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Nano-scale study of the nucleation and growth of calcium phosphate coating on titanium implants

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Abstract

The nucleation and growth of a calcium phosphate (Ca-P) coating deposited on titanium implants from simulated body fluid was investigated by using atomic force microscopy (AFM) and environmental scanning electron microscopy (ESEM). Forty titanium alloy plates were assigned into two groups. One group with a smooth surface having a maximum roughness $R_{\max} < 0.10\,\mu\text{m}$ (s-Ti6Al4V) and a group with a rough surface with an $R_{\max} < 0.25\,\mu\text{m}$ (r-Ti6Al4V) were used. Titanium samples were immersed in SBF concentrated by five (SBF × 5) from 10 min to 5 h and examined by AFM and ESEM. Scattered Ca-P deposits of approximately 15 nm in diameter appeared after only 10 min of immersion in SBF × 5. These Ca-P deposits grew up to 60–100 nm after 4 h on both s- and r-Ti6Al4V substrates. With increasing immersion time, the packing of Ca-P deposits with size of tens of nanometers in diameter formed larger globules and then a continuous Ca-P film on titanium substrates. A direct contact between the Ca-P coating and the Ti6Al4V surface was observed. The Ca-P coating was composed of nanosized deposits and of an interfacial glassy matrix. This interfacial glassy matrix might ensure the adhesion between the Ca-P coating and the Ti6Al4V substrate. In the case of s-Ti6Al4V substrate, failures within this interfacial glassy matrix were observed overtime. Part of the glassy matrix remained on s-Ti6Al4V while part detached with the Ca-P film. The Ca-P coating detached from the smooth substrate, whereas the Ca-P film extended onto the whole rough titanium surface over time. In the case of r-Ti6Al4V, the Ca-P coating covered evenly the substrate after immersion in SBF × 5 for 5 h. The present study suggested that the heterogeneous nucleation of Ca-P on titanium was immediate and did not depend on the Ti6Al4V surface topography. The further growth and mechanical attachment of the final Ca-P coating strongly depended on the surface, for which a rough topography was beneficial.

Keywords: Biomimetic coatings; Amorphous calcium phosphate; Ti6Al4V; Atomic force microscopy (AFM); Environmental scanning electronic microscopy (ESEM); Nucleation; Crystal growth; Adhesion

1. Introduction

The nucleation and growth of calcium phosphates (Ca-P) on titanium and its alloys have been extensively investigated because of their relevance in orthopaedic applications. In vivo, a direct bone contact has been often observed between the host bone and titanium implants. This bone apposition was explained by the surface calcification of implants resulting from (1) the supersaturation in calcium and phosphate of the body fluids, and (2) the surface properties of titanium (Ti) [1–8]. Regarding the surface chemistry, titanium is naturally covered by an oxide layer, which is hydrated in aqueous media. It has been shown that hydroxyl groups on the titanium surface favoured the chemical bonding with calcium and phosphate ions [1,2,4,5,8]. The degree of supersaturation in the solution influences also the calcification ability of titanium and titanium oxides [3,7]. Regarding the surface morphology of titanium implants, it has been found that the nucleation and

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growth of Ca-P film can be affected by the substrate roughness, when immersed in Hank's Balanced Salt Solution, i.e. a simulated physiological fluid [6].

Resulting from these physico-chemical properties, the idea to create a Ca-P coating under simulated physiological conditions has risen. A so-called biomimetic coating process has been developed [8–13]. It is based on the heterogeneous nucleation of Ca-P from simulated body fluids (SBFs), which are supersaturated towards hydroxyapatite at $\mathrm{pH}=7.25$ and at 37°C [8]. By this technique, bone-like apatite coatings could be deposited on titanium implants within 7 days [9]. Recently, we have found that the deposition of biomimetic Ca-P coatings could be greatly accelerated by decreasing pH with carbon dioxide gas and simultaneously increasing concentrations by five ($\mathrm{SBF}\times 5$) [10]. Our previous studies have shown that uniform and well-attached Ca-P coatings could be deposited on titanium implants within 5 h [11]. It has been found that the precipitation of Ca-P onto titanium substrates was related to the out-gassing of carbon dioxide resulting in a pH increase and thus, the rise of supersaturation in the $\mathrm{SBF}\times 5$ solution [10,11]. In addition, the phase composition, crystallinity and adhesion of biomimetic Ca-P coatings on titanium were strongly related to the ionic strength, carbonate and magnesium contents in the $\mathrm{SBF}\times 5$ [12,13]. The magnesium content in the solution was found to be particularly critical for the formation of a Ca-P coating on Ti6Al4V substrate [13].

The goal of the present study was to investigate the early events in the nucleation and growth of Ca-P films on the Ti6Al4V surface from immersion time of 10 min to 5 h in $\mathrm{SBF}\times 5$. For this purpose, atomic force microscopy (AFM) and environmental scanning electronic microscopy (ESEM) have been used in order to detect at the nanometer scale the primary Ca-P nucleating on titanium substrate. The bonding and growth of Ca-P deposits onto the Ti6Al4V substrates were investigated for short periods of immersion and prior to any visible precipitation in solution.

2. Materials and methods

2.1. Materials

Forty Ti6Al4V plates of $10\times 10\times 1\,\mathrm{mm}^3$ were used for these experiments. The samples were mechanically polished for further AFM and ESEM observations. The Ti6Al4V plates were first polished with series of SiC papers (P320, P1200 and P4000) until a maximum roughness peak-to-valley $R_{\max}<0.25\,\mu\mathrm{m}$ [6]. Twenty Ti6Al4V plates were then polished with metallographic tissue and 1 μm diamond paste until a maximum roughness peak-to-valley of $R_{\max}<0.1\,\mu\mathrm{m}$. Depending on surface finishing, the batch polished up to $R_{\max}<0.25\,\mu\mathrm{m}$ was called rough Ti6Al4V (r-Ti6Al4V), and the batch polished up to $R_{\max}<0.1\,\mu\mathrm{m}$ was called smooth Ti6Al4V (s-Ti6Al4V). Thereafter, the Ti6Al4V plates were ultrasonically cleaned in acetone, ethanol 70% and demineralized water, 15 min for each step. The plates were finally dried at 50°C for few hours.

