 shows the atomic concentrations for detected elements for an immersion series with the (110) surface. For comparison the corresponding data for a (001) surface immersed in PBS for 24 h is also shown. The data shows a continuous increase of the Ca and P concentrations, as well as Ca/P ratio, with immersion time. The Ca/P ratio is in all cases far below the expected for hydroxyapatite (nominal Ca/P ratio of 1.67). Comparison between the (110) and (001) surfaces after 24 immersion shows a higher Ca and P values for the latter. It should be noted though, that the Ca levels measured are close to the detection limit of XPS, and that the P concentrations measured are too low for reliable chemical state determination. Further details about these aspects are obtained from the TOF-SIMS data presented below.

4 Time of flight secondary ion mass spectrometry (TOF-SIMS)

Figure 3 shows positive secondary ion mass spectra from a non-immersed TiO_2 (110) surface and from a TiO_2 (110) immersed in PBS for 24 h. The non-immersed samples show similar spectra, irrespective of the crystal direction. They are dominated by signals from TiO_2 giving rise to ions such as

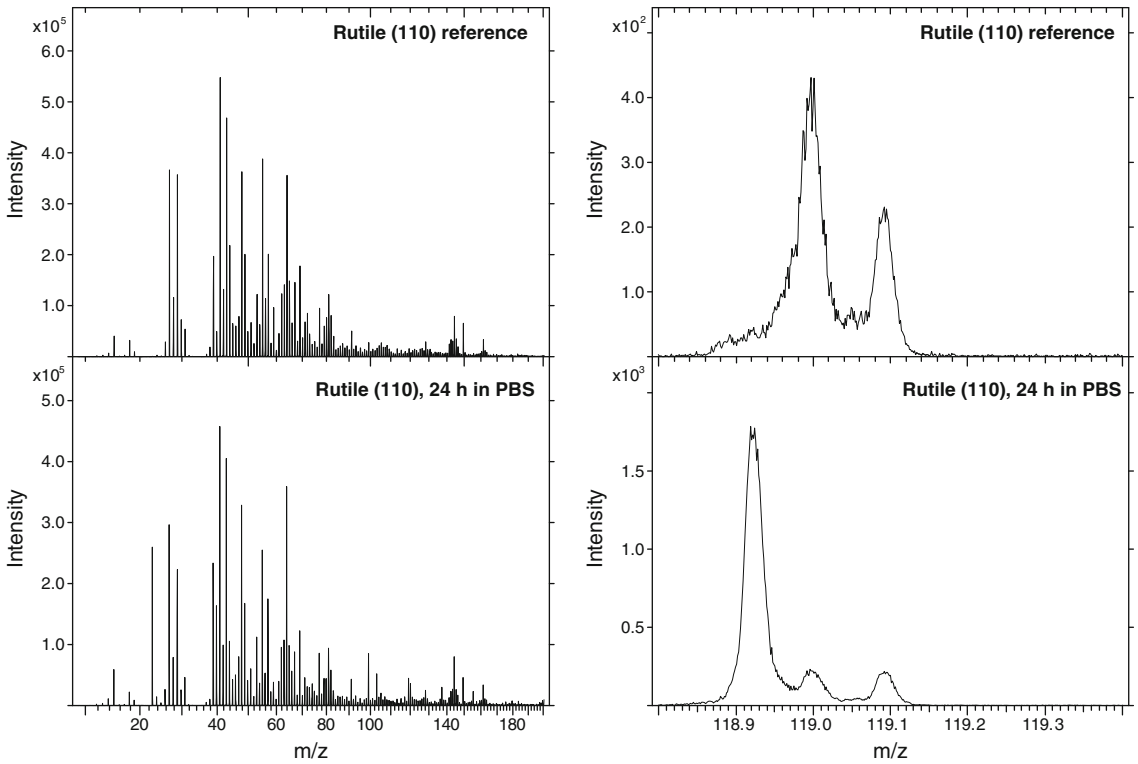

Fig. 3 Positive TOF-SIMS spectra of non-immersed (110) surface and (110) surface soaked in PBS for 24 h. Left spectra show *m/z* range 9–200, right spectra show peaks around *m/z* 119, with the $CaPO_3^+$ peak at *m/z* 118.9

