

Wettability of nanotubular titania layers for biomedical applications developed by electrochemical anodization

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Abstract. The titania nanotubes (TNT) were synthesis on Ti6Al4V alloy planar surfaces with different initial preparation: polished, turned, acid etched, and SLA (sand blasted with large grits and acid etched), by electrochemical anodization performed in phosphate/fluoride based electrolyte, using anodization potentials of 20 V or 24 V. The morphology of developed layers was investigated by scanning electron microscopy (SEM) and consists in highly ordered, self-arranged TiO₂ nanotubes of 25-110 nm in diameter. The initial and modified surfaces' wettability was evaluated by static contact angle method and drop shape analysis. The contact angle measured on modified surfaces with TNT developed on polished surfaces is 70° showing moderate hydrophilic properties, while the contact angle measured on TNT layers developed on micro rough surfaces (turned, acid etched, SLA) is lower, showing the enhancement of surfaces wettability. Turned surfaces covered with TNT exhibits a contact angle of 60°, on SLA surfaces modified with TNT layers contact angle is 47°, while the best wettability, demonstrated by a contact angle of 35°, is showed by acid etched surfaces covered with TNT layers.

1. Introduction

The most used metallic materials for medical implants are titanium-based materials, since they provide good mechanical strength, outstanding chemical stability, and excellent biocompatibility. Their unique biocompatibility relies on the formation of a native TiO₂ compact layer, with a thickness of 2...10 nm, on material surface.

The process of implants' osseointegration can be enhanced by modifying their surface at the micro level, in order to develop a microrough surface with a large bone-to-implant contact area. Among other methods, this can be done by acid etching and mechanical sand blasting, or their combinations, resulting acid etched surfaces (AE) and sand blasted whit large grits and acid etched surfaces (SLA). Furthermore, recent studies show that surface modification at nano scale level, by producing ordered nanostructured surfaces, promotes enhanced bone apposition, as titania nanotubes (TNT) with inner diameter of 15-100 nm combine very well with osseous tissue and can be the perfect basis for osteoblasts integration during the process of bone regeneration [1-6].

Electrochemical anodization (EA) method employed in surface modification of titanium and titanium alloys attracted wide scientific interest, as it can control the morphology, structure, and chemical composition of TiO₂ layers [7, 8]. The growth mechanism of nanotubes by EA is very



complex and still under debate [7-12]. The most popular theory that explains it is the dissolution equilibrium theory [7], which indicates that the equilibrium between the formation of oxide at the metal-oxide interface and its etching at the oxide-electrolyte interface is the major cause of nanotubular structure. In a very recent study evidence against this theory were published [11], authors concluding that nanotubes' formation results from plastic flow around the oxygen bubbles. Nevertheless, in experimental research, by optimizing the process parameters of anodization, highly order and self-arranged nanostructured (nanoporous/nanotubular) surfaces can be developed. In the literature there are many reports on nanotubular TiO₂ synthesis on polished surfaces of pure titanium. However, the formation of self-ordered nanotubes on multi-phase titanium alloys is a more demanding process, due to the preferential dissolution and different reactions rates of different phases of the alloy. TNTs synthesis on micro rough surfaces is another challenge that isn't properly addressed until now.

Wetting of the implant surface and rapid adsorption of biologically active molecules, such as proteins, occurs in the very first moments of a medical implantation procedure, followed by enlisting of the osteoprogenitor cells that would regenerate the tissue [13]. But the effects of surface wettability on key biological aspects suffers from a lack of consistent investigation.

The wettability of a surface can be quantified by sessile drop technique, where a drop of a desired wetting liquid is placed on the surface of the specimen, and the angle between the tangent of the drop at the solid/liquid/gas three-phase boundary and the horizontal baseline of the solid surface is measured. This angle is called contact angle (θ) and characterizes the hydrophilicity of the surface if water is used as the wetting agent. Water contact angles lower than 90° designate surfaces as hydrophilic and indicate that wetting of the surface is favorable, and the fluid will spread over a large area on the surface. Surfaces with water contact angles above 90° are considered hydrophobic and generally means that wetting of the surface is unfavorable [14-16]. In contact with blood and biological fluids hydrophilic surfaces promote protein adsorption and enhance cell adhesion. In contrast, hydrophobic surfaces can partially denature proteins, causing cell-binding sites to be less accessible, which results in diminished cell adhesion [17]. Several recent hydrophilized implant systems favor superhydrophilicity, but still the optimal degree of hydrophilicity for best biological and clinical outcomes remains unclear. A very recent study suggests that moderately hydrophilic surfaces (20-40° water contact angle) promote the highest level of cell attachment [18].