2.2. Biomimetic coating

Titanium samples were attached to specially designed holders and placed into a 1500 ml glass vessel. A five times concentrated simulated body fluid solution was prepared by dissolving reagent grade chemicals NaCl (40 g), $\mathrm{CaCl_2\cdot 2H_2O}$ (1.84 g), $\mathrm{MgCl_2\cdot 6H_2O}$ (1.52 g), $\mathrm{NaHCO_3}$ (1.76 g) and $\mathrm{Na_2HPO_4\cdot 2H_2O}$ (0.89 g) salts in 1000 ml of demineralized water under constant bubbling of carbon dioxide gas, magnetic stirring at 200 rpm and thermo stating at 37°C [13]. The supply of $\mathrm{CO_2}$ led to a pH of ~6 and to the complete dissolution of salts within 20 min. A clear $\mathrm{SBF}\times 5$ solution was obtained under these conditions. The onset of immersion time in $\mathrm{SBF}\times 5$ corresponded to the removal of $\mathrm{CO_2}$ supply. The pH and temperature of $\mathrm{SBF}\times 5$ were continuously recorded over time using a combined polymer membrane electrode (Knick, Portamess 913). In order to check the absence of precipitation in solution, a laser beam pointer of 1 mW was directed in the $\mathrm{SBF}\times 5$. The scattering of the red laser beam indicated the precipitation of Ca-P. The Ti6Al4V plates were immersed into $\mathrm{SBF}\times 5$ for various immersion times ranging from 10 min to a maximum of 5 h. After the different times, two samples were taken out and thoroughly rinsed with demineralized water to remove any loose Ca-P, dried overnight at 50°C in air and finally stored in closed plastic vials in desiccators to avoid any transformation of the Ca-P deposits due to air moisture. Subsequently, the samples were observed by field emission gun environmental scanning electron microscopy (FEG-ESEM, XL-30 Philips, Eindhoven, The Netherlands) after gold sputtering. Energy dispersive X-ray analysis (EDX), X-ray diffraction (XRD Rigaku Miniflex, CuK_α, 30 kV, 15 mV, scan range: 3–60°, scan speed: 2°/min, scan step: 0.02°) and Fourier transform infrared spectroscopy (FTIR, Perkin-Elmer Analytical Instruments, model Spectrum 1000) were performed to analyse the surface of titanium and Ca-P deposits over time. In addition, the samples were studied by AFM (Nanoscope III Multimode, Digital Instruments, Santa Barbara, CA). The measurements were performed in constant force mode in air with oxide-sharpened tips with a spring constant of 0.12 N/m as specified by the manufacturer (Digital Instruments). All images shown were flattened raw data. The cross-sections were used to determine the diameter of the observed Ca-P deposits. All experiments have been repeated at least 3 times in order to check the reproducibility and reliability of data.

3. Results

As shown in Fig. 1, the pH of SBF × 5 increased steadily from 6.05 to 7.3 in 15 h. This increase of pH resulted from the out gassing of CO_2 and the buffering capacity of phosphate and carbonate in SBF × 5. While pH increased, SBF × 5 solution gradually reached supersaturation and thereafter, the labile state at which the Ca-P precipitated. This precipitation in solution occurred around pH 6.8 or 5 h of immersion in SBF × 5 [12]. After 5 h, uniform and thin ($<3\,\mu$m) calcium phosphate coatings covered all the Ti6Al4V substrates. The EDX spectra (not shown) exhibited calcium and phosphate peaks with traces of magnesium, sodium and chlorine. Small peaks of the titanium substrate were also detected [11]. XRD and FTIR analyses showed a halo without diffraction peaks and featureless phosphate and carbonate bands, respectively [12]. These XRD and FTIR features were characteristic of the deposition of an amorphous carbonated calcium phosphate phase onto the titanium substrates.

The early events in the nucleation and growth of Ca-P films on the Ti6Al4V surface from immersion time of 10 min to 5 h in SBF × 5 were investigated by using AFM and ESEM. Prior to the immersion, the surfaces of both the r-Ti6Al4V and the s-Ti6Al4V substrates were examined by AFM (Fig. 2). Scratches due to the mechanical polishing were visible in both cases. But the different polishing techniques led to numerous and deep scratches on the r-Ti6Al4V surface (Fig. 2a) while the s-Ti6Al4V appeared more flat than the former (Fig. 2b). The roughness values averaging the peak-to-valley distances were $R_a < 0.25$ and 0.1 µm for r- and s-Ti6Al4V substrates, respectively.

After only 30 min of immersion in SBF × 5 (pH = 6.18), some Ca-P deposits were visible on the r-Ti6Al4V substrates (Fig. 3a). These Ca-P globules scattered on the whole titanium surface even if no precipitation was detected in solution using a laser beam pointer. The diameter of these Ca-P deposits varied from 50 up to 100 nm (Fig. 3a). The globules of about 100 nm in size seemed to be composed of aggregated smaller entities. After 2 h of immersion (pH = 6.45), part of the r-Ti6Al4V substrate surface was covered with a Ca-P film (Fig. 3b) and precipitation in solution was still not detected. This Ca-P film was composed of deposits with an approximate size of 100–500 nm and these deposits have merged to form a continuous layer. At this stage, the thickness of this layer was about 100 nm. Again, the Ca-P deposits appeared to be formed of aggregated entities with an approximate diameter of few tens of nanometres. With increasing immersion time, the film covered a larger part of r-Ti6Al4V substrate. After 3 h of immersion in SBF × 5 (pH = 6.60), the film was composed of large Ca-P globules linked to each other (Fig. 3c). In other areas, individual globules have deposited on the r-Ti6Al4V substrates. The diameter of these globules varied approximately from 500 to 1000 nm. By comparing Figs. 3a–c, one can see that the size of these Ca-P globules has grown from 50 to 1000 nm together with the thickness of the Ca-P layer. After 4 h of immersion in SBF × 5 (pH = 6.71), the surface of the r-Ti6Al4V substrate was completely covered by Ca-P deposits forming a continuous film (Fig. 3d). At this time, we could not detect any precipitation in solution while the scattering of the laser beam was observed 1 h later or after 5 h of immersion (pH = 6.80). On the top of this Ca-P film, bigger globules were visible (Fig. 3d). In all cases, the Ca-P deposits on r-Ti6Al4V were composed of two entities: (1) more or less large globules of few hundreds of nanometres depending on the immersion time in SBF × 5 and (2) these globules were formed by the aggregation of smaller Ca-P entities of approximately tens of nanometres.

The AFM observations corroborated the previous findings with ESEM. As shown in Fig. 4, the r-Ti6Al4V surface topography evolved with immersion time in SBF × 5. At the first measured point (30 min, pH = 6.18), the r-Ti6Al4V sample exhibited already scattered deposits on its surface suggesting the heterogeneous nucleation of Ca-P (Fig. 4a). The size of these deposits was a few nanometres in diameter. By increasing immersion time to 1 h (pH = 6.27) the Ca-P deposits became bigger and covered homogeneously the whole observed area (Figs. 4b–d). The presence of these Ca-P deposits is evidenced by a quick comparison with AFM images of the bare r-Ti6Al4V substrates shown in Fig. 2. The diameter of these Ca-P deposits reached approximately 50–100 nm after 4 h of immersion. This increase in diameter of deposits confirmed the previous ESEM observations where artefacts due to gold sputtering could be suspected. One can also see that the Ca-P deposits followed initially the topography of substrates (Fig. 4c) but masked entirely the scratches as their size increased with time (Fig. 4d).

