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Surface modification of Ti-6Al-4V alloy for biomineralization and specific biological response: Part I, inorganic modification

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Abstract

Titanium and its alloys represent the gold standard for orthopaedic and dental prosthetic devices, because of their good mechanical properties and biocompatibility. Recent research has been focused on surface treatments designed to promote their rapid osteointegration also in case of poor bone quality. A new surface treatment has been investigated in this research work, in order to improve tissue integration of titanium based implants. The surface treatment is able to induce a bioactive behaviour, without the introduction of a coating, and preserving mechanical properties of Ti6Al4V substrates (fatigue resistance). The application of the proposed technique results in a complex surface topography, characterized by the combination of a micro-roughness and a nanotexture, which can be coupled with the conventional macro-roughness induced by blasting. Modified metallic surfaces are rich in hydroxyls groups: this feature is extremely important for inorganic bioactivity (in vitro and in vivo apatite precipitation) and also for further functionalization procedures (grafting of biomolecules). Modified Ti6Al4V induced hydroxyapatite precipitation after 15 days soaking in simulated body fluid (SBF). The process was optimised in order to not induce cracks or damages on the surface. The surface oxide layer presents high scratch resistance.

Keywords: titanium alloys, surface modification, bioactivity, nanotextured surface, osteointegration

Introduction

The goal of prosthetic surgery is to obtain implants able to reproduce the natural functions of healthy tissues, with adequate mechanical properties, stability, reliability, good bone integration and regeneration of health tissue at the damaged site. Titanium is the most widespread metal for orthopaedic implants intended for bone integration. It presents high fatigue strength (600- 700 MPa) and a comparatively low modulus of elasticity (70-130 GPa), respect to other metals [1, 2]. So it is able to support loads and distribute them to bone, limiting stress shielding. Besides titanium and its alloys are characterized by a thin natural oxide layer on the surface that limits ion release and reactivity, making the surface almost inert and biocompatible. On the other hand this surface inactivity often causes the development of a layer of fibrous tissue, around the implanted device, hampering bone integration [3].

Several surface modifications have been proposed in literature in order to promote osteointegration of titanium implants. The easiest strategy is to modify the surface morphology in order to improve mechanical adhesion between implant and bone. In this field chemical etching, blasting, Ti particles sintering, Ti plasma spray coatings and micro-patterning could be listed [2, 4]. On the other hand the deposition of a bioactive coating (for example of hydroxyapatite or bioactive

glass) could be employed in order to improve the adhesion between implant and bone [2, 5, 6]. Coatings are very effective in terms of bioactivity improvement, but some problems related to their adhesion could thwart their utility [5, 6]. Different treatments have been proposed in literature, in order to induce a bioactive behaviour directly on the metal surface, without the deposition of a coating on it. One of these is the Kokubo treatment that provides samples soaking in NaOH solution and eventually a subsequent thermal treatment [7-10]. On the other hand Osaka, Tsuru et al. proposed a series of possible processes based on hydrogen peroxide, eventually added with TaCl, HCl or $TiOSO_4$, in order to induce bioactive behaviour onto titanium surfaces [11-15]. These processes are not easily applicable on industrial scale, they sometimes induce surface cracking and low fatigue resistance of the treated materials is reported [16]. So actually there is still the need to develop new processes for this purpose.

An innovative patented [17] modification treatment for bioactive titanium surfaces is discussed in this research work. It is a thermo-chemical process and it includes firstly an acid etching in diluted hydrofluoric acid, subsequently a controlled oxidation in hydrogen peroxide and a thermal treatment. Results of the modification are both a micro and nano-patterned surface and a modified surface chemistry. A tight control of the treatment is required in order to get a surface free of cracks and a high adhesion of the modified surface to the substrate. The treatment was tested also on sandblasted surfaces in order to get a micro and nano-texture superimposed onto a macro-roughness. The modified titanium alloy promotes in-vitro hydroxyapatite precipitation and it is suitable for further biological functionalization [18, 19] by grafting biomolecules on it, so it is promising in order to promote healing by stimulating the growth of both inorganic and organic component of bone. The inorganic thermo-chemical treatment is essential in order to obtain an active surface for the direct grafting of biomolecules on the metal, so polymeric coatings and toxic spacer (such as glutaraldehyde) can be avoided.

Materials and methods

Ti6Al4V has been employed as substrate. Specimens with a thickness of 2mm have been cut from cylindrical bars (10mm in diameter) with an automatic cutter (Struers Accutom 5) provided with an alumina blade (356 CA). Each sample has been signed with a number on one side and polished on the opposite one with abrasive SiC papers (up to 4000 grit).

Some samples have been blasted with corundum for one minute at different pressures (4-6-8 atm) in a blaster (BLAST0/f, Blastline) and named SB4, SB6 and SB8 respectively. Samples have been twice ultrasonically washed in double distilled water for 15 minutes after blasting. No organic solvents have been employed for cleaning them, in order to avoid surface contamination.

The thermo-chemical treatment includes a first acid etching in hydrofluoric acid, intended for the removal of the native oxide layer, and a subsequent controlled oxidation in hydrogen peroxide [17], so treated samples will be named HF+H_2O_2. Some samples have been treated only with acid (named HF samples) and others only with hydrogen peroxide (named H_2O_2) for comparison.

Different times of acid etching have been tested for the treatment of 6 atm blasted surfaces (SB6), in order to evaluate the opportunity to preserve sandblasting macro-roughness after the thermochemical treatment. The blasting pressure for this test has been selected as the most commonly used in industrial production of medical devices. Increasing etching time are indicated as HF(1), HF(2) and HF(3) respectively.

Some samples have been thermally treated at 300°C (TT300 samples), 400°C (TT400 samples), 500°C (TT500) and 600°C (TT600 samples) for 1 hour in air and 400°C under vacuum (TT400v), in order to stabilize the oxide layer generated on the surface. Samples have been posed in an alumina holder and closed with an aluminium foil in order to prevent contaminations. Samples have been let cool gradually to room temperature in the furnace at the end of treatments.

Samples morphology has been investigated by means of Scanning Electron Microscopy (SEM – FEI, QUANTA INSPECT 200, EDS - EDAX PV 9900) and Field Emission Scanning Electron Microscopy analysis (FESEM - SUPRATM 40, Zeiss)

Crystallographic structure has been analysed by means of X-ray diffraction measurements (XRD - Philips X'Pert) in parallel beam configuration (with an incident angle fixed to 1°) in order to analyze only the outermost layer of the sample.

Hydroxyls exposition has been studied by means of a FTIR microscope (IR Hyperion 2000, Tensor 27 - Bruker S.p.A) and X-ray Photoelectron Spectroscopy - XPS (Al source, Surface Science Instruments, M-Probe) analysis. In this work vibrations of hydroxyls groups at 3000-3600 cm^{-1} and of adsorbed water at 1600 cm^{-1} have been investigated by FTIR in reflection configuration [20, 21] . Oxygen region (524-540 eV) and titanium region (457-459 eV) have been analysed by XPS in order to evaluate hydroxyls exposition at different steps of the treatment. The XPS energy scale was calibrated with reference to the 4f7/2 level of a freshly evaporated gold sample, at 84,00 +/- 0,1 eV, and with reference to the 2p3/2 and 3s levels of copper at 932,47+/- 0,1 and 122,39 +/- 0,15 eV, respectively.

The thickness of the modified layer has been measured by means of XPS analysis equipped with Ar gun. Surface elements have been monitored in function of the etching time in order to detect the transition point between metallic substrate and surface oxide layer. Some samples have also been sent to Filmetrics (San Diego –CA- USA) for measurement of the oxide layer thickness by means of spectral reflectance technique (Filmetrics F20 instrument).

Scratch tests have been performed in Revetest mode (CSM Revetest machine) in order to study the mechanical adhesion of the modified oxide. A load has been applied to the sample surface through a Rockwell C diamond stylus with 200 μm diameter, increasing progressively from 1N to 50N along a 4.91 mm track. Scratch test parameters have been set according to the standard [22]. Scratch track has been optically observed and critical load determined as the ones at which coating damages appears.

Fatigue tests were carried out by rotating bending tests (2600 rpm – room temperature) and by applying a simplified stair-case method (6 samples).