In this context, recently we reported efficient anodization process that leads to the formation of ordered nanotubular TiO₂ layer, superimposed onto micro rough topography, resulting by turning, of two phases ($\alpha+\beta$) Ti6Al4V alloy [19], presenting novelty compared with most of studies reporting TNTs growth on polished surfaces. The aim of present paper was the development of nanotubular titania layers superimposed onto micro rough AE and SLA surfaces, results that also have degree of novelty compared to the state of the art. The wettability of titania nanotubular layers developed on polished, turned, acid etched, and SLA surfaces was investigated in comparison with the initial ones, in order to find if the modified surfaces exhibit enhanced hydrophilicity.

2. Methodology

The experiments were performed on planar surfaces of Ti6Al4V alloy (Ti grade 5). The specimens have disc shape of diameter $D = 16$ mm and height $H = 3$ mm.

Prior to electrochemical anodization specimens were subjected to different initial processing: turning, polishing, acid etching, and sand blasting and acid etching. Specimens are designated accordingly to their surface preparation procedure: T (turned surface), P (polished surface), AE (acid etched surface), SLA (sand blasted and acid etched surface).

Turning (T) was performed on Cincom K16 (Citizen) CNC turning machine. Polishing (P), preceded by wet grinding with 320-grit and 1200-grit SiC papers (ATM), done for 5 minutes, was performed using 6 μm diamond suspension (ATM). Acid etching (AE) was done by using a combination of HCl 1n and H₂SO₄ 1n (1:1) at 60°C, in BOV T25F (Biobase) furnace, for a duration of 12 hours. Sand blasting and acid etching (SLA) procedure consisted in a sand blasting step, performed by using SiO₂ particles with an average dimension of 200-250 μm , at a pressure of 4 bar, for 10 min,

in a Basic Eco (Renfert) sand blasting machine, followed by an acid etching step performed in above mentioned conditions. After each of preparation step the specimens were carefully cleaned in distilled water, ethanol, and dried in hot air using Elmadry TD 30 (Elma) drying unit. The surfaces' roughness was measured using SJ-310 (Mitutoyo) roughness tester, and the samples' weight was measured using AEA-100G (Adam Equipment) analytical balance with an accuracy of ± 0.1 mg.

The modification at nano scale level of as prepared surfaces (T, P, AE, and SLA) was done by electrochemical anodization (EA) in a custom built electrochemical cell controlled and monitored by our originally designed software - Nanosource 2. The nanostructured surfaces that were synthesis are designated as: NT-T (nanotubes on turned surface), NT-P (nanotubes on polished surface), NT-AE (nanotubes on acid etched surface), and NT-SLA (nanotubes on sand blasted and acid etched surface). During anodization, carried out at room temperature, the specimen was connected to the anode, and the cathode was a pure copper disc of 16 x 3 mm, placed at a distance of 15 mm from specimen. The electrolyte was a mixture of 1M H₃PO₄ and 0.4 wt% HF or 0.5 wt% HF, prepared from reagent grade chemicals (Chemical Company) and deionized water. The anodization potential U was 20 V or 24 V, applied with a potential ramp U_r of 0.1 V/s or 0.08 V/s. The potentiostatic stage of anodization experiments had a duration of 30 min. After anodization, the samples were rinsed in deionized water, cleaned in ethanol, and dried in hot air using an Elmadry TD 30 (Elma) drying unit.