The s-Ti6Al4V specimens were studied in the same experimental conditions. As shown in AFM images

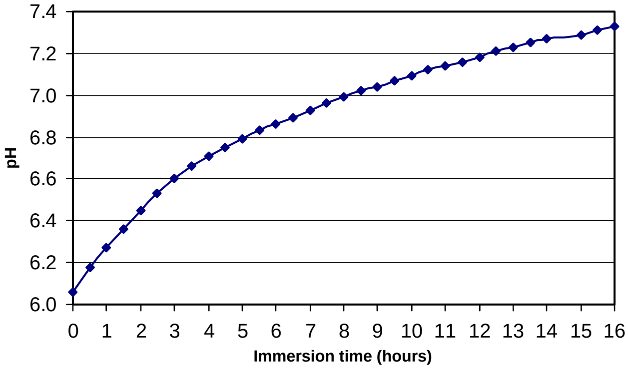

Fig. 1. pH versus immersion time in SBF × 5.

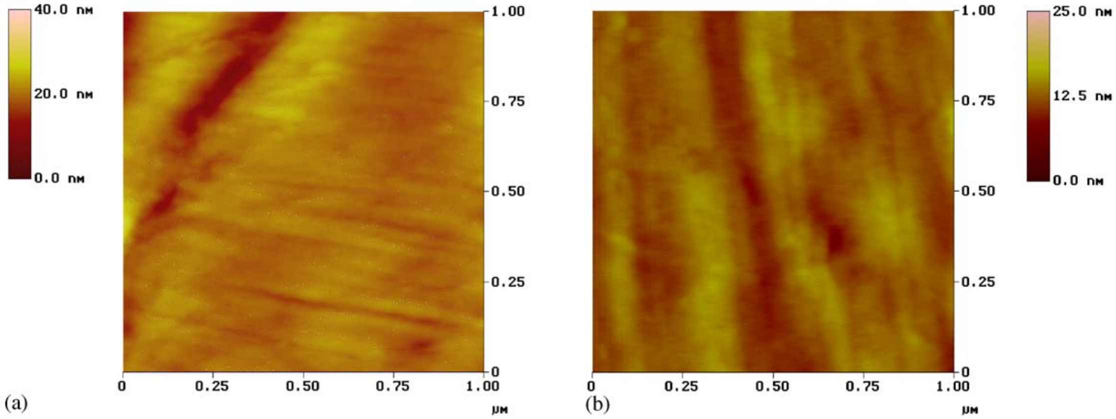

Fig. 2. AFM images of (a) r-Ti6Al4V and (b) s-Ti6Al4V; window 1 µm.

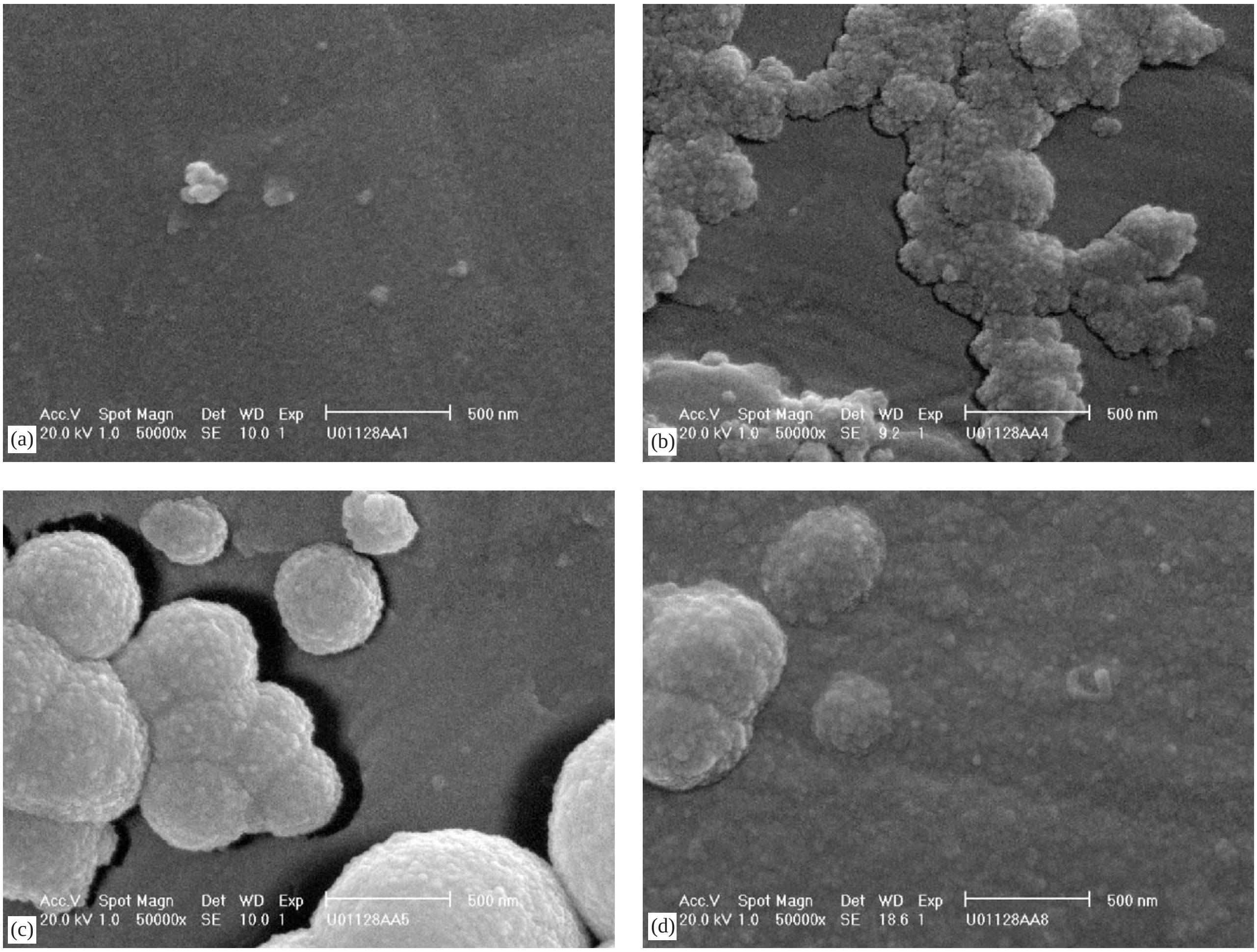

Fig. 3. ESEM micrographs of r-Ti6Al4V versus soaking time in SBF × 5 (magnification × 50 000). (a) 30 min, (b) 2 h, (c) 3 h and (d) 4 h of immersion.