Surface wettability has been determined by means of static contact angle measurements with water before and after the thermo-chemical process.

Samples have been analysed by means of a contact profiler (KLA-Tencor P15) at different steps of the process (polished, blasted, acid etched, chemically oxidised, thermally treated samples), in order to determine the surface roughness. Scan length has been set to 500 μm and 2 μm and cantilever speed at 50 and 2 μm/s respectively. Three measurements have been carried out on each sample in three different points. Average roughness (R_a) has been evaluated.

Atomic Force Microscopy observations (AFM – XE-100, Park Systems) have been carried out in order to better characterize surface topography and roughness at the nano-scale.

Inorganic bioactivity has been investigated in-vitro by soaking samples in simulated body fluid (SBF) for 2 weeks at 37°C. SBF has been prepared according to Kokubo protocol [23]. Solution refresh has been performed every two days, in order to mimic physiological turnover of body fluids. The pH has been measured after each refresh. Samples have been gently washed with double distilled water at the end of the soaking time and let to dry at room temperature. Finally they have been observed at SEM to look for hydroxyapatite precipitation onto their surface.

Ion release from Ti6Al4V surfaces before and after $HF+H_2O_2$ treatment has been evaluated in simulated body fluid up to 1 week, in order to investigate whether the proposed treatment can reduce metallic ion release from Ti6Al4V surfaces. In fact ion release from metallic surface reaches its maximum value after few days in simulated physiological fluids [16]. Three just polished samples and three modified samples with the optimized treatment ($HF+H_2O_2$) and thermally treated at 300°C for 1h have been soaked in each in 30 ml of SBF at 37°C up to 1 week. 1 ml of the solution has been spiked up at several time steps (3h, 1, 3, 7days) and analysed by means of graphite furnace atomic absorption spectroscopy (GFAAS). Any solution refresh have been performed during the soaking period, a cumulative release has been finally obtained. All values have been referred to sample surface area, only the modified surface has been considered.

Results and discussion

Surface morphology

The interaction between implant materials and the physiological environment play mainly at the interface. Surface topography and chemistry are the most important characteristics that affect cell behaviour on artificial materials and that, at the end, pilot the entire tissue integration process [24]. A lot of literature works underline that both shape and dimensions of topographical features can affect cell adhesion, proliferation and differentiation onto material surface [24, 25]. So the peculiar surface topography (multiscale roughness at the micro- and nano- levels) and chemistry (hydroxylation), of the treated and modified materials analysed in this work, have been investigated in details by means of several techniques (SEM, FESEM, contact profilometry, AFM, XPS and FTIR).

Polished and treated surfaces

Figure 1 shows the surface morphology of samples treated by acid etching (Figure 1a), by oxidation in hydrogen peroxide (Figure 1b) or by the complete chemical treatment (etching in HF as first and then oxidation in H_2O_2) (Figure 1c). The etching in hydrofluoric acid readily removes the natural oxide from the all the surface and dissolves titanium according to the reaction (1):

$$Ti + 3HF \rightarrow Ti^{3+} + 3/2H_2 + 3F^- \qquad (1)$$

Ti^{3+} can be further oxidized by atmospheric oxygen or it can be complexed by F^- [26]. This treatment induces a micro rough surface, due to the preferential dissolution of the alpha phase of the biphasic alloy by HF (figure 1.a).

On the contrary, hydrogen peroxide causes pitting corrosion on the untreated alloy, where it acts on the native passivation layer that is not removed by H_2O_2. The TiO_2-H_2O_2 interaction was widely studied in literature [27, 28] and it can be described as follows. Hydrogen peroxide is decomposed to oxygen and water; Ti oxidation and eventually corrosion take place according to the pH and concentration of the solutions. The formation of Ti(IV)-H_2O_2 complex occurs in case of surface corrosion with the appearance of a typical yellow colour of the solution. It should be underlined that, considering the Ti-Al-V alloy, the alpha phase (rich in Al) is covered by a more efficient passivation film (since both Ti and Al occur in its formation) while the beta phase (rich in V) is passivated by a simple TiO_2 film. A localized corrosion at the grain boundary between the alpha phase and beta one can be noticed (figure 1.b), causing surface beta crystal detachment and pitting effect.

A peculiar surface can be obtained by the complete chemical treatment, etching in hydrofluoric acid and then controlled oxidation in hydrogen peroxide. The natural oxide is firstly removed by acid etching and the nude titanium alloy is then exposed to the action of hydrogen peroxide, which causes a re-oxidation of the metal surface at the nanoscale. The reaction between the metal and the hydrogen peroxide is different respect to what previously described for the titania layer. The treated surface presents a micro rough aspect and a nanoporous pattern (figure 1 c.). Figure 2 shows a high magnification image of the spongy structure on a nanoscale of the oxide layer produced by the complete treatment. Corrosion of the titanium alloy does not occur in all the tested conditions as it can be deduced from the colourless appearance of the hydrogen peroxide solution at the end of the treatment. The last step of the treatment is a thermal consolidation of the surface oxide layer (300°C-1h in air). It does not cause any variation of the surface morphology. SEM observations (not reported) verified the maintenance of nanostructured spongy surface also after thermal treatments.

Surface roughness of the treated surfaces has been evaluated using a contact profiler. Results are reported in figure 3a. The acid etching induces a micro roughness (about 0.2 micron) as observed by SEM observations. Chemical oxidation in hydrogen peroxide and the following thermal treatment do not alter the surface micro roughness. On the other hand the roughness on a nanoscale, induced by the chemical oxidation in hydrogen peroxide, can not be detected by this technique

So AFM analysis was employed in order to measure characteristic parameters for the nanotextured surfaces. In particular it has been observed that the nano-scale roughness R_a can be estimated as about 10 nm and that the surface phase is homogeneous.

Several studies dealing with cells interactions with random or ordered nano-patterned surfaces (produced by different methods, such as photolithography or chemical etchings) are reported in literature [29] and they are confirming that cells are sensitive to nano-textures and that osteoblast cells show a lot of filopodia protrusion into the direction of motility over a nanopattern. Moreover, nano-pores are reported as a determining factor for the chemical equilibrium needed for biomineralization and apatite precipitation [24, 30, 31] and it was reported that micro and nano-patterns improve mechanical adhesion of precipitated hydroxyapatite on metallic surface [32]. So the presence of this morphology is quite relevant for the purpose of this study.

It must be underlined that a crack free surface is obtained at the end of the process by a severe control of the chemical oxidation of the metallic surface. This is quite relevant in order to maintain the good fatigue resistance of the titanium alloy and to increase the adhesion of the

oxidised surface layer to the substrate. So this treatment seems to be very promising compared to other bioactive surfaces described in literature [13, 16].

XPS measurements, with surface Ar-etching, have been performed in order to determine the thickness of the oxide layer. Samples treated with the complete chemical treatment (called $HF+H_2O_2$) and also samples chemically and then thermally treated (300°C for 1 h in air) (called $HF+H_2O_2$+TT300) have been tested. It has been observed that increasing the Ar-etching time the surface oxygen content decreases, while the aluminium and titanium ones increase.

Figure 4 reports surface elemental composition detected by XPS in function of Ar etching time for a chemically and thermally treated sample. The situation is almost the same for both types of samples analyzed. The intersection between the O1s curve and Al2p and Ti2p ones has been considered as index of the transition between the surface oxidized layer and the metallic substrate. The cited point has been observed at about after 50 min etching for the $HF+H_2O_2$ sample and after at about 55 min for the TT300 one.

Considering that the etching rate of titanium oxide is at about 6.375 nm/min (from instrument tabulates) the oxide thickness can be estimated as about 320 nm for the $HF+H_2O_2$ sample and 350 nm for the $HF+H_2O_2$+TT300 one. These results are not so far from what obtained by Filmetrics trough spectral reflectance technique (225-230 nm for the $HF+H_2O_2$ sample). It must be noted that the chemical treatment induces an oxide layer with a thickness comparable to that induced by anodizing. The thermal treatment does not induce a significant increase in the oxide thickness, but it improves the mechanical resistance of the modified layer, as shown in the following.

