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Contact Angle Measurement on Medical Implant Titanium Based Biomaterials

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Abstract

The aim of present research was to study the effect of some surface modification processes on wettability of Ti6Al4V surfaces for medical implants quantified by using the measurement of contact angle. The surface modification procedures of several disc samples were performed by sand blasting, acid etching, passivation, and their combinations. Sand blasting was done with 250...300 μm SiO_2 large grits. The acid etching process was performed in a dual bath of H_2SO_4 1n and HCl 1n (1:1) at different elevated temperatures (60°C, 80°C, and 100°C) using different process durations (1 h, 3 h, 6 h, 12 h, and 24 h). Passivation was done in 30% HNO_3 at room temperature. On modified surfaces we evaluated topography, by measuring roughness, and analyzing scanning electron micrographs. Contact angle was measured using static method and direct measurement of the tangent angle at the three-phase contact point on a sessile drop profile. We found that the influence of sand blasting prior to acid etching at low temperature (60°C) on contact angle is beneficial when using long acid etching times (12 h and 24 h). Increasing the process time for acid etching leads to a decrease in contact angle which is a desired result. Increasing the temperature of acid etching bath leads to an increase in contact angle, thus decreasing the wettability. The passivation treatment leads to an increase of contact angle on sand blasted surfaces, lowering the hydrophilic properties of surfaces, and does not influence significantly the contact angle on surface without sand blasting treatment. The best preparation procedure, in terms of wettability measured by contact angle, was found to be sand blasting, followed by acid etching in above mentioned dual acid bath at 60°C for 24 hours. In this case we measured a contact angle of 46° showing good hydrophilic properties.

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1. Introduction

Titanium based materials are nowadays considerred valuable biomaterials, due to specific combination of mechanical strength and biocompatibility which makes them suitable for medical applications such as artificial bones, joint replacements and dental implants. The high corrosion resistance of titanium based materials in vivo environments is due to their ability to form a chemically stable, highly adherent and continuous protective oxide layer on their surface. The nature, composition (usually based on TiO_2, Ti_2O_3 or TiO), and thickness of this oxide layer depend on the environmental conditions and the preparation procedure. In orthopaedics and dentistry the clinical goal that measures the success of bone implants is achieving osseointegration. This means to establish a strong and long-lasting connection between the implant surface and peri-implant bone, leading to a stable mechanical attachment of the implant at the site of the implantation. Kulkarni et al show that as soon as the implantation procedure occurs, several biological reactions take place in a specific order: in the first stage, there will be wetting of the implant surface and rapid adsorption of biologically active molecules, such as proteins, followed by enlisting of the osteoprogenitor cells that would regenerate the tissue [1].

In order to enhance osseointegration many methods (mechanical, chemical, physical, or their combination) of surface modification have been developed. The roles of surface properties such as roughness and chemistry have been thoroughly evaluated in osseointegration. However, the effects of surface wettability on key biological aspects suffers from a lack of consistent investigation. As a result there is an increasing interest in understanding the wettingmechanisms of implant surfaces and the role of wettability on the biological response at theimplant/bone or implant/soft tissue interface.

The wettability of a surface is quantified by sessile drop technique, in which 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, the so-called contact angle (θ_c) characterizes the hydrophilicity of the surface if water has been used as the wetting agent. Water contact angles lower than 90° designate surfaces as hydrophilic and indicates that wetting of the surface is favorable, and the fluid will spread over a large area on the surface, while surfaces with water contact angles above 90° are considered hydrophobic and generally means that wetting of the surface is unfavorable [2, 3].Vogler defined hydrophobic surfaces already as those exhibiting water contact angles higher than 65° [4].When a liquid is placed on a lower-energy surface metal, the contact angle will be higher as compared with a higher-energy surface metal [5].