(Fig. 5), the surface topography evolved with immersion time in SBF × 5. Already at the first measured point of 10 min, s-Ti6Al4V sample exhibited scattered globules on its surface suggesting the heterogeneous nucleation of Ca-P (Fig. 5a). Again, the topography was completely different than the bare s-Ti6Al4V substrate shown in Fig. 2b. The diameters of these Ca-P deposits were around 10–80 nm. Both the size and the density per surface area of deposits increased with immersion time from 30 min to 1 h (Figs. 5b and c). For longer

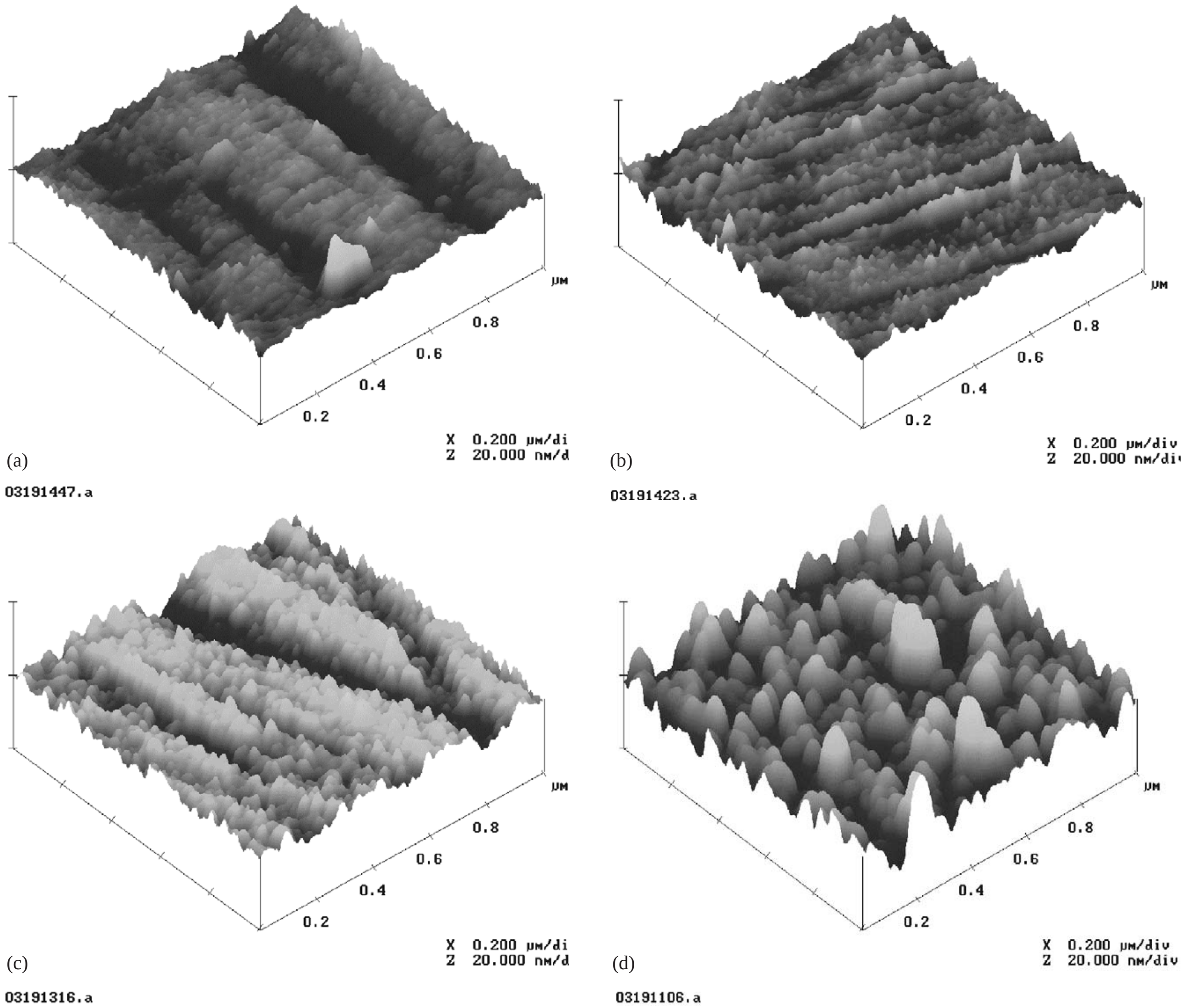

Fig. 4. AFM images r-Ti6Al4V substrate immersed for (a) 30 min, (b) 1 h, (c) 2 h and (d) 4 h.

immersion time, the Ca-P deposits were not homogeneously present on the titanium surface, while their size remained approximately 50 nm in diameter (Fig. 5d).

A similar behaviour was observed for s-Ti6Al4V under ESEM. After 20 min, some Ca-P deposits were scattered on the s-Ti6Al4V substrate (Fig. 6a). Their diameter varied from tens of nanometres to 300 nm and they were in direct contact with the s-Ti6Al4V surface. In few areas of about 5 μm^2, a continuous Ca-P film covered the s-Ti6Al4V substrate (Fig. 6b). This film was composed of spherical globules of 200–1000 nm (Fig. 6b). At higher magnification, we noticed that the film is formed of smaller and flatter globules in direct contact with the titanium substrate (Fig. 6c). There, the Ca-P entities of tens of nm in diameter were lying between s-Ti6Al4V and the Ca-P film. After 40 min and 1 h of immersion in SBF × 5, the Ca-P film has developed on s-Ti6Al4V surface. In Fig. 6d, the Ca-P film was composed of large globules in which relatively tiny entities of tens of nanometres aggregated. In addition, beneath and surrounding the Ca-P film, a glassy interfacial matrix was observed. At higher magnification (Figs. 6e and f), this glassy interface appeared to be partially composed of entities apparently in direct contact with s-Ti6Al4V. The diameter of these entities varied between tens of nanometres to 100 nm. In other areas, the Ca-P film started to detach from the s-Ti6Al4V substrate (Fig. 7). A failure was observed at the glassy interface: part of the glassy interface remained on Ti6Al4V, and the other part remained attached to the Ca-P film (Figs. 7a–c). For longer immersion times up to 2 h, the Ca-P film developed and covered larger areas of the s-Ti6Al4V substrate (Fig. 7d). In other areas, the globules appeared however rougher than in previous observations, and less bonded to each other's (Fig. 7e).