Blasted and treated surfaces

As expected blasting causes a macro-roughness of 1.2-1.5 microns which is directly proportional to the applied pressure (figure 3a). Moreover blasted surfaces have been submitted to the complete chemical treatment. It was noted that the typical micro texture of the treated samples is reproduced on the blasted surfaces and at higher magnifications (data not reported) it is possible to observe the characteristic nanotexture, presenting the same morphology obtained on polished samples. The roughness of sandblasted samples submitted to acid etching and controlled oxidation is reported in Figure 3. It can be observed that the macro roughness due to blasting is removed by the acid etching (HF3).

EDS analysis underlines that, as expected, blasting causes a significant oxidation (the O/Ti ratio is 0.24 before blasting and it is 1.55 after it) and increase in surface Aluminium content (from 9.03 to 14.47 at%) is observed, due to residual alumina particles. The acid etching retrieves it to the expected value for Ti6Al4V alloy and it removes the oxidation layer induced by blasting.

So, it can be concluded that the complete chemical treatment removes alumina contamination from blasted surfaces and it induces surface micro and nano-structures, but reducing macro-roughness. Moreover it can be suggested that the treatment removes the atomic disordered layer induced by blasting, in fact it is thinner than the roughness induced dimension [33]

Figure 3b reports surface roughness of 6 atm blasted samples treated with different acid etching times, increasing from HF(1) to HF(3), and then oxidized in hydrogen peroxide. It can be noted that the reduction in roughness is proportional to the acid etching time and that it is possible to properly preserve macroroughness by reducing the duration of acid etching. The micro and nanotextures are obtained on all these samples.

SEM observations (figure 5) confirm these results.

Hydroxyls exposition

Figure 6 shows FTIR spectra of samples after different steps of the treatment. It is interesting to note that untreated samples (curve a), samples treated only with HF (curve b) or only with hydrogen peroxide (curve c) do not present any signal in hydroxyls characteristic region, while surfaces after the complete chemical treatment (acid etching and the chemical oxidation) show both a broad band between 3000 cm-1 and 3600cm-1 (d track) characteristic of “H-bonded” OH stretching and also the band at 1600 cm-1 related to the bending of adsorbed water molecules [34-38]. Both regions have been underlined on figure 6 through circles. Water signal decreases or disappears after the thermal treatment (tracks e and f), as expected, while hydroxyls band is only slightly reduced. This can be explained considering that chemisorbed water is almost completely removed up to 130°C about while elimination of hydroxyl groups bonded to surface cations occurs at higher temperature (also more than 600 °C, depending on the Lewis acidity strength of the surface cations) [34-38]. There are no differences between samples treated at 300°C or 400°C.

The nature of the exposed hydroxyl groups was also investigated by means of XPS analysis. The observed titanium signal at 458.5 eV corresponds to TiO_2, while signals at about 457-455-453 eV, corresponding to Ti_2O_3, TiO or Ti were never detected.

Figure 7 includes XPS spectra (O 1s region) of a polished sample (a) and a sample after complete chemical treatment (b). All the samples show a broad region that can be fitted with two/three peaks. All the samples have a first, intense peak at low bonding energy (530.0 eV) and a second peak at about 531.5 eV, less intense than the first except for the sample with the complete chemical treatment (polished + HF + H_2O_2, Fig. 7b). Simply polished sample (Fig. 7a) also shows a small peak at high binding energy (BE).

It is difficult to precisely attribute XPS peaks to oxygen species except for the one at about 530 eV, attributable to bulk oxygen in titanium oxide. A debate is present in literature on the attribution of the other two peaks at about 531.5 eV and at BE higher than 532 eV. In fact the first peak at about 531.5 eV can be attributed to generic surface OH groups [39] and ref [40] therein or to acidic hydroxyl groups together with adsorbed water [41]. Last peak at BE higher than 532 eV is attributed to adsorbed water [40] or to basic hydroxyl groups [41], respectively.

Notwithstanding this uncertainty, it is possible to observe unequivocally that the sample treated with both HF and H_2O_2 shows a very intense peak related to hydroxyl groups at 531.5 eV. (figure 7b) which is less intense on the untreated one (figure 7a), as also shown by FTIR. A significant reduction in OH signal is also observed after thermal treatments, both at 300°C (figure 7c) and 400°C (data not shown). In this case, the reduction underlined by XPS analysis is higher than the one detected by FTIR measurements, this phenomenon could be explained considering that the two techniques explore different depth of the surface. XPS analyses only the very first surface layer (2-10 nm) [42] while FTIR goes more in depth (some microns) [42].

The reduction of OH signals on thermally treated samples has been widely investigated in literature. it is well known that OH disappearing at lower temperature are only the weakly adsorbed ones, while the OH strongly bound with Ti can remain on the surface up to 600°C or more [34-38]. Moreover it has been observed that only strongly bound hydroxyls groups are effectively responsible of titanium bioactivity [40, 43].

Observing FTIR and XPS data, it can be concluded that the thermo-chemical treatment modifies also the chemistry of the surface. The presence of a high density of OH groups on the surface is interesting also for its further functionalization with biomolecules [18] in order to combine inorganic and biological bioactivity. The hydroxylated surface is reactive and biomolecules can be directly linked to it, without the use of a polymeric coating or toxic spacers.

Crystallographic structure

XRD measurements have been carried out in order to determine the crystallographic structure of the formed oxide layer. In fact it has been suggested in literature that the nature of titanium oxide can affect both bioactivity and cellular interaction [39].

Figure 8 reports XRD patterns of samples treated with the complete chemical treatment and then thermally treated at different temperatures (from 300°C to 600°C) in air and at 400°C under vacuum.

All patterns present reflexes typical of metallic titanium (at 35.4°, 38.4° and 40.5°) due to the sample substrate. These signals are present because X-rays penetrate in the sample for a depth higher than the oxide layer, despite of the small angle technique employed.

Two Ti reflexes have almost the same height on the sample treated by the complete chemical treatment; this is an index of preferential orientation of crystalline planes, probably due to the polishing process.

The height of the 40.5° reflex growths up to about four times the 35.4° one, after thermal treatments, this condition is the typical one for randomly oriented crystals. Probably thermal treatments act as an annealing able to report the natural casual orientation of crystalline planes.

Reflexes at 25° and 48° are characteristic of crystalline titanium oxide, and their height is a measure of the oxide thickness. A broad and very weak diffraction peak related to TiO_2 can be observed for the sample treated at 300°C, indicating the formation of a highly disordered TiO_2 layer. It can be noted that increasing the temperature of the air thermal treatment from 300°C to 600°C diffraction peaks of anatase (TiO_2) gradually increase, as sign of increase crystallinity and thickness of the oxide layer. On the other hand the spectra of the sample treated at 400°C under vacuum is almost analogous to the one of the untreated sample, in fact the inert environment limit the oxidative process that take place for thermal treatments performed in air.

XRD patterns show also that none of the considered chemical and thermal treatments induce hydrides formation.

Mechanical properties of the modified samples

As described before, titanium and its alloys are widely employed thanks to their favourable mechanical properties, so surface modifications should not alter this peculiarity. Fatigue and scratch resistance of the treated samples have been analysed for this purpose.

Since coating thickness and removal mechanisms were almost the same for all the samples, critical loads observed as a consequence of scratch tests can be used as an indication of adhesion and scratch resistance. The results suggested that the thermal treatment increases mechanical adhesion of the modified layer to the substrate. In fact the first failing load is at about 3N for the sample chemically treated (HF and H_2O_2), while it is in the range 12-14 N for the chemically and thermally treated ones. The increasing in adhesion can be related to the structure changes in the substrate, as indicated from the XRD spectra. Furthermore, since the test also provides the entity of the scratch resistance, the higher initial critical load observed for the sample thermally treated could be also an indication of the formation of a harder layer, probably related to an order increase of the TiO_2 layer as a consequence of the thermal treatment. In fact, in the XRD patterns the level of disorder in the

film only chemically treated is so high that it is really hard to observe the related peak, while it can be slightly distinguished in the case of the material thermal treated at 300°C. There are not significant differences in adhesion between samples treated at 300°C or 400°C. The thermal treatment at 300°C was selected for the following tests, considering that it is able to assure a good adhesion and that treatments at lower temperature have less effect on the fatigue resistance.