Contact angle was assessed by using static method. The tangent angle at the three-phase contact point on a sessile drop profile (figure 1a) was measured using high resolution photographs of pure water drops of 4 μ l, placed on the surface with a 0.5-10 μ l micropipette (DLAB Scientific). The images were taken with DSLR D3400 (Nikon) camera. For each specimen 10 droplets were placed, not all 10 in the same time, but in several series of examination. For an examination two, or three drops were placed on the surface (figure 1a). Between two series of measurements the surfaces were cleaned by rinsing in ethanol, drying at 80°C for 15 min in BOV T25F (Biobase) furnace, and letting them to cool down to the room temperature. An open source graphical image processing software (Gimp) was used to measure contact angle on as taken images. On each drop 5 readings of each side of the drop were made. The results presented in the paper are the average values of the measurements made using above described protocol.

The morphology of specimens' planar surfaces was investigated by scanning electron microscopy (SEM) performed in a JSM 5200 (JEOL) scanning electron microscope operated at 25 kV. SEM micrographs were collected at magnifications of 500 X, 1500X, 20000X, and 35000X. Open source graphical image processing software (Gimp) was used for the assessment of nanotubes dimensions. On each micrograph 50 nanotubes were selected, and their features were measured, the results presented in the paper being the average values of these measurements.

3. Results and discussion

Ti6Al4V is a two phase ($\alpha+\beta$) alloy, where hexagonal close packed (hcp) α phase is stabilized by aluminium, and body centred cubic (bcc) β phase is stabilized by vanadium.

The main parameters of specimens' processing by anodization to synthesis ordered TiO₂ nanotubes are listed in table 1. The table shows also the results of the experiments regarding the roughness R_a and contact angle θ of initial surfaces, prior to EA, and the inner diameter D_i of the nanotubes and contact angle θ measured on the modified surfaces consisting in nanotubular titania developed by EA. Contact angles measured on initial surfaces (P, T, AE, SLA) show their moderate hydrophilicity. Modification of the surfaces at nano scale level by synthesis of nanotubular morphologies on specimens' surfaces enhances the wetting properties for all types of surfaces (NT-P, NT-T, NT-AE, NT-SLA) compared to the initial ones, as the decreasing of contact angles show (table 1).

On polished surface (P) the contact angle is 78.13° (inset of figure 1a). Anodization of polished specimen in 1M H₃PO₄ + 0.5 wt% HF, anodization potential U = 20 V, potential ramp U_r = 0.1 V/s, for a duration of 30 min leads to the surface modification and the development of uniform, highly arranged TiO₂ nanotubes with tube openings in 30-90 nm range and 67.88 nm average inner diameter

(figure 1b). On this surface (NT-P) the contact angle is 70.55° (inset of figure 1b), lower than on initial polished surface, showing a slight enhancement of wettability.

Table 1. Surface processing, process parameters and results.

Surfaces prior to electrochemical anodization						
Specimen	Surface processing			Roughness R_a [μm]	Contact angle θ [$^\circ$]	
P	polishing			0.033	78.13	
T	turning			0.535	77.19	
AE	acid etching			1.202	67.60	
SLA	sand blasting and acid etching			2.488	64.89	
Modified surfaces by electrochemical anodization						
Specimen	Process parameters			Nanotubes' inner diameter D_i [nm]		Contact angle θ [$^\circ$]
	% wt. HF	U [V]	U_r [V/s]	Range	Average	
NT-P	0.5	20	0.1	30-90	67.88	70.55
NT-T	0.5	20	0.1	25-90	64.27	60.29
NT-AE	0.4	24	0.08	40-110	81.36	34.56
NT-SLA	0.4	24	0.08	40-110	82.48	46.82