Regarding the biological response to changes in biomedical surfaces wettability in a recent review paper Gittens et al. summarize several studies that suggest a general stimulating effect of higher surface hydrophilicity on hard and soft tissue integration with the implant, yielding accelerated healing and early osseointegration. He found that „Surface wettability can affect four major aspects of the biological system: (1) adhesion of proteins and other macromolecules onto the surface (conditioning), (2) hard and soft tissue cell interactions with the pre-conditioned surfaces, (3) bacterial adhesion and subsequent biofilm formation, and (4) rate of osseointegration in the clinic (in vivo)" [6]. In contact with blood and biological fluids hydrophilic surfaces promote protein adsorption and enhances cell adhesion. In contrast, hydrophobic surfaces can partially denature proteins, causing cell-binding sites to be less accessible, which results in diminished cell adhesion. Gittens concude that the optimal degree of hydrophilicity for best biological and clinical outcomes remains unclear. While several recent hydrophilized implant systems favor superhydrophilicity, it is unclear if a more moderate hydrophilicity would further optimize interfacial reactions" [6].

Based on our previous results [7, 8] current work is focused on surface modification methods that involve mechanical sand blasting and chemical acid etching. Recently was reported that sand blasting induces some changes of surface-charge of medical implants, generating a small amount of negative electric charge on thetitanium material's surface, which seems to lead to enhancement in osseointegration, along with already known roughening effect[9, 10].

Our major goal is to design, perform and asses an effective procedure for Ti6Al4V medical implants preparation that assures proper surface morphology, roughness and wettability for a successful osseointegration. In this context, the aim of present research is to elucidate the effect of surface preparation procedure and subsequent passivation treatment on contact angle of Ti6Al4V surfaces for medical implants.

2. Methodology

For present set of experiments we prepared thirty samples of titanium alloy Ti6Al4V (Ti grade 5).The substrates are in form of disc samples manufactured by turning on CNC machine, each sample having dimensions of 16 mm in diameter and 3 mm in height.

The surface modification procedure of samples surface was design in order to allow us to conclude which is its influence on roughness, morphology, and wettabilily. Based on our previous results [7] we selected as corrosion agent a dual bath of H_2SO_4 1n and HCl 1n (1:1). The acid etchig (AE) process was performed at different elevated temperatures T (60°C, 80°C, and 100°C) using different process durations t (1 h, 3 h, 6 h, 12 h, and 24 h). Prior to acid etching half of samples were sand blasted (SB) with 250...300 μm SiO_2 large grits for 10 min. The remaining half was acid etched without sand blasting their surfaces. After acid etching procedure some samples were passivated (P) in 30% HNO_3 at room temperature for 15 min. After sand blasting and etching the samples were subjected to a carefull cleaning process step in distilled water followed by alcohol and drying.

Humbold 30GC furnace was used for hot acid etching process step. Kern ARJ 220-4M laboratory balance with a reproducibility of 0.1 mg was used for assesment of samples mass loss Δm [g]. The roughness R_a [μm] of samples was measured using a Surftest SJ-210 (Mitutoyo) roughness tester. The surface topography was evaluated using scanning electron microscopy performed in a JEOL JSM 5200 scanning electron microscope operated at 25 kV SEM. Micrographs were collected at magnifications of 100X, 500X, and 2000X, using secondary electrons and backscattered electrons. Contact angle was measured using static method and direct measurement of the tangent angle at the three-phase contact point on a sessile drop profile, using high resolution photographs of pure water drops and a graphical image processing software (Gimp). On each sample we perform 5 sets of contact angle measurements. The volume of the drops was 0.05 ml and the distance of dropping was 10 mm. On each set we measured contact angle from both sides (right and left), each reading being made for 5 times. The following results present the average of these measurements.

3. Results and discussion

Initially, after CNC turning, the samples exhibit an average roughness Ra of 1.2 μm. On sand blasted samples we measured an average roughness of R_a = 3.3 μm. Two samples, one turned and one sand blasted, were kept as control samples. Table 1 summarizes the surface modification procedure, process parameters, and results of present set of experiments.