Figure 9 reports the scratch track for a chemically and thermally treated (300°C) sample with detailed images of the surface at the critical loads. It can be observed that at about 11 N some white point appears on the track as first signs of coating delamination. Delamination of the modified layer increases with the applied load and reach an almost complete removal only at about 44 N.

The fatigue strength ($\sigma_{D50\%}$= 675 MPa) of the thermo-chemically (300°C) treated alloy is slightly lower than the untreated one (683 MPa). So it can be concluded that the treatment does not damage the surface and good mechanical performance of the titanium alloy is retained.

Wettability

Wettability tests have been performed in order to better understand the interaction between modified surfaces and physiological fluids. In fact it can affect both protein absorption and cellular adhesion.

Static water contact angle on polished samples results 81.4° ± 0.77° while after the complete chemical treatment (HF and then H_2O_2) it is 76.00° ± 5.59°. So, it seems that the modification process slightly increases surface wettability, according to the higher surface area exposed, but in any case the surface is very weakly hydrophilic.

The role of the wettability of an implant surface on its osteointegration ability is not so clear. Surface hydrophilicity influences the adsorption of cell adhesion proteins containing Arg–Gly–Asp (RGD) sequences, such as fibronectin and thus enhances the adhesion and spreading of osteoblast precursors on implant surfaces [44]. Furthermore, hydrophilic surfaces stimulate the biomineralization process: rapid calcium phosphate nucleation was achieved on highly wettable Ti surfaces [44]. On the other side the hydrophobic character of osteoblastic cells is often reported [45], so it can be supposed that hydrophilicity of the implant surface must not be increased excessively.

The weakly hydrophilic behaviour of the treated surface, described in this paper, is in agreement both with its biomineralization ability and good osteoblastic differentiation [19], but a further investigation of this topic is required.

In vitro bioactivity

Finally inorganic bioactivity of Ti6Al4V alloy, in simulated body fluid, has been investigated in order to verify the effectiveness of the proposed treatment.

Samples have been observed at SEM after 15 day soaking in SBF. Figure 10 shows micrographies and EDS spectra of sample area and of precipitates on different samples: HF+H_2O_2 (figure 10a), HF+H_2O_2+TT300 (figure 10b) and HF+H_2O_2+TT400 (figure 10c), after 15 days in SBF. Particles with dimension of about 5μm and the typical morphology of hydroxyapatite precipitate can be noted on all the considered samples (figure 10 a2, 10 b2, 10 c2). EDS analysis on particles (figure 10 a3, 10 b3, 10 c3) confirm that they are rich in calcium and phosphorous in a ratio close to the one of hydroxiapatite (at about 1.7). Sodium and chlorine traces derive from SBF soaking that contains NaCl. XRD analysis does not detect these precipitates because of their reduced dimension and amount. EDS analysis on the whole surface (figure 10 a1, 10 b1, 10 c1) underlines that the bioactivity of all the treated surfaces is comparable. An enrichment in Ca and P was also detected on the surface free from precipiated, showing a diffuse interaction of the treated surfaces with physiological fluids.

The pH measurements of the SBF soaking solution show that values remain in the range 7.1 – 7.6 during the entire soaking time for all tested samples. These variations are included in physiological tolerability range (7.00 – 7.80).

So it can be concluded that the proposed process is able to induce inorganic bioactivity on titanium alloy and it is very promising in order to induce in-vivo biomineralization on the implant.

The thermal treatment does not alter this property despite of the apparent reduction in OH amount on the surface.

Figure 7d reports XPS detailed analysis of the oxygen region for a sample HF+H_2O_2+TT400 after 15 days in SBF. It could be noted that the oxygen region is modified and an OH signal attributable to hydroxyapatite appears [46]

The inorganic bioactivity must be coupled with a good cell response of a biomaterial in order to be really profitable. A synergistic effect between inorganic and biological bioactivity of the titanium surface after the described thermo-chemical treatment was previously reported by the authors [19, 47]. The thermo-chemically treated surface shows a better cell response, with a higher early and late differentiation of osteoblastic cells. Furthermore, a higher and specific biological response of the cells to the surface can be stimulated by grafting biomolecules on it, as reported in the part II of this work [18].

Ion release evaluation

The amount of Al and V ions released from treated ($HF+H_2O_2$+TT300) Ti6Al4V samples after 7 days soaking in simulated body fluid is reported in figure 11. A reduction in their release can be observed for surface modified samples. So it can be assumed that the proposed inorganic treatment is able to reduce metallic ion release, due to the higher thickness of the formed oxide layer, compared to the native one, improving biocompatibility.

Conclusions

The application of a new patented thermo-chemical treatment, based on hydrofluoric acid etching, hydrogen peroxide controlled oxidation and a subsequent thermal treatment allows the preparation of a bioactive Ti6Al4V alloy. The modified surface induces in-vitro apatite precipitation, so it is promising for the promotion of in-vivo biomineralization processes. It shows a particular surface chemistry (high amount of hydroxyls groups exposed), so it is suitable for functionalization with biomolecules, in order to induce a specific cell response. It has a characteristic surface morphology (a micrometric roughness coupled with a nano-metric texture). It can be applied also to blasted surfaces, resulting in a multiscale topography (macro, micro and nano-levels) So these materials combine inorganic bioactivity with a multiscale texture on the same surface. Finally titanium modified with the described process possesses is crack free, it has good scratch resistance and the starting fatigue resistance of the metal is maintained. This feature is often compromised in others treatments reported in literature.

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Figure legends

Fig 1: SEM micrographies of samples a) polished + HF, b) polished + H_2O_2, c) polished + HF + H_2O_2

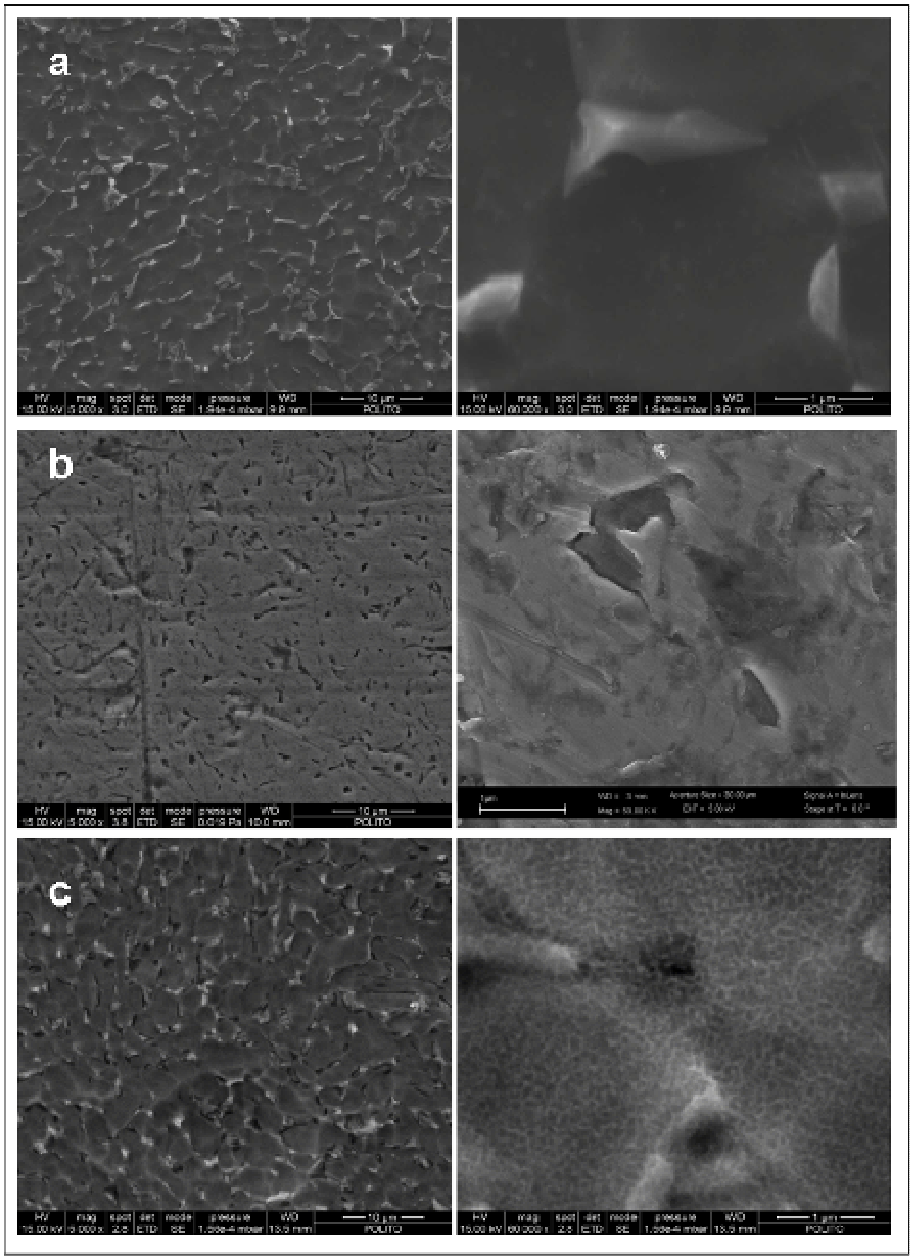

Fig 2: High magnification image of a polished + $HF+H_2O_2$ treated sample (FESEM)