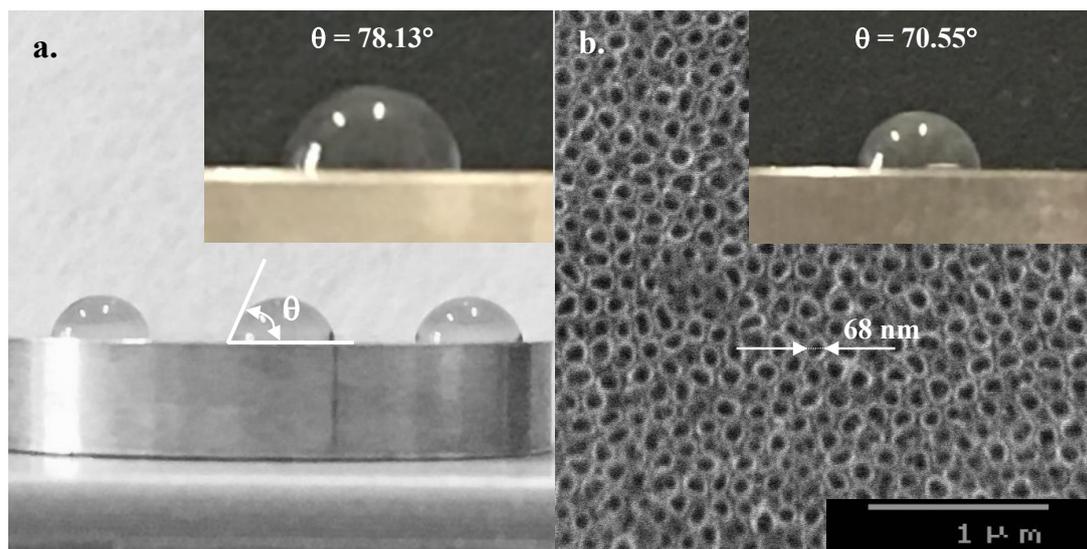


Figure 1. a) Water droplets placed on P (polished) surface and contact angle θ measurement; b) SEM micrograph of modified surface NT-P (nanotubes on polished surface); insets: water droplet and contact angle value on respective surface.

Turned surface's (T) morphology is a minimally rough one (R_a is in 0.5-1 μm range) consisting in regular machining grooves apparent on the surface, as the SEM micrograph taken at low magnification of 500X shows (figure 2a). Its contact angle is 77.19° (inset of figure 2a). By EA in 1M H_3PO_4 + 0.5 wt% HF electrolyte, anodization potential $U = 20$ V, potential ramp $U_r = 0.1$ V/s, for a duration of 30 min, the turned surface is covered with a TiO_2 nanotubular layer, nanotubes having inner diameters in 25-90 nm range (average $D_i = 64.27$ nm), as SEM micrograph, taken at magnification of 20000X, presented in figure 2b, demonstrates. On this surface (NT-T) the contact angle is 60.29° (inset of figure 2b), lower than on initial turned surface, showing enhancement of hydrophilicity.

The development by EA of ordered nanotubular TiO₂ layer, superimposed onto micro rough topography resulting by turning, on two phases ($\alpha+\beta$) Ti6Al4V alloy was recently optimized by us [19] and has novelty compared with most of studies reporting TiO₂ nanotubes' growth, studies that are made on polished surfaces. Moreover, in present paper we report the successful synthesis of nanotubular titania layers superimposed onto moderately rough (R_a in 0.5-1 μm range) AE and rough ($R_a > 2 \mu\text{m}$) SLA surfaces, results that also have degree of novelty compared to the state of the art.