Ti^+ (isotope cluster of five peaks between *m/z* 46 and 50, with the main isotope at *m/z* 48), TiO^+ (main isotope at *m/z* 64), TiO_2H^+ (*m/z* 81), Ti_2O^+ (*m/z* 112), $Ti_2O_3^+$ (*m/z* 144) in positive spectra, and TiO_2^- (*m/z* 80), TiO_3H^- (*m/z* 97) and $Ti_2O_5H^-$ (*m/z* 177) in negative spectra. In addition, the spectra contained signals from organic substances, mainly in the form of fragment ions of the type $C_xH_y^+$. Except for a relative weak fragment signal from phthalates at *m/z* 149 u, no specific organic contaminant species were identified.

The spectra from the samples immersed in PBS were qualitatively similar irrespective of crystal direction and immersion time. In addition to the above mentioned TiO_2 signals and organic signals, the immersed samples showed clear signals from ions due to calcium and phosphates, giving rise to peaks at Ca^+ (*m/z* 40), $CaOH^+$ (*m/z* 57), $CaPO_2^+$ (*m/z* 103) and $CaPO_3^+$ (*m/z* 119 u) in positive spectra, and PO_3^- (*m/z* 79) in negative spectra. These signals were observed only at very low intensities, close to the detection level, on the non-immersed samples. The relative intensities of the calcium and phosphate signals varied for the different crystalline directions and PBS immersion times, as shown in Fig. 4, upper panel. For the calcium phosphate signal $CaPO_3^+$ (at *m/z* 119) they are similar for all directions after 10 min soaking time. After 24 h the $CaPO_3^+$ intensities are higher for the (001) and (100) surface than for the (110) surface. The same was observed for the phosphate peak PO_3^- (*m/z* 79) in the negative spectra (Fig. 4, lower panel). The intensities were about the same level at 10 min and 1 h, and after 24 h the PO_3^- intensities were higher for the (001) and (100) surface than for the (110) surface. These observations are in agreement with the XPS results, and indicate that the adsorption of calcium and phosphate is faster on the (001) and (100) face than on the (110) surface.

Ion images (Fig. 5) of the lateral distribution of calcium phosphate signals showed a homogeneous distribution for all crystal directions and immersion times. This indicates that the initial deposition of calcium and phosphate ions occurs homogeneously on a length scale that can be resolved in the analysis (2–3 μm). However, we can not exclude that the deposition is inhomogeneous on a smaller scale.

5 Discussion

Previous work has shown that there are differences in the growth direction of hydroxyapatite on different rutile crystals (001), (100) and (110) [7]. In that study the authors' state that for understanding the growth mechanism of hydroxyapatite on single rutile faces the interaction between the calcium and phosphate ions at the substrate surface would be necessary to investigate [7]. In this study