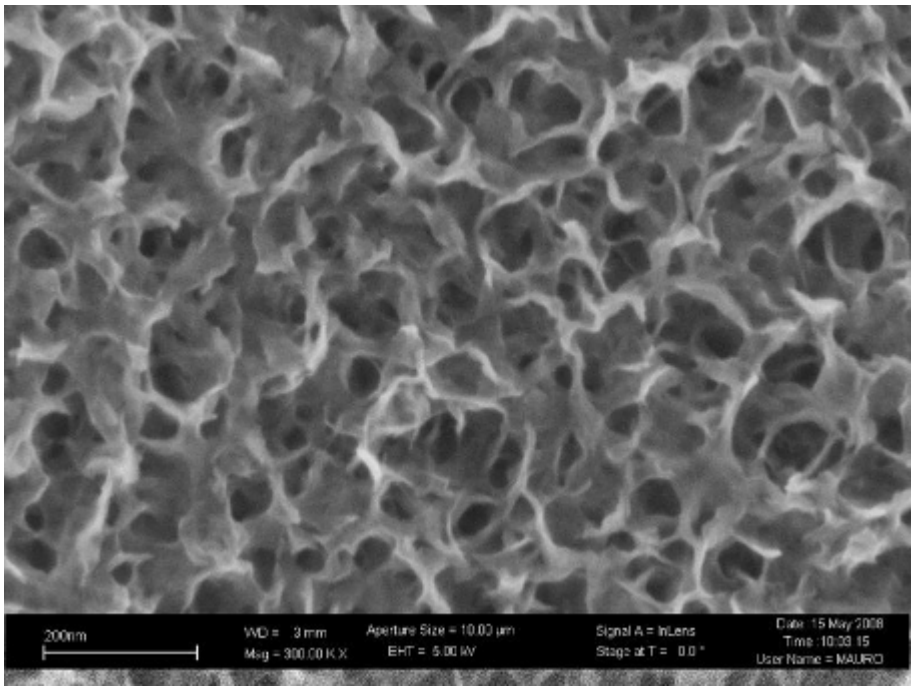

Fig 3: Surface roughness of samples with different surface treatments

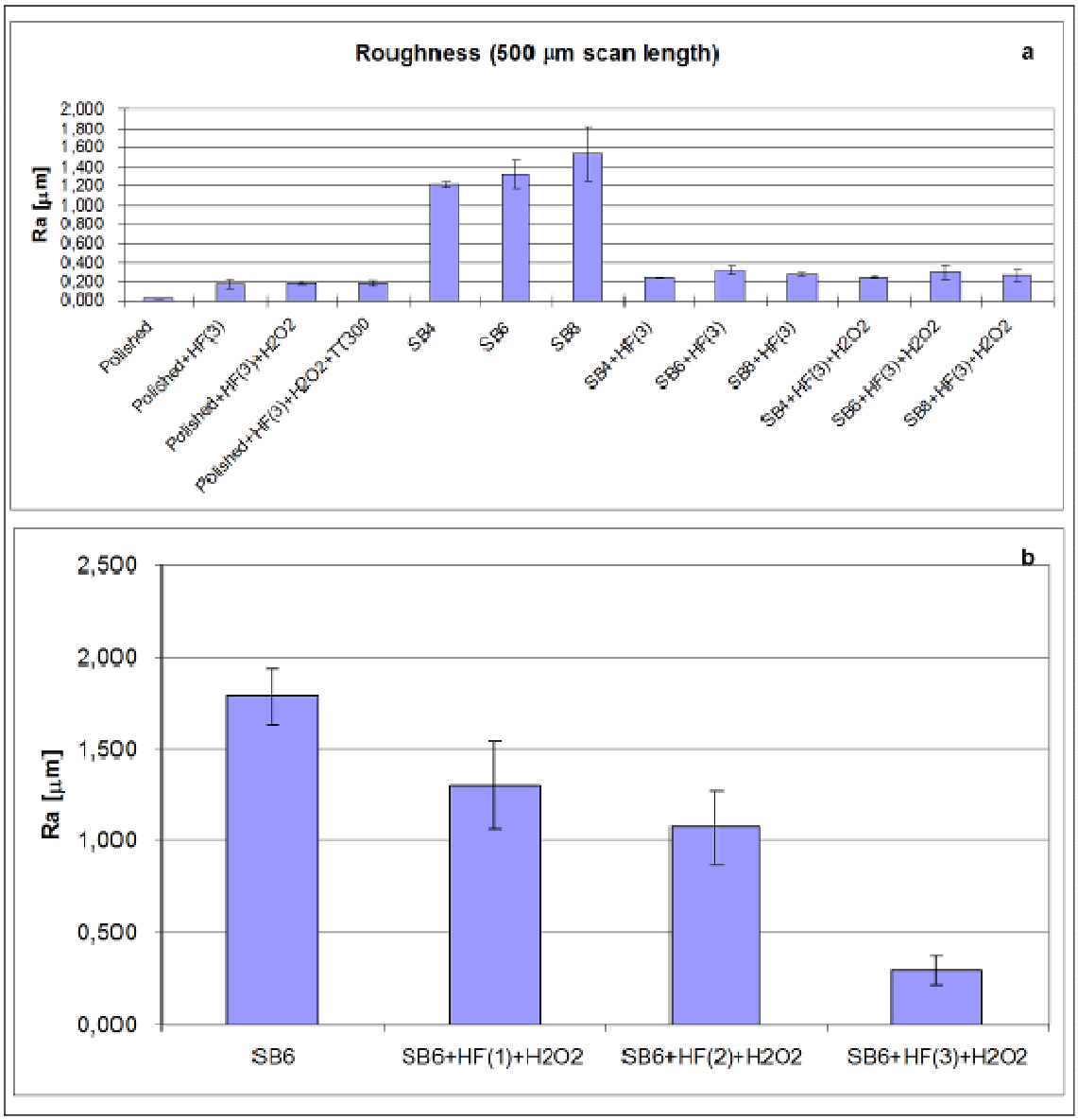

Fig 4: XPS atomic composition of HF+H_2O_2+TT300 sample surface in function of the Ar-etching time