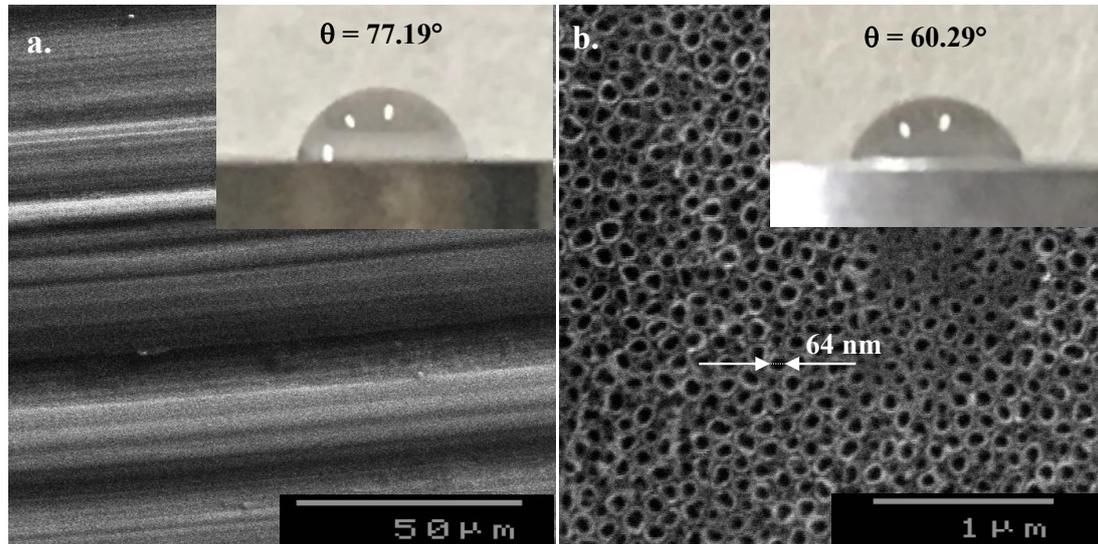


Figure 2. SEM micrographs showing the morphology of: a) T (turned) surface; b) modified surface NT-T (nanotubes on turned surface); insets: water droplet and contact angle value on respective surface.

By optimizing the electrochemical anodization process parameters (1M H₃PO₄ + 0.4 wt% HF electrolyte, $U = 24 \text{ V}$, $U_r = 0.08 \text{ V/s}$) the TiO₂ layer that is developing on EA and SLA surfaces has a self-ordered nanotubular morphology, as the SEM micrographs presented in figure 3 and figure 4 show. Micrographs collected at magnification of 1500X (main images in figure 3 and 4) show that the nanostructured oxide layer is covering rough topography of the surfaces. The insets of the figures 3 and 4 showing SEM micrograph taken at magnification of 35000X, top view, clearly demonstrate the highly ordered, self-arranged nanotubular morphology of the titania layers.

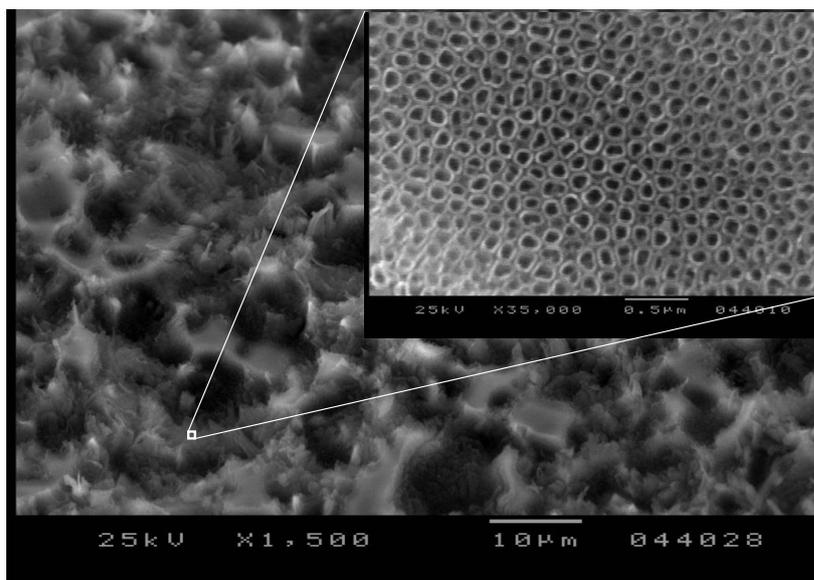


Figure 3. SEM micrographs of TiO₂ nanotubular layer developed on AE surface; main figure – 1500X magnification; inset 35000X magnification.