Table 1. Surface modification procedure, process parameters and results

Sample	Surface preparation	Temperature T [°C]	Time t [h]	Roughness R_a [μm]	Mass loss Δm [g]	Contact angle θ_c [°]
30	sand blasting + acid etching	60	6	3.331	0.0049	64.09
31	sand blasting + acid etching + passivation	60	6	3.436	0.0043	74.35
40	acid etching	60	6	1.202	0.0030	61.46
41	acid etching + passivation	60	6	1.074	0.0027	60.91
33	sand blasting + acid etching	60	12	2.706	0.0119	54.08
34	sand blasting + acid etching + passivation	60	12	2.594	0.0126	77.61
42	acid etching	60	12	1.102	0.0096	69.04
43	acid etching + passivation	60	12	1.066	0.0090	67.42
36	sand blasting + acid etching	60	24	2.289	0.0207	46.22
37	sand blasting + acid etching + passivation	60	24	2.516	0.0218	64.46

44	acid etching	60	24	1.292	0.0206	61.22
45	acid etching + passivation	60	24	1.226	0.0181	62.36
51	sand blasting + acid etching	80	6	2.053	0.0133	65.12
52	sand blasting + acid etching + passivation	80	6	2.083	0.0138	72.63
26	acid etching	80	6	1.126	0.0098	67.89
27	acid etching + passivation	80	6	1.196	0.0093	70.34
53	sand blasting + acid etching	80	24	2.021	0.0878	59.87
54	sand blasting + acid etching + passivation	80	24	2.292	0.0839	72.02
28	acid etching	80	24	1.525	0.0839	62.89
29	acid etching + passivation	80	24	1.548	0.0772	61.39
55	sand blasting + acid etching	100	1	2.244	0.0081	69.75
56	sand blasting + acid etching + passivation	100	1	1.920	0.0070	81.44
46	acid etching	100	1	1.101	0.0046	75.43
47	acid etching + passivation	100	1	1.135	0.0046	64.00
57	sand blasting + acid etching	100	3	2.093	0.0247	67.66
59	sand blasting + acid etching + passivation	100	3	2.013	0.0286	61.76
48	acid etching	100	3	1.457	0.0222	66.31
49	acid etching + passivation	100	3	1.282	0.0240	70.47
83	control sample - turned	-	-	1.221	-	60.76
58	control sample - sand blasted	-	-	2.694	-	82,23

The influence of surface modification procedure on surface roughness and topography was analyzed and reported elsewhere [8]. Present paper is focused on surface hydrophilic properties enhancements, demonstrated by its wettability improvement, measured by the decreasing of contact angle θ_c. After initial CNC turning, the samples exhibit a relatively good wettability, having a contact angle θ_c of 60.76° (Table 1 and Fig. 1). But these surfaces aren't proper from the point of view of surface morphology [8]. Review studies [11, 12] revealed that a good osseointegration requires a proper surface topography with micropores (<10 μm) opened on the surface and a surface roughness R_a in 1...2.5 μm range. In these micropores osteoblasts and supportive connective tissue can migrate, by this enhancing medical implants bioadhesion.

By sand blasting the surface morphology is enhanced, but the process is detrimental on wettability. Our results show an increase of contact angle after sand blasting to 82.23° (Table 1 and Fig. 1), this being the highest value for present set of experiments. Sand blasted surface has the lowest hydrophilic properties compared with turned surfaces or modified surfaces. By applying the acid etching procedure on sand blasted surfaces the wettability is improved. The process of acid etching is a very complex one, being influenced by the titanium oxide layer from the surface of the material. Also etching procedure may create titanium hidrides (TiH_2, TiH_3, TiH_4, or combinations) in addition to the titanium oxide. The influence of titanium hidrides is mainly unknown. Our results show that the best hydrophilic properties are exhibited after surface modification by sand blasting followed by acid etching at 60°C for 24 hours, where we measured a θ_c of 46.22° (Table 1 and Fig. 1).