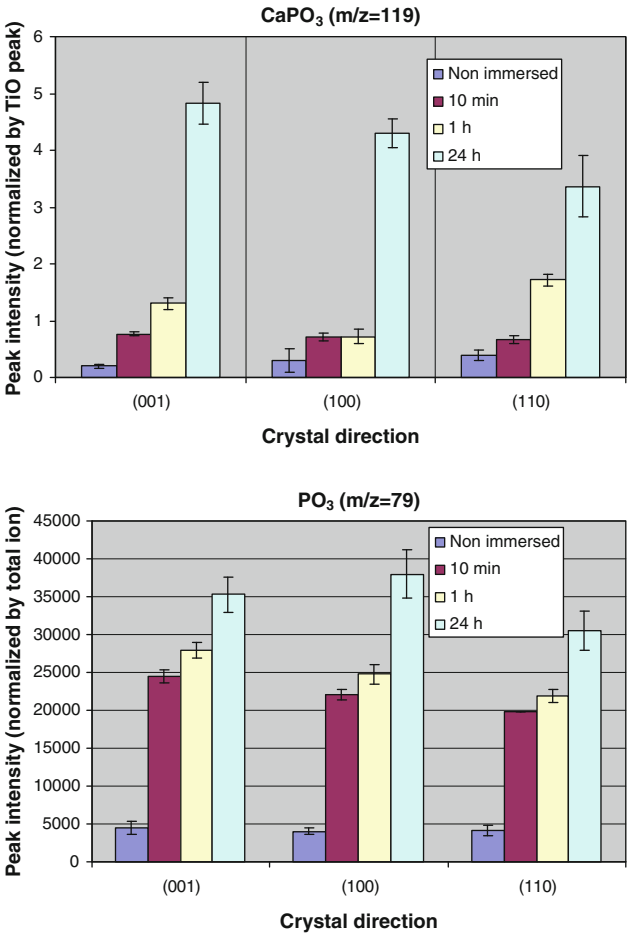

Fig. 4 Relative peak intensities $CaPO_3^+$ and PO_3^- for different crystal surfaces and PBS immersion times. The $CaPO_3^+$ peak intensities are normalized against the TiO^+ signal from TiO_2 and the PO_3^- signal is normalized against total ion intensity. Values shown are mean ± standard deviation for three measurement areas on each sample

we therefore used two highly surface sensitive techniques, XPS and ToF-SIMS, to study the early phenomena taking place at well defined TiO_2 single crystal surfaces upon immersion in PBS. The adsorption of Ca and phosphate ions is believed to the first crucial step in the nucleation of HA on single crystalline substrates. According to theoretical studies the driving force for adsorption is the electrostatic interaction between the Ca^{2+} complexes and the negatively charged deprotonated sites present on the surface of TiO_2 (110) [8]. In recent studies on nucleation there is a strong support for two features of the nucleation mechanism. The initial step is the deposition of Ca^{2+} ions on the substrate. The process is then followed by binding of the phosphate groups to form calcium phosphate [8]. Our data does not fully support such a mechanism, since the results show that the amount of Ca adsorbed at the surfaces is very low compared to phosphate. The results do, however, show that there are some differences in the adsorption behaviour of the three studied crystal faces. At shorter soaking times (10 min and 1 h) the intensities for the positive ion peaks $CaPO_3^+$ are quite similar for all three directions. In contrast, after 24 h the amount of adsorbed ions are higher for the (001) and (100) surfaces than for the (110) surface. Even after 24 h immersion time the Ca/P ratios are far below that of hydroxyapatite. The lateral distribution of adsorbed species was also found to be homogeneous (within the lateral resolution of the analysis, 2–3 μm). It thus appears that no nucleation or formation of hydroxyapatite, or other calcium phosphates such as octacalcium phosphate or tricalcium phosphate, has taken place at these conditions and immersion times. It should, however, be noted that these observations are for quite well-defined, low-index plane single crystal surfaces. It may well be that earlier nucleation will occur on less ordered