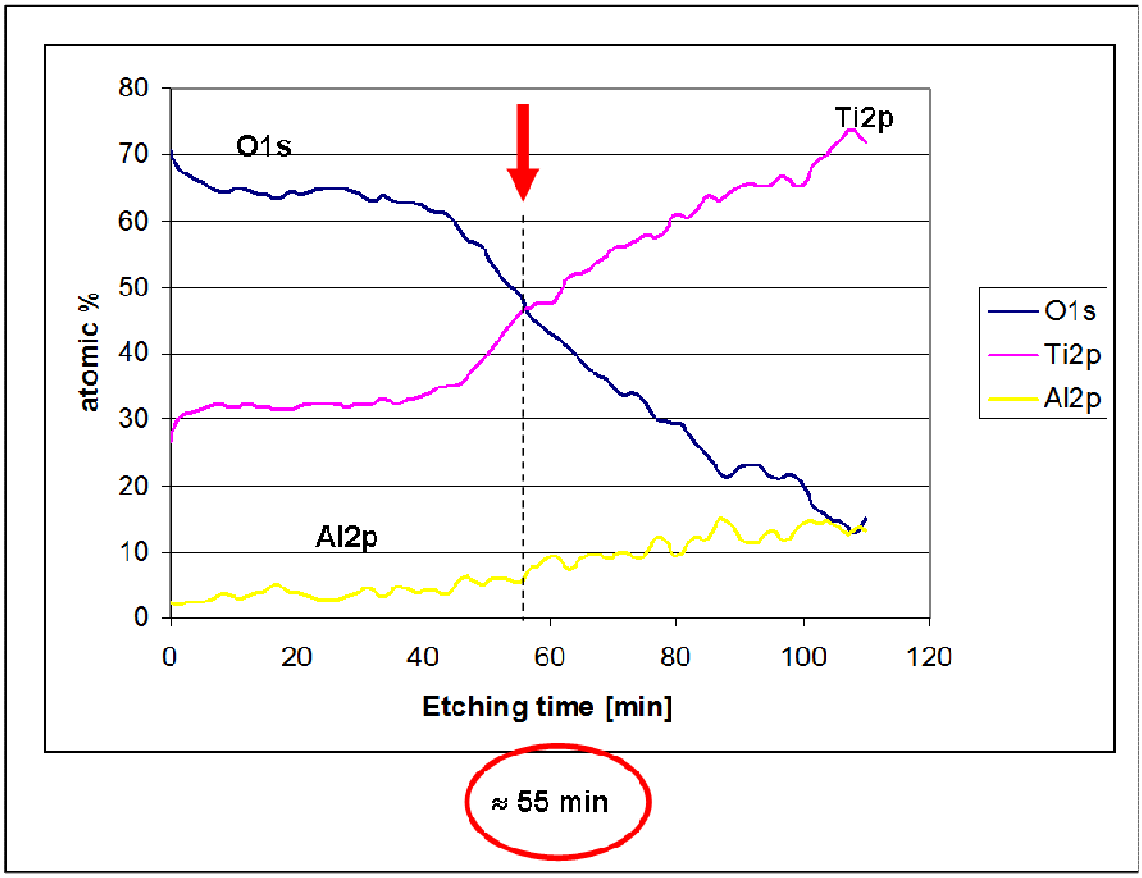

Fig 5: Morphology of Ti6Al4V samples after 6 atm blasting (control a) and inorganic treatment with increasing acid etching time from picture b to d. Scale bar: 50 μm.

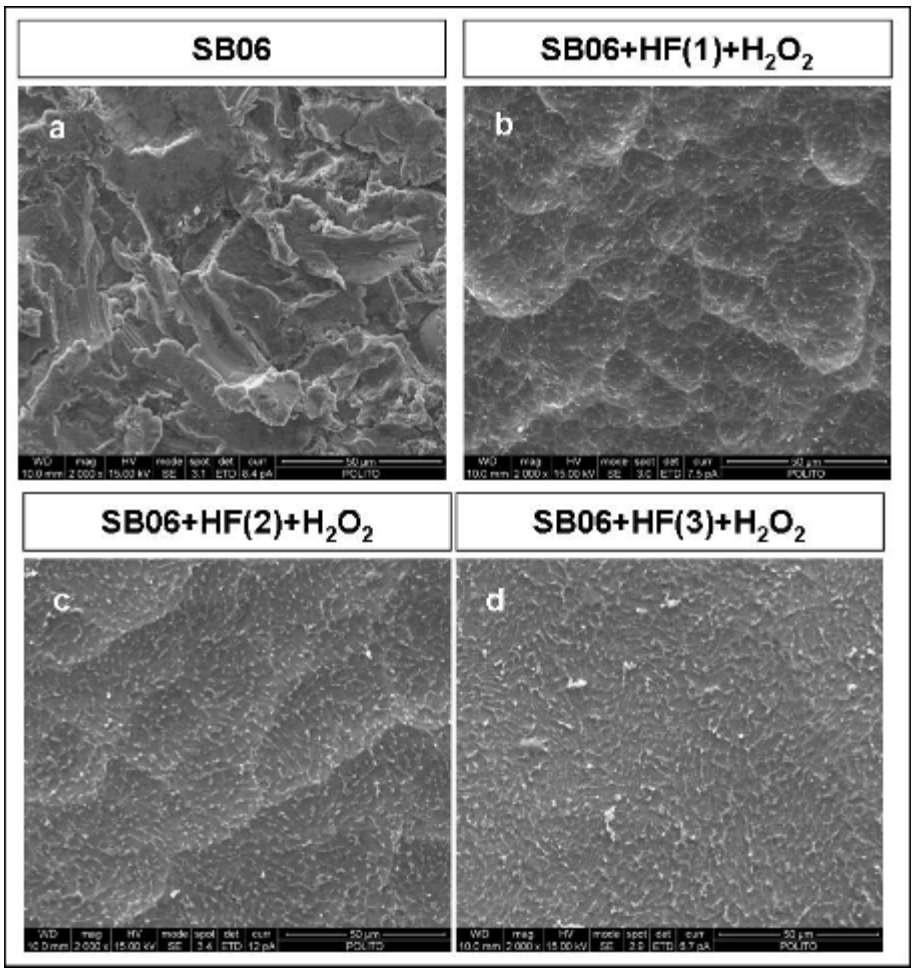

Fig 6: FTIR spectra of samples with different surface treatments

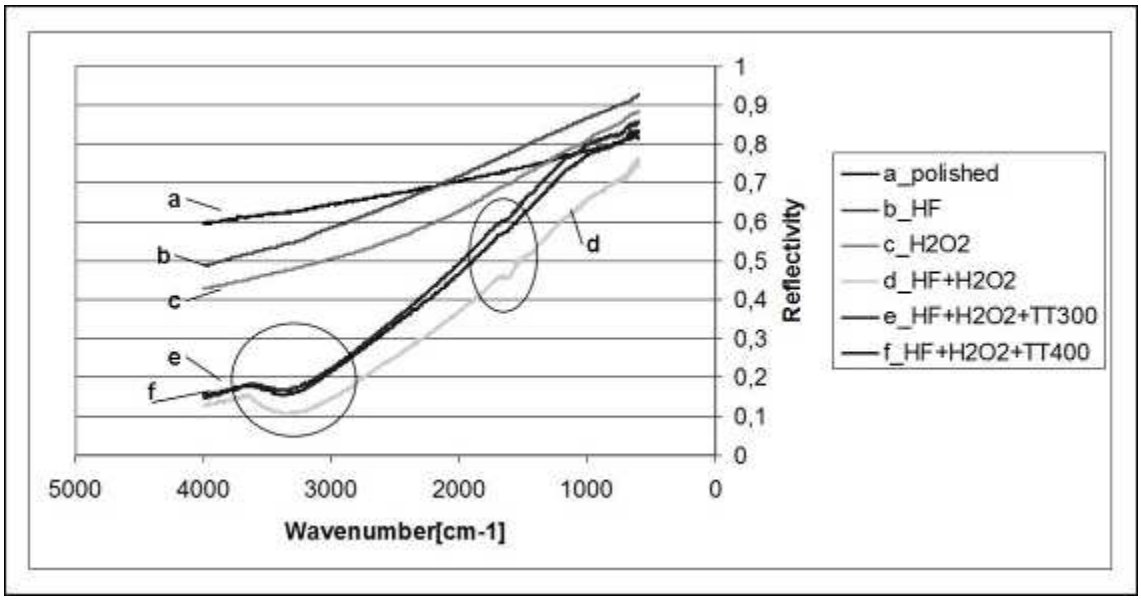

Fig 7: XPS spectra of a) polished, b) polished+HF+H_2O_2 sample; c) polished+HF+H_2O_2+TT300 sample and d) polished+HF+H_2O_2+TT400 sample after 15 days in SBF