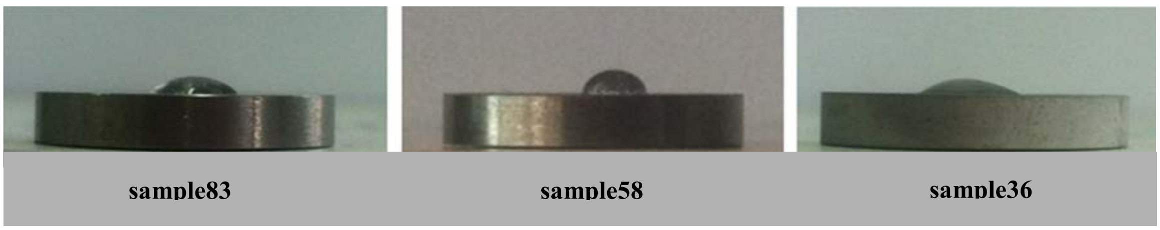


Fig. 1. Wettability of turned (sample 83), sand blasted (sample 58), and sand blasted and acid etched (sample 36) Ti6Al4V surfaces, showing the enhancement of surface hydrophilic properties by surface modification procedure

Good wettability was found also on samples prepared by sand blasting followed by acid etching at 60°C for 12 hours (θ_c = 54.08°) and at 80°C for 24 hours (θ_c = 59.87°). These surface modification processes – sand blasting followed by acid etching - allows also the development of proper surface morphology, detailed results on this aspect being reported by us elsewhere [8].

From Table 2 we can analyze and conclude which is the *influence of process parameters on contact angle*. For each set of process parameters we arrange the data from lowest contact angle, and thus the most favorable, to the highest.

Table 2. The influence of process parameters on contact angle

Process parameters		**Process steps (AE – acid etching; SB – sand blasting; P – passivation)**			
		Contact angle θ_c [°]			
Temperature	**Time**	**Contact angle decreasing**			
T [°C]	**t [h]**	**lowest** ←			**highest**
60°C	6	AE + P	AE	SB + AE	SB + AE + P
		61°	61°	64°	74°
	12	SB + AE	AE + P	AE	SB + AE + P
		54°	67°	69°	78°
	24	SB + AE	AE	AE + P	SB + AE + P
		46°	61°	62°	64°
80°C	6	SB + AE	AE	AE + P	SB + AE + P
		65°	68°	70°	73°
	24	AE + P	SB + AE	AE	SB + AE + P
		59°	61°	62°	72°
100°C	1	AE + P	SB + AE	AE	SB + AE + P
		64°	70°	75°	81°
	3	SB + AE + P	AE	SB + AE	AE + P
		62°	66°	68°	70°

The results show that when the acid etching is performed at 60°C the influence of sand blasting before acid etching is beneficial on wettability when we used long corrosion durations (12 hours, and 24 hours). The θ_c is 54° compared to 69° at 12 hours, and 46° compared to 61° at 24 hours. Using short acid attack times (6 hours) the contact angle is higher in the case of samples that were sand blasted compares with those without sand blasting processing step (θ_c is 64° compared to 61°).

Comparing the evolution of contact angle with those of roughness and mass lost we can say that at short process time there is a good similarity between them. This means that the samples that exhibited low roughness and mass loss have also low contact angle. At long process times this similarity in evolution is lost and we measured the lowest contact angle in the case of samples with roughness higher than others. For example, at 12 hours of acid attack, the lowest θ_c is for the sample with the higher R_a. So, we can conclude that there is no direct and simple influence of R_a on contact angle, the wettability being influenced by a complex of changes in chemistry of surface, surface energy and residual stresses that can lead to a change from a hydrophobic surface to a hydrophilic one.