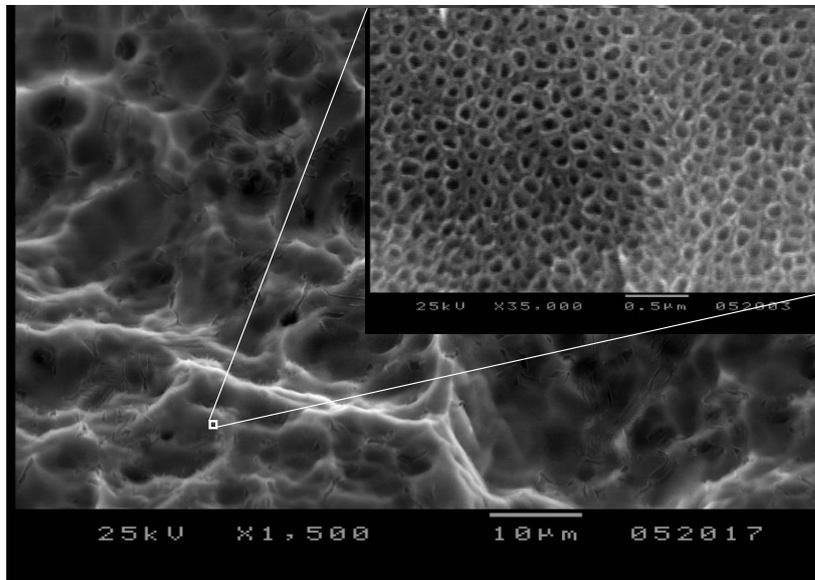


Figure 4. SEM micrographs of TiO₂ nanotubular layer developed on SLA surface; main figure – 1500X magnification; inset 35000X magnification

On initial acid etched surface's (AE) the morphology is a fragmented one, resulting from material removal process due to acid attack, as the SEM micrograph taken at low magnification of 500X shows (figure 5a). On this surface contact angle is 67.60° (inset of figure 5a). By EA in 1M H₃PO₄ + 0.4 wt% HF electrolyte, anodization potential $U = 24$ V, potential ramp $U_r = 0.08$ V/s, for a duration of 35 min, the AE surface is covered with a TiO₂ nanotubular layer. The nanotubes exhibit inner diameters in 40-110 nm range, as SEM micrograph, taken at magnification of 20000X, shows (figure 5a). On this surface (NT-EA) we measured a low contact angle of 34.56° (inset of figure 5b), showing an important enhancement of hydrophilicity and very good wettability of nanostructured surface.

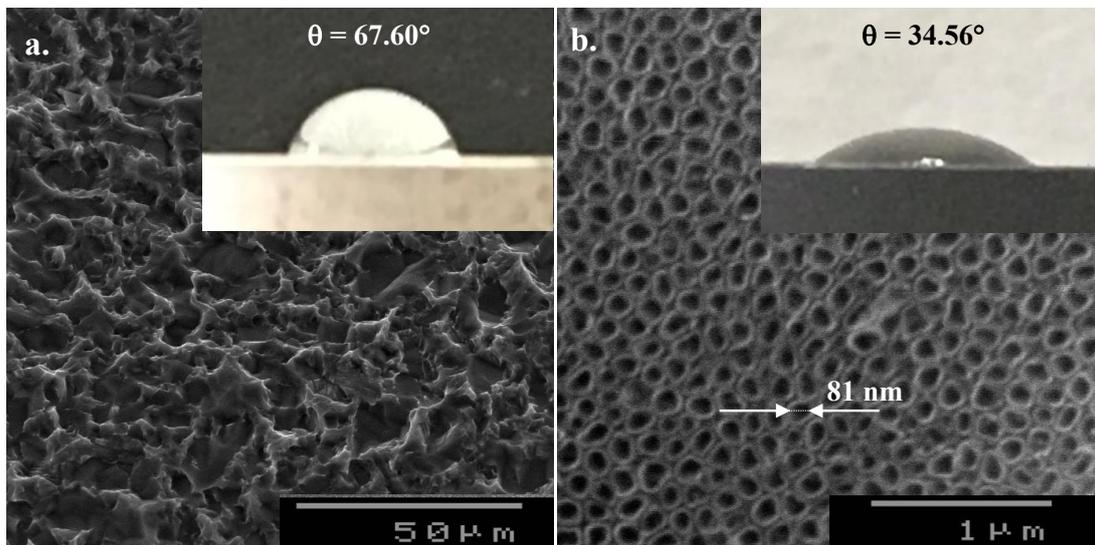


Figure 5. SEM micrographs showing the morphology of: a) AE (acid etched) surface; b) modified surface NT-AE (nanotubes on acid etched surface); insets: water droplet and contact angle value on respective surface.