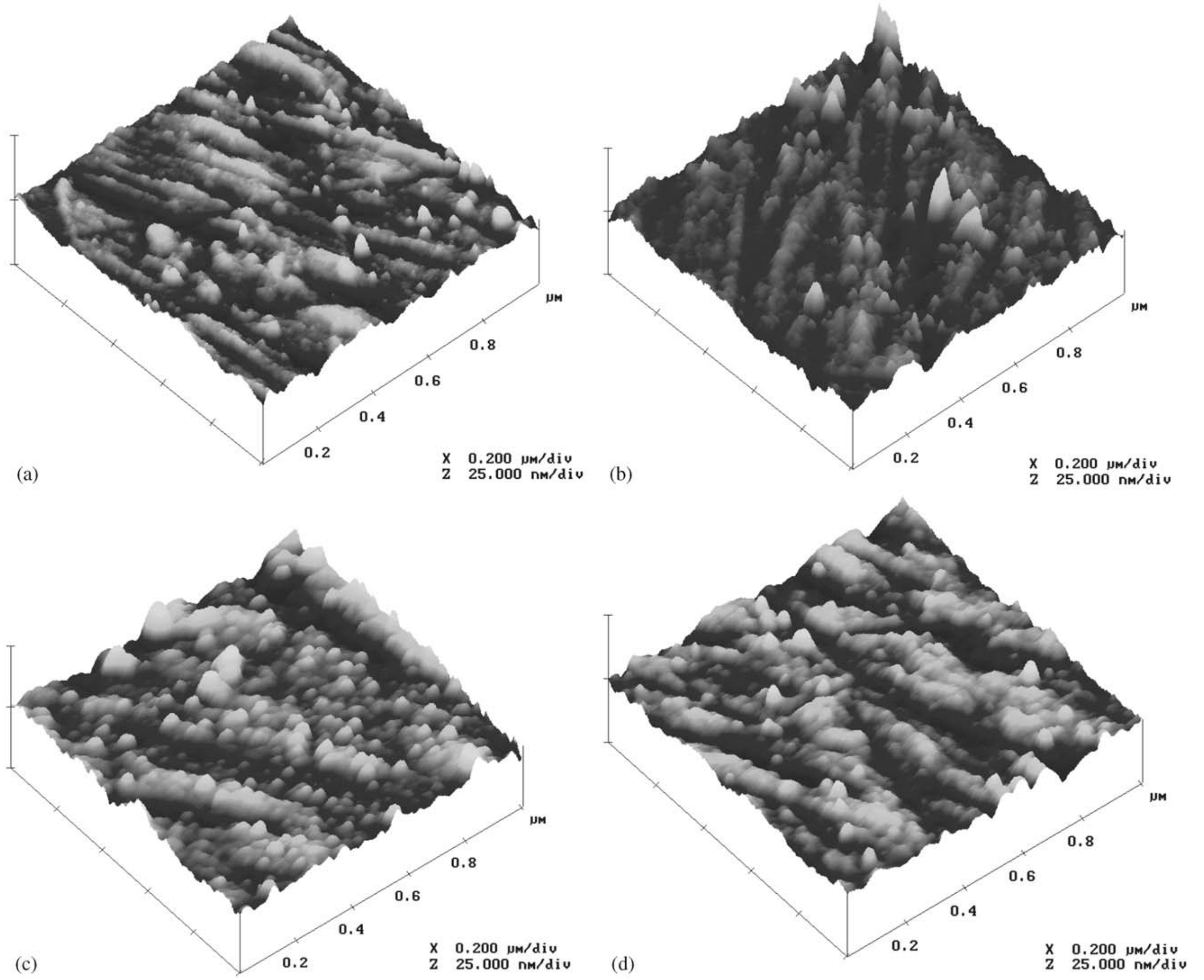

Fig. 5. AFM images r-Ti6Al4V substrate immersed for (a) 10 min, (b) 30 min, (c) 1 h and (d) 4 h.

After 4 h of immersion in SBF × 5, a Ca-P film was observed (Fig. 7f) but this layer was solely present in some areas of the s-Ti6Al4V substrate. In opposition to the earlier immersion periods, the Ca-P coating appeared flat, but always composed of entities of tens of nm in diameter.

4. Discussion

We have previously shown that SBF × 5 solution reached the limits of supersaturation at pH ~6.8 after ~5 h of immersion. At a pH higher than 6.8, the SBF × 5 reached the labile state leading to the precipitation of Ca-P in the solution, and to the homogeneous deposition of an amorphous Ca-P coating on the whole Ti6Al4V substrate. This coating was composed of an amorphous carbonated calcium phosphate phase with traces of magnesium. After 24 h in SBF × 5, the Ca-P coating on titanium implants was dense and uniform, has a thickness of approximately 3 μm and exhibited some cracks [13]. This thin and amorphous layer serves as a seed surface on titanium implants to further precipitate more thick and crystalline coatings [14].

The supersaturation of SBF × 5 solution continuously increased with time, out gassing of CO_2 and pH. As the partial pressure of CO_2, pH and concentrations of ions would not be accurately and simultaneously measured in the course of experiments, it was not possible to calculate the degree of supersaturation in SBF × 5. Further, the SBF × 5 changed its supersaturation state over time with respect to calcium carbonate, DCPD, OCP and finally HAP.[1] Nevertheless, none of these phases has been detected on titanium due to the high inhibiting effects of magnesium and carbonate and high

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[1] The terms of heterogeneous nucleation and secondary nucleation must be referred to the definitions established by Mullin: heterogeneous nucleation is induced by a foreign substrate, and secondary nucleation is induced by crystals present in the system [15].