The same good influence of sand blasting is exhibited by the samples prepared at 80°C, but the enhancement of wettability is lower than in the case of samples prepared at 60°C with long acid etching times.

In the case of samples prepared at 100°C the influence of sand blasting is not so clear, the contact angles are high, unfavorable from the point of view of a good wetting. More of that, at this temperature, the phenomena of acid solution evaporation leads to an increase on its concentration and a poor control of the acid attack process, so it is advisable to avoid working at this high temperature.

At high temperatures (80°C, and 100°C) there is no similarity between evolution of contact angle and roughness. This shows that not R_a is the main parameter that influences the hydrophilic properties of modified surfaces of medical implants.The results from Table 2 show the same influence of surface modification procedure on contact angle performed at 60°C for 24 hours as the one performed at 80°C for 6 hours: the lowest θ_c is for SB + AE procedure, next for AE, followed by AE + P, the highest being for SB + AE + P procedure.A similarity is also shown by the modification procedures performed at 80°C for 24 hours and performed at 100°C for 1 hour.

The passivation treatment leads to an increase in contact angle in the case of sand blasted samples (except the procedure developed at 100°C for 3 hours), and appears to have no clear and significant influence in the case of samples without sand blasting. Our results show that, when acid attack is performed at a certain temperature, the wettability is enhanced in the case of samples corroded for longer durations. In the case of the surface modification procedure by sand blasting and acid etching, which is the most favorable from surface morphology point of view, this enhancement is shown by the following results: at 60°C - θ_c is decreasing 64°/54°/46° for 6 h/12 h/24 h; at 80°C - θ_c is decreasing 65°/61° for 6 h/24 h; and at 100°C - θ_c is decreasing 70°/68° for 1 h/3 h.The best results on enhancing the hydrophilic properties of the surface are for sand blasting and acid etching procedures performed at 60°C for 24 hours (θ_c = 46°), and at 60°C for 12 hours (θ_c = 54°).

The influence of *surface modification procedure on contact angle* can be analyzed using tables (not shown here), and Fig. 2 and 3. In the tables the results were arranged in the order in which contact angle is increasing, and in the same way in the figures, for AE and SB + AE. By this we can conclude which are the differences in enhancing wettability for different procedures, and which is the best combination of process parameters for each procedure.

Regarding acid etching procedure, performed on all samples, the results show that the contact angle is higher than the contact angle on the samples after turning (θ_c = 60.76° for control sample turned CS-T). Figure 2 shows that by increasing the time of acid etching, the contact angle is decreasing. Increasing the temperature of acid etching leads to an increase in contact angle. The values for contact angle, at different preparation parameters aren't too disperse (61° to 75°), and there is no similarity in evolution between contact angle and roughness.

By applying a passivation treatment after acid etching, in order to stabilize the titanium oxide layer formed on the surface, the results (Fig. 2) show that there is a relatively low influence on contact angle. The influence of time and temperature on contact angle exhibited after acid etching is maintained after passivation.