In the case of sand blasted and acid etched (SLA) surfaces, after sand blasting the surface morphology become a fragmented one and by acid etching a material removal process occurs, the surface becomes smoother, with large valleys and high hills of $\sim 50 \mu\text{m}$, interrupted by micro pores of $5\text{-}10 \mu\text{m}$ (figure 6a). These are typical SLA surfaces, as they are present on many commercial dental implants available today on the market. Our measurements show contact angle on SLA surface being 64.89° , the lowest value for initial surfaces that were subjected to anodization. By EA in $1\text{M H}_3\text{PO}_4 + 0.4 \text{ wt\% HF}$, $U = 24 \text{ V}$, $U_r = 0.08 \text{ V/s}$, for a duration of 35 min, on the SLA surface TiO_2 nanotubes with inner diameters in $40\text{-}110 \text{ nm}$ range are developed, majority having $D_i = 80 \text{ nm}$, as SEM micrograph, taken at magnification of 20000X, shows (figure 6a). On this surface (NT-SLA) we measured a contact angle of 46.82° (inset of figure 6b), showing the enhancement of surface hydrophilic properties.

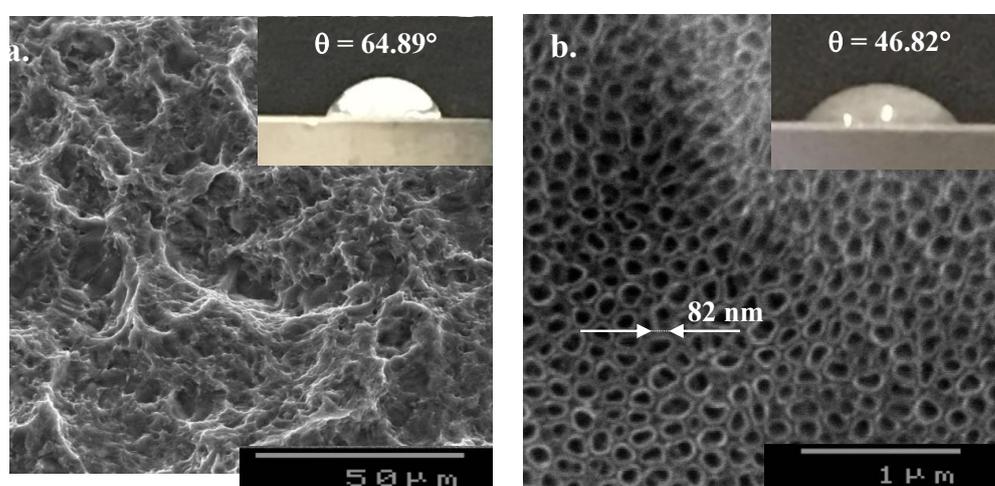


Figure 6. SEM micrographs showing the morphology of: a) SLA (sand blasted and acid etched) surface; b) modified surface NT-SLA (nanotubes on sand blasted and acid etched surface); insets: water droplet and contact angle value on respective surface.

4. Conclusions

Our results show successful development of highly ordered, self-arranged nanotubular TiO_2 layers on planar surfaces of Ti6Al4V alloy for biomedical applications (orthopaedic and dental implants), by using electrochemical anodization in $\text{H}_3\text{PO}_4/\text{HF}$ electrolytes. Nanostructured morphologies were synthesized on polished (P), turned (T), acid etched (AE), and sand blasted and acid etched (SLA) surfaces. The development of titania nanotubes on two phase ($\alpha+\beta$) titanium alloy micro rough AE and SLA surfaces presents degree of novelty compared with the state of the art, where the most results are reported as carried out on extra polished surfaces.

The wettability of initial surfaces (P, T, AE, SLA) is a moderate one, all surfaces exhibit hydrophilicity demonstrated by the contact angle in the range of $65\text{-}78^\circ$. Modification of the surfaces at nano scale level by synthesis of nanotubular morphologies on specimens' surfaces enhances the wettability for all types of surfaces (NT-P, NT-T, NT-AE, ST-SLA) compared to the initial ones, as the decreasing of contact angles in the range of $34\text{-}60^\circ$ show. The best hydrophilicity is exhibited by acid etched surface covered with nanotubular titania (contact angle $\theta = 34.56^\circ$). The sand blasted and acid etched surface modified with nanotubular titania has also good wettability (contact angle $\theta = 46.82^\circ$). These results demonstrate the enhancement of hydrophilicity in the case of nanotubular TiO_2 layers developed on micro rough surfaces making them favorable for cell attachment and proliferation, adding one more reason to their use in medical implants applications.

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