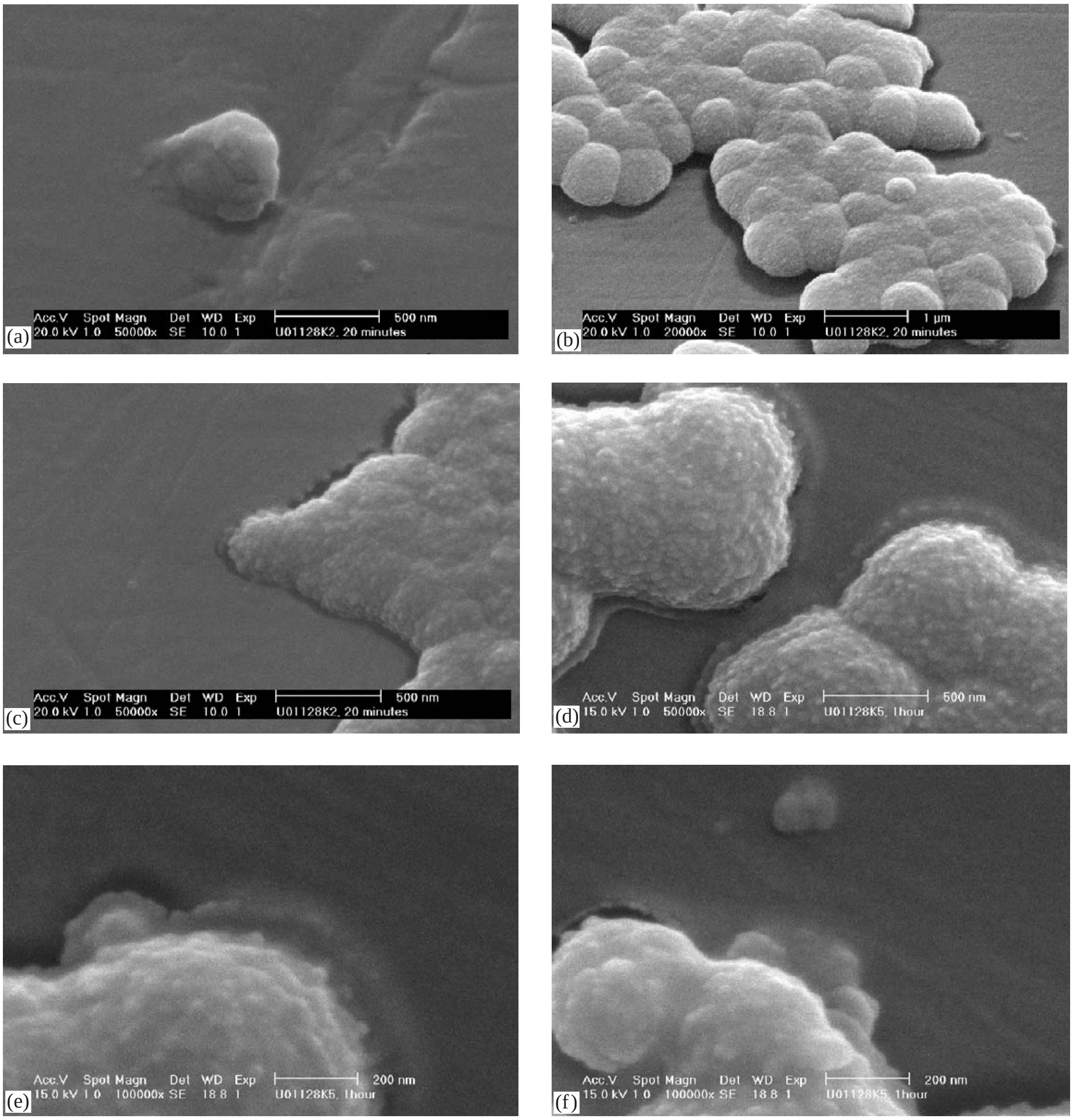

Fig. 6. ESEM micrographs of r-Ti6Al4V specimens immersed in SBF × 5 for different periods. After 20 min: (a) single Ca-P globule growing on r-Ti6Al4V (magnification × 50 000), (b) agglomerated Ca-P globules forming a continuous film on titanium (× 20 000), (c) bonding of Ca-P film on titanium (× 50 000). After 1 h of immersion: (d) Ca-P film and its glassy halo on r-Ti6Al4V substrate (× 50 000), (e) and (f) details of the glassy interface between Ca-P globules and titanium (× 100 000).

ionic strength [11–14]. Indeed, the Ca-P film was only composed of amorphous carbonated calcium phosphate and crystalline phases were not detected in both the coating and the precipitate even after immersion for 24 h [10–13]. These studies have indicated the high stability of the amorphous Ca-P film which is in equilibrium with the SBF × 5 solution.

In the present study, the formation of Ca-P on Ti6Al4V has been investigated for short immersion periods. The early events occurring on titanium substrates have been studied with two complementary microscopic techniques, AFM and ESEM. It has been found that the Ca-P deposition occurred already after 10 min of immersion in SBF × 5 solution, i.e. at pH ~6.0, while no precipitation was detected in solution at this stage. The heterogeneous nucleation of Ca-P on Ti6Al4V substrate occurred before SBF × 5 attained the labile state indicated by the formation of a Ca-P precipitate in the solution. The high chemical affinity of Ca-P for titanium might explain this early heterogeneous nucleation [1,2,4]. It might also result from the high concentrations of calcium and phosphate in SBF × 5. The Ca-P deposited at early immersion times by the heterogeneous nucleation of Ca-P entities scattered on Ti6Al4V substrate. The average diameter of these primary entities was a few nanometres in diameter. With an increasing immersion time, these entities aggregated into large globules of approximately