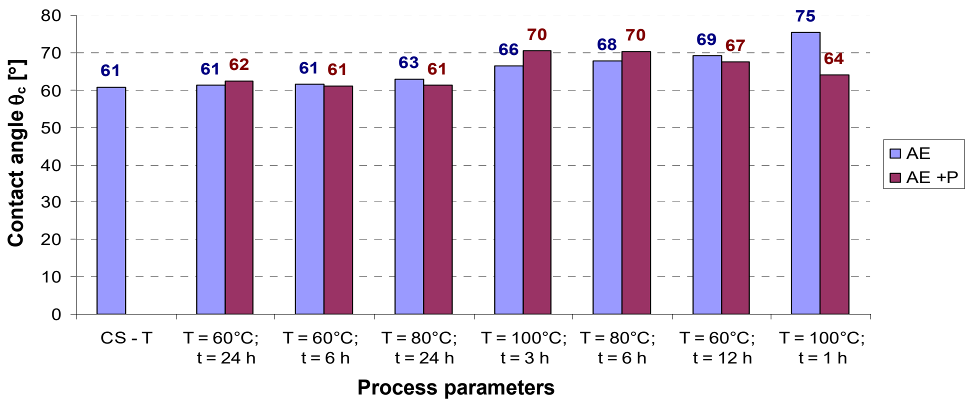


Fig. 2. The influence of process parameters on contact angle for acid etching (AE) and acid etching followed by passivation (AE +P) surface modification procedures, showing the enhancement of wettability by increasing the time, and decreasing the temperature of the acid etching

Sand blasting induces changes in surface wettability, the contact angle is higher than after turning (θ_c = 82.23° for control sample sand blasted CS-SB compared withθ_c = 60.76° for control sample turned CS-T). Acid etching procedure enhances not only the morphology of the surface, but also its wettability, inducing changes in surface chemistry, residual stresses and surface energy. Our results show low contact angles for SB + AE procedure performed at relatively low temperatures and long process times (Fig. 3). By corrosion in dual acid bath at 60°C for

24 hours or 12 hours, or at 80°C for 24 hours our modified titanium based surfaces for medical implants exhibit the best contact angle.

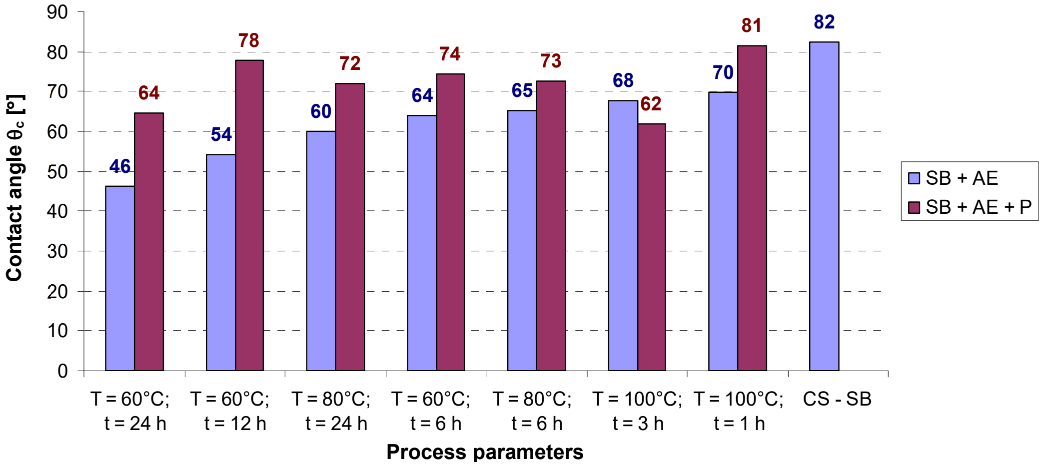


Fig. 3. The influence of process parameters on contact angle for sand blasting followed by acid etching (SB +AE) and sand blasting followed by acid etching and passivation (SB + AE + P) surface modification procedures, showing the enhancement of wettability by increasing the time, and decreasing the temperature of the acid etching

Figure 3 shows that by increasing the time of acid etching, the contact angle is decreasing. Increasing the temperature of acid etching leads to an increase in contact angle. The values for contact angle, at different preparation parameters are relatively dispersed (46° to 70°), and there is no similarity in evolution between contact angle and roughness. By applying a passivation treatment after sand blasting and acid etching the results (Fig. 3) show that the contact angle is increased. The influence is higher on the samples prepared at low temperatures.

Figure 4 presents the scanning electron micrographs collected from the surfaces that exhibits the lowest contact angle (sample 36) and the highest contact angle (sample 56). Sample 36 was prepared by sand blasting and acid etching at 60°C, for 24 hours, and sample 56 was prepared by sand blasting, acid etching at 100°C, for 1 hour, followed by passivation.