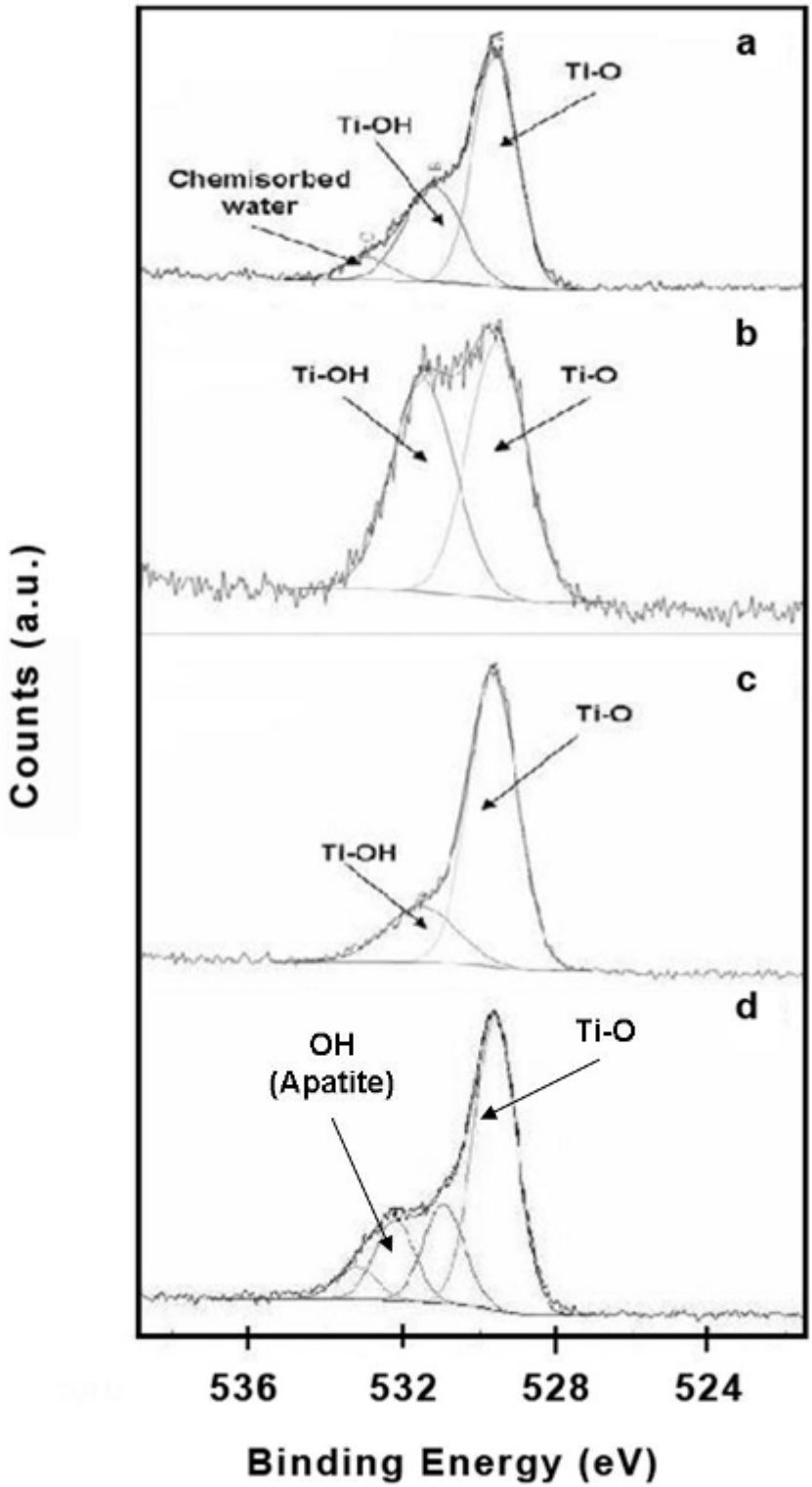

Fig 8: XRD spectra of Ti6Al4V samples treated with HF and H_2O_2 and with different thermal treatments

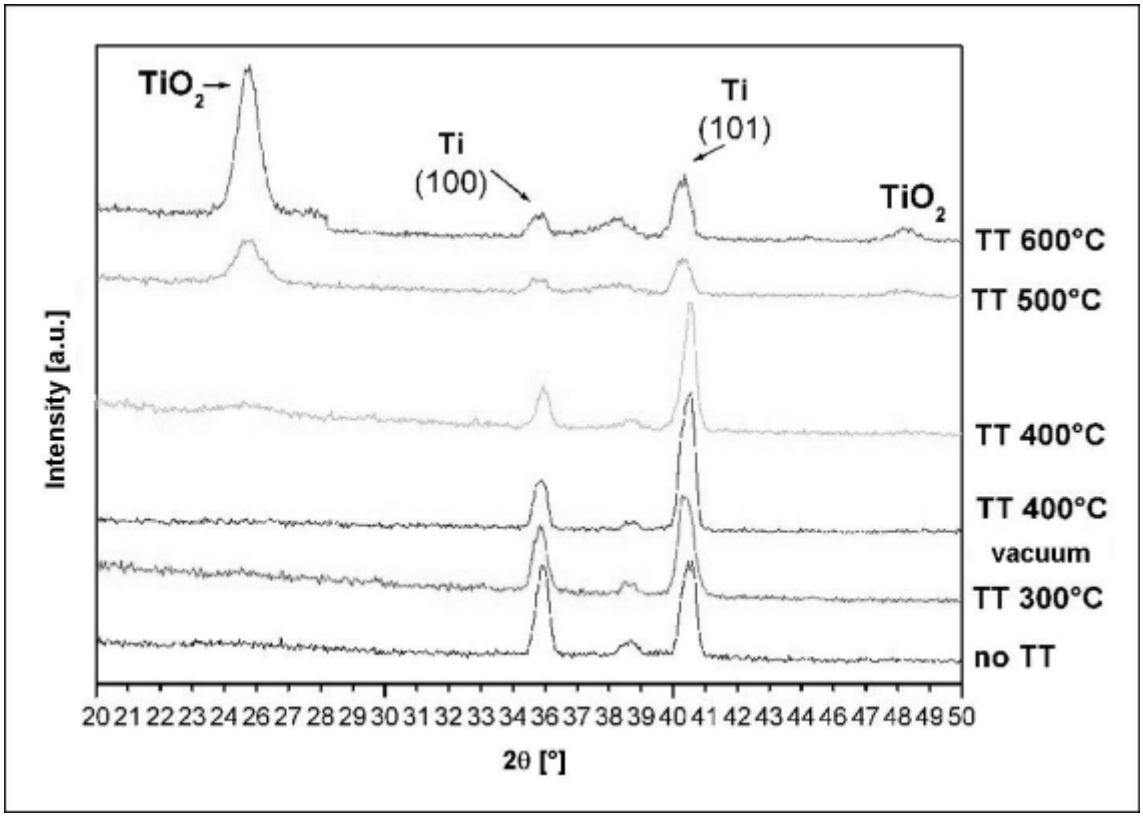

Fig 9: Scratch track and magnification of critical loads point for Ti6Al4V+ HF+H_2O_2+TT300 sample.

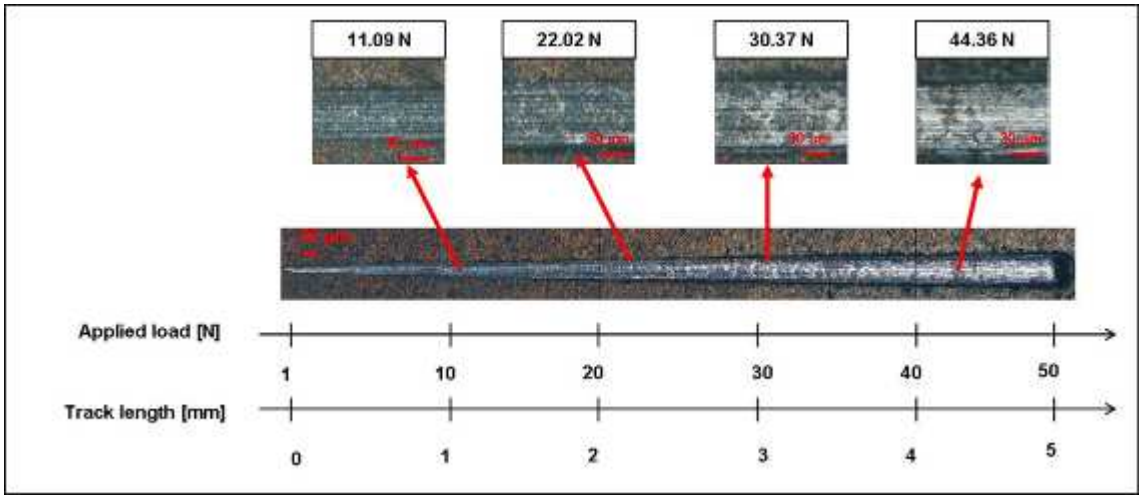

Fig 10: SEM images and EDS spectra of a) polished + HF + H_2O_2, b) polished + HF + H_2O_2 + TT300, c) polished + HF + H_2O_2 + TT400 samples after 15 days in SBF