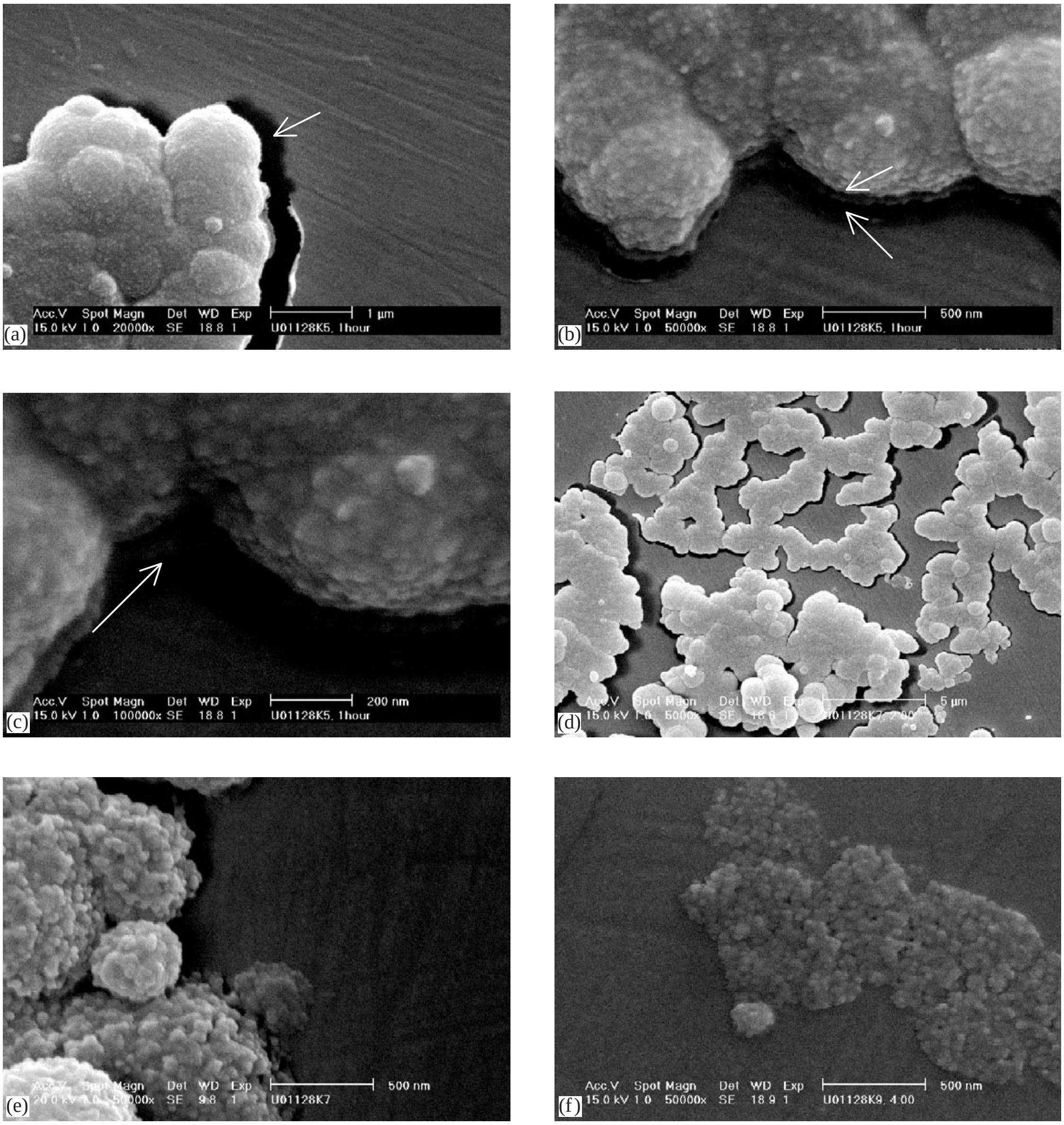

Fig. 7. ESEM micrographs of s-Ti6Al4V immersed in SBF × 5 (a), (b) and (c) display the detachment of the Ca-P film from s-Ti6Al4V substrate within the glassy interface (arrows) at magnification (a) × 20 000, (b) × 50 000, (c) × 100 000. After 2 h of immersion: (d) development of the Ca-P film (× 5000), (e) morphology of the Ca-P film (× 50 000). After 4 h of immersion: (f) morphology of the Ca-P film.

50–100 nm which expanded on the whole Ti6Al4V after 4 h of immersion (Figs. 4 and 5). These nanometre-scale entities were hardly detected as an individual item by ESEM, but were more likely aggregated into large globules. This suggests that the large globules might have been formed by a secondary nucleation mechanism onto nanosized Ca-P entities. The nucleation of calcium phosphate should be easier on a surface of the same nature (Ca-P) compared to the nucleation on a chemically different surface like titanium oxide. [15]. Nevertheless, some Ca-P nuclei have directly formed on Ti6Al4V substrate while other clustered on the surface of the primary nuclei forming large globules and then a continuous Ca-P film on titanium. The growth of these secondary globules seems to be limited to a diameter of approximately 1000 nm. These secondary globules could be found individually or bond to each other's creating a film that extended on the Ti6Al4V substrate. The globular shape and the diameter in the nanometre range of the Ca-P entities are characteristics of an amorphous Ca-P phase [16–19].

Besides these globular amorphous Ca-P entities, a glassy matrix was observed at the interface between Ti6Al4V substrate and the globular Ca-P film (Figs. 6d–f). This interfacial glassy matrix partially contained nuclei of tens of nanometers in diameter and appeared glassy and unstructured for another part. The chemical composition of this interfacial glassy matrix could not be established by EDX. Nevertheless, we can hypothesize that this matrix might be composed of smaller Ca-P entities stabilized by magnesium ions and water [18–20]. According to Posner, the formation of amorphous Ca-P starts with the packing of spherical clusters $Ca_9(PO_4)_6$ surrounded by water molecules and other labile ions.[18] Recently, the existence and the stability of these clusters have been demonstrated in a magnesium-free and carbonate-free SBF solution. From $pH = 5.28$ to 7.40, their diameter was constant between 0.7 and 1.0 nm [20]. In a regular SBF solution containing magnesium and carbonate, the diameter of the clusters was not significantly affected by these two apatite crystal growth inhibitors, but their diameter was slightly higher in a 1.5 more concentrated SBF solution (SBF × 1.5) [20]. In the present study, one can expect that the unstructured matrix might be composed of similar packed clusters, although certainly larger in diameter due to the higher supersaturation of SBF × 5. The presence of magnesium ions at the Ca-P coating/Ti6Al4V substrate interface may stabilize these tiny amorphous Ca-P entities and ensure the chemical bonding of Ca-P clusters to the substrate [16,21].

The interfacial glassy matrix remarkably illustrates the way in which the Ca-P film formed on Ti6Al4V and bond to the surface. In some parts, this unstructured matrix insured a direct contact between the Ca-P globules and the Ti6Al4V substrate (Fig. 6d). The presence of an interface composed of very small entities may create an efficient adhesion of the final Ca-P coating and favour secondary nucleation of calcium phosphate [8,9,11,13,16]. Nanosized and aggregated Ca-P entities observed under both AFM and ESEM are probably well bound to titanium. Those amorphous and tiny Ca-P globules are certainly less sensitive to mechanical stresses as compared to more crystalline and large Ca-P deposits [22–24]. The importance of the nanometre size of the interfacial nuclei corroborates the fact that magnesium is a critical element in the deposition of Ca-P coating [11,13]. In preventing the growth of the Ca-P nuclei, magnesium acted as a gluing agent between the Ca-P film and the Ti6Al4V substrate. In some other zones, Ca-P was found detached from the substrate and the failure appeared to be within this interfacial matrix and not in the Ca-P layer (Figs. 7a–c). Part of the interfacial glassy matrix remained attached to Ti6Al4V, and the other part detached with the large Ca-P globules. This failure in the middle of the interface indicates that the bonding between the matrix and the Ti6Al4V substrate was as strong as the matrix and the Ca-P globules, suggesting a chemical bonding between Ca-P and titanium involving magnesium.

Although a comparable initial Ca-P growth was observed for s-Ti6Al4V and r-Ti6Al4V, the final surface coverage of the Ca-P coating differed for the two substrates. With regard to r-Ti6Al4V, Ca-P entities grew with time, and they significantly spread all over the substrate between 2.5 and 3 h. On the other part, the Ca-P film has significantly disappeared from s-Ti6Al4V substrate after 2 h of immersion in SBF × 5 solution. Only few parts remained covered at long immersion time, and the morphology of the Ca-P film had changed. The behaviour of r-Ti6Al4V plates is in accordance with the usual Ca-P coating process previously described [11]. The Ca-P coating grew over time on Ti6Al4V with an average roughness of 0.8 μm, i.e. rougher than the bare r-Ti6Al4V and s-Ti6Al4V substrates. The initial heterogeneous nucleation of Ca-P seems not to be affected by the substrate topography. As illustrated in Fig. 6a, the Ca-P globule did not grow specifically in a scratch but on an apparently flat area of the substrate. However, surface topography might have affected the reactivity of the substrate, exhibiting more active nucleation sites for Ca-P as the roughness of titanium increased. In addition, surface topography affected the mechanical stability of the Ca-P coating. This is illustrated with the delamination of Ca-P coating on the very smooth s-Ti6Al4V. This smooth substrate might not be able to anchor sufficiently the growing Ca-P globules to withstand shear stresses. Since the thickness of the Ca-P film increased with immersion time, it might provoke a failure in the interfacial glassy matrix, especially when the film grows on a smooth surface [23,24]. Resulting from the low roughness and liquid flow, the Ca-P coating detached from s-Ti6Al4V substrate (Figs. 7a–c). The relative irregular topography of r-Ti6Al4V might stabilize the deposited Ca-P film, and its continuous growth over time. The formation of Ca-P globules took place on s-Ti6Al4V, but not sufficiently to cover the whole substrate. On the other hand, the r-Ti6Al4V was suddenly and completely covered by a layer of small Ca-P globules, indicating that SBF × 5 reached the labile state. Therefore, two distinct events occurred on the Ti6Al4V substrate. First, a heterogeneous nucleation of Ca-P on Ti6Al4V is initiated spontaneously on Ti6Al4V certainly because of chemical affinities between the Ca-P nuclei and the titanium substrate [1,2,4] followed by a secondary nucleation from the initial Ca-P globules already deposited on the substrate. This event appeared to be independent of the topography of the substrate. Second, the labile state of SBF × 5 solution is attained, and a marked Ca-P nucleation and growth takes place. The initial Ca-P film already developed on r-Ti6Al4V substrate acts as a stable basis for the development of the final spreading and the complete covering of r-Ti6Al4V. Thereby, the surface roughness of the substrate has a secondary effect on the Ca-P coating formation: a smooth surface does not preclude the initial Ca-P nucleation but the further mechanical stability of the Ca-P coating.