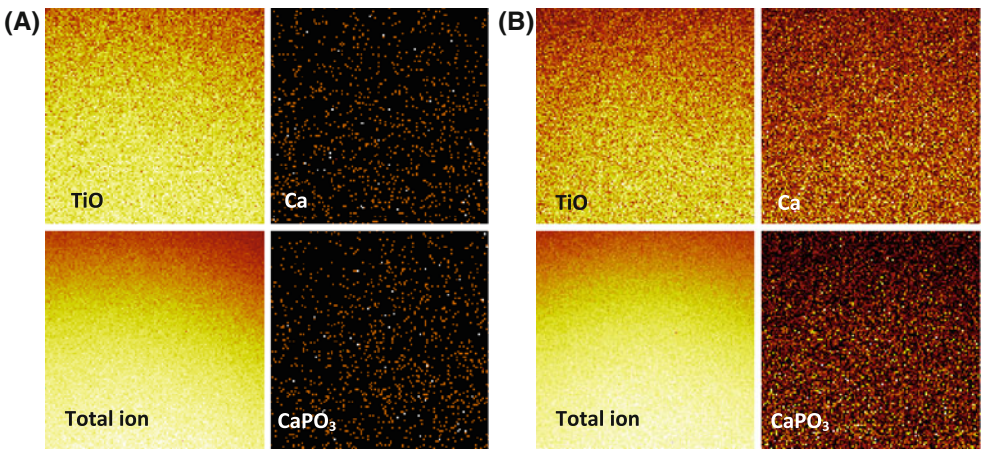

Fig. 5 TOF-SIMS ion images of **a** clean (001) surface **b** (001) surface soaked in PBS for 24 h

polycrystalline surfaces which have a much higher density of structural defects that can act as active nucleation sites.

6 Conclusions

In this study the adsorption of calcium and phosphate ions from PBS solution on single crystal rutile substrates (001), (100), (110) was investigated using a thorough experimental protocol without intervening air exposure between surface preparation and cleaning, and analysis by the surface sensitive techniques XPS and TOF-SIMS. Differences were observed in the adsorption of calcium- and phosphate ions, depending on crystal direction and soaking times. The adsorption of calcium and phosphate ions was faster on the (001) and (100) surfaces than on the (110) surface. The measured Ca/P ratios were in all cases far below that of hydroxyapatite and other calcium phosphates, showing that no crystal formation had taken place at the surfaces after the studied immersion times. These results could be used to further increase the understanding of HA formation on bioactive surfaces.

References

1. Yang B, Uchida M, Kim HM, Zhang X, Koboku T. Preparation of bioactive titanium metal via anodic oxidation treatment. Biomaterials. 2004;25:1003–10.
2. Wang XX, Yan W, Hayakawa S, Tsuru K, Osaka A. Improvement of the bioactivity of H_2O_2/TaCl5-treated titanium after subsequent heat treatments. J Biomed Mater Res. 2000; 52:171–6.
3. Uchida M, Kim HM, Kobuko T, Fujibayashi S, Nakamura T. Structural dependence of apatite formation on Titania gels in a simulated body fluid. J Biomed Res. 2003;64A:164–70.
4. Kuboku T, Kusitani H, Sakka S, Kitsugi T, Yamamuro T. Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramics A-W. J Biomed Mater Res. 1990;24: 721–34.
5. Li P, Kangasniemi I, de Groot K, Kobuko T. Bonelike hydroxyapatite induction by gel-derived titania on titanium substrate. J Am Ceram Soc. 1994;77(5):1307–12.
6. Li P, Ohtsuki C, Kuboku T, Nakaishi K, Soga K, de Groot K. The role of hydrated silica, titania and aluminia in inducing apatite on implants. J Biomed Mater Res. 1993;28(1):7–15.
7. Lindberg F, Heinrich J, Ericsson F, Thomsen P, Engqvist H. Hydroxyapatite growth on single-crystal rutile substrates. Biomaterials. 2008;29:3317–23.
8. Svetina M, Ciacchi C, Sbaizero O, Meriani S, De Vita A. Deposition of calcium ions on rutile (110): a first-principles investigation. Acta Mater. 2001;49:2169–77.
9. Vickerman JC, Briggs D. TOF-SIMS: surface analysis by mass spectrometry. Chichester: Surface Spectra, Manchester and IM Publications; 2001. p. 789.
10. Belu AM, Graham DJ, Castner G. Time-of-flight secondary ion mass spectrometry: techniques and applications for the characterization of biomaterial surfaces. Biomaterials. 2003;24: 3635–53.
11. Benninghoven A. Chemical analysis of inorganic and organic surfaces and thin films by static Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS). Angew Chem Int Ed Eng. 1994;33(10):1023–43.
12. Pacholski ML, Winograd N. Imaging with mass spectrometry. Chem Rev. 1999;99:2977–3006.
13. Eriksson C, Malmberg P, Nygren H. Time-of-flight secondary ion mass spectrometric analysis of the interface between bone and titanium implants. Rapid Commun Mass Spectrom. 2008;22(7): 943–9.