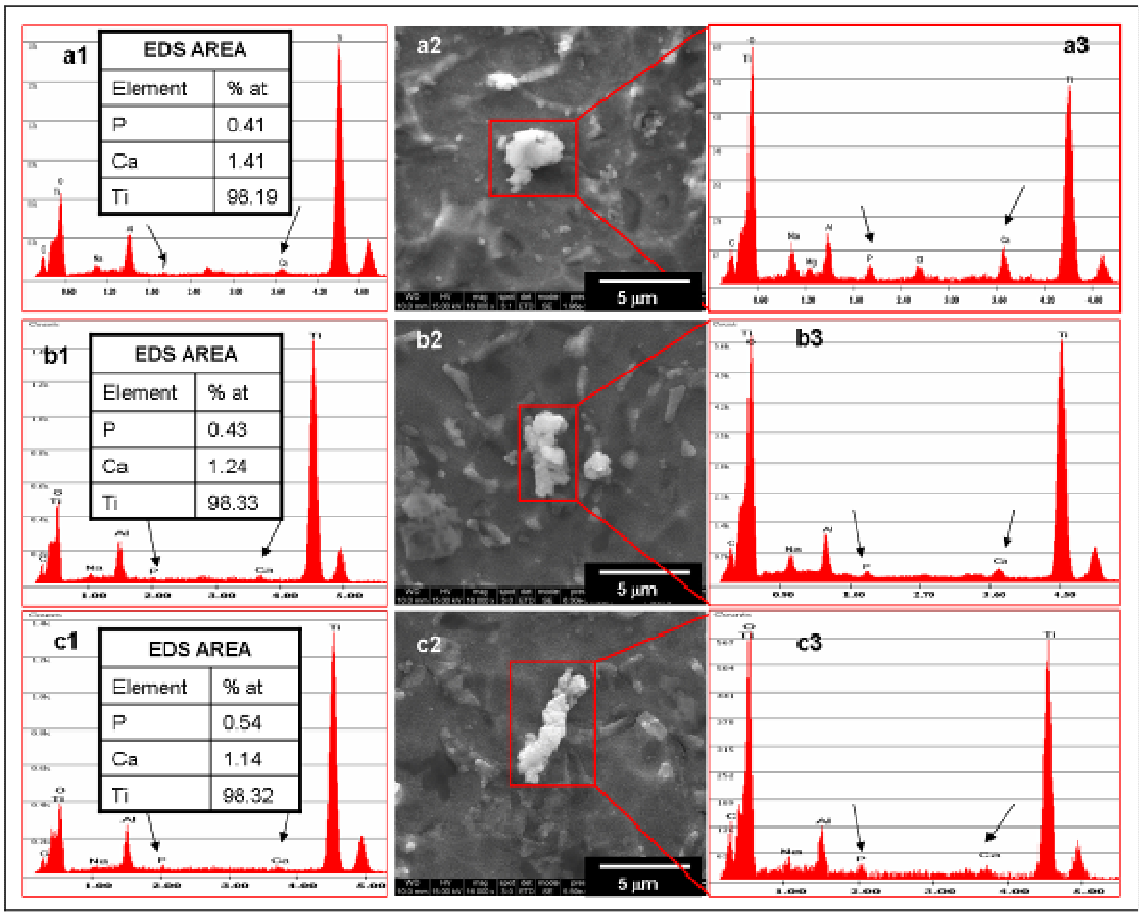
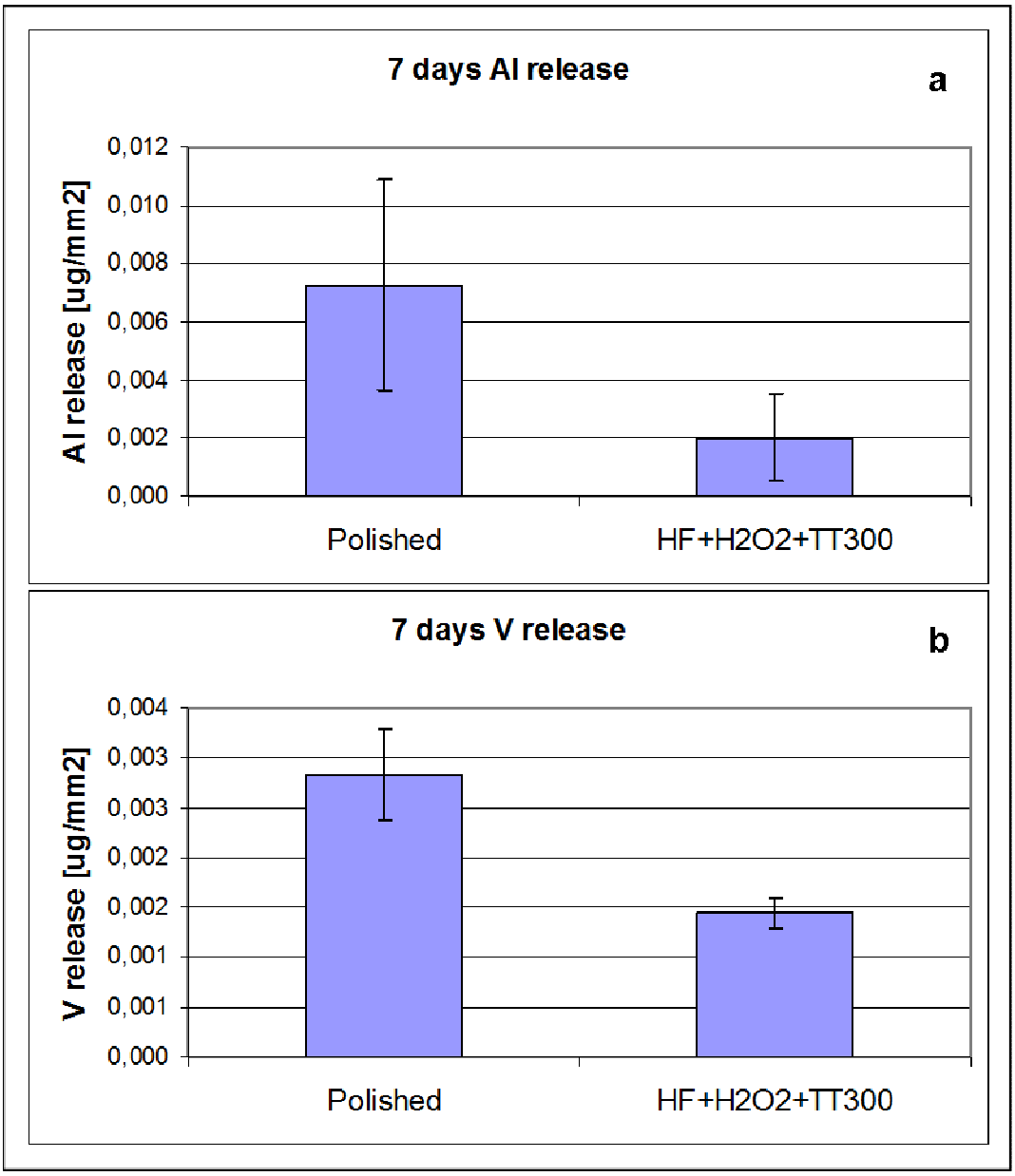
Fig 11: Ion release after 1 week in SBF from Ti6Al4V samples before and after HF+H_2O_2+TT300 treatment . a) Al, b) V.