5. Conclusion

The formation mechanism of the Ca-P coating from SBF × 5 solution can be divided in two subsequent steps. First, the heterogeneous nucleation of Ca-P is initiated at the earliest point of the immersion in SBF × 5. Second, the coating develops all over the substrate when the labile state of SBF × 5 solution is attained. The heterogeneous nucleation and growth of Ca-P on titanium surface is initiated by chemical bonding of nanosized clusters forming an interfacial unstructured matrix, which was certainly stabilized by the presence of magnesium ions. The surface roughness did not affect the development of the Ca-P globules. However, a rougher substrate allowed the final Ca-P coating to remain on the surface, whereas a smoother substrate was not efficient for the anchorage of the final Ca-P coating. In summary, the heterogeneous nucleation of Ca-P did not depend on the surface morphology, whereas the mechanical stability of the Ca-P coating requires a Ti6Al4V substrate of a roughness higher than 0.10 μm.

References

[1] Tengvall P, Lundstrom I. Physico-chemical considerations of titanium as a biomaterial. Clin Mater 1992;9(2):115–34.

[2] Healy KE, Ducheyne P. Hydration and preferential molecular adsorption on titanium in vitro. Biomaterials 1992;13(8):553–61.

[3] Combes C, Freche M, Rey C. Nucleation and crystal growth of dicalcium phosphate dehydrate on titanium powder. J Mater Sci Mater Med 1995;6:699–702.

[4] Combes C, Rey C, Freche M. XPS and IR study of dicalcium phosphate dehydrate. Colloids Surf B: Biointerfaces 1998;11:15–27.

[5] Li P, Ohtsuki C, Kokubo T, Nakanishi K, Soga N, de Groot K. Role of hydrated silica, titania and alumina in forming biologically active bone-like apatite on implants. J Biomed Mater Res 1994;28:7–15.

[6] Leitao E, Barbosa M, de Groot K. Influence of substrate material and surface finishing on the morphology of the calcium phosphate coating. J Biomed Mater Res 1997;36:85–90.

[7] Wu W, Nancollas GH. Kinetics of nucleation and crystal growth of hydroxyapatite and fluoroapatite on titanium oxide surfaces. Colloids Surf B: Biointerfaces 1997;10:87–94.

[8] Kokubo T, Kushitani H, Abe Y, Yamamuro T. Apatite coating on various substrates in simulated body fluids. Bioceramics 1989;2:235–42.

[9] Li P, Ohtsuki C, Kokubo T, Nakanishi K, Soga N, Nakamura T, Yamamuro T. Effects of ions in aqueous media on hydroxyapatite induction by silica gel and its relevance to bioactivity of bioactive glasses and glass-ceramics. J Appl Biomater 1993;4(3):221–9.

[10] Barrere F, Layrolle P, van Blitterswijk, de Groot K. Fast formation of biomimetic Ca-P coating on Ti6Al4V. Mater Res Soc Symp Proc 2000;599:135–40.

[11] Habibovic P, Barrere F, van Blitterswijk CA, de Groot K, Layrolle P. Biomimetic hydroxyapatite coating on metal implants. J Am Ceram Soc 2002;85(3):517–22.

[12] Barrere F, van Blitterswijk CA, de Groot K, Layrolle P. Influence of ionic strength and carbonate on the Ca-P coating formation from SBF × 5 solution. Biomaterials 2002;23(9):1921–30.

[13] Barrere F, van Blitterswijk CA, de Groot K, Layrolle P. Nucleation of biomimetic Ca-P coatings on Ti6A14V from a SBF × 5 solution: influence of magnesium. Biomaterials 2002;23(10):2211–20.

[14] Barrere F, Layrolle P, van Blitterswijk CA, de Groot K. Biomimetic calcium phosphate coatings on Ti6Al4V: a crystal growth study of octacalcium phosphate and inhibition by Mg^{2+} and HCO^{3-}. Bone 1999;24:107S–11S.

[15] Mullin JW. Crystallization, 3rd ed. Oxford, UK: Butterworths-Heinemann Ltd; 1993.

[16] Abbona F, Baronnet A. A XRD and TEM study on the transformation of amorphous calcium phosphate in the presence of magnesium. J Crystal Growth 1996;165:98–105.

[17] Christoffersen J, Christoffersen MR, Kibalczyc W, Andersen FA. A contribution to the understanding of the formation of calcium phosphates. J Crystal Growth 1989;94:767–77.

[18] Posner AS, Betts F. Synthetic amorphous calcium phosphate and its relation to bone mineral structure. Acc Chem Res 1975;8: 273–81.

[19] Onuma K, Ito A. Cluster growth model for hydroxyapatite. Chem Mater 1998;10:3346–51.

[20] Oyane A, Onuma K, Ito A, Kokubo T. Clustering of calcium phosphate in SBF and in the system $CaCl_2$-H_3PO_4-KCl-H_2O. Bioceramics 1999;12:157–60.

[21] Boskey AL, Posner AS. Magnesium stabilization of amorphous calcium phosphate: a kinetic study. Mater Res Bull 1974;9: 907–16.

[22] Wolke JGC, van Dijk K, Schaeken HG, de Groot K, Jansen JA. Study of the surface characteristics of magnetron-sputtered calcium phosphate coatings. J Biomed Mater Res 1994;28: 1477–84.

[23] Cleries L, Martinez E, Fernandez-Pradas JM, Sardin G, Esteve J, Morenza JL. Mechanical properties of calcium phosphate coatings deposited by laser ablation. Biomaterials 2000;21:967–71.

[24] Fernandez-Pradas JM, Cleries L, Martinez E, Sardin G, Esteve J, Morenza JL. Influence of thickness on the properties of hydroxyapatite coatings deposited by KrF laser ablation. Biomaterials 2001;22:2171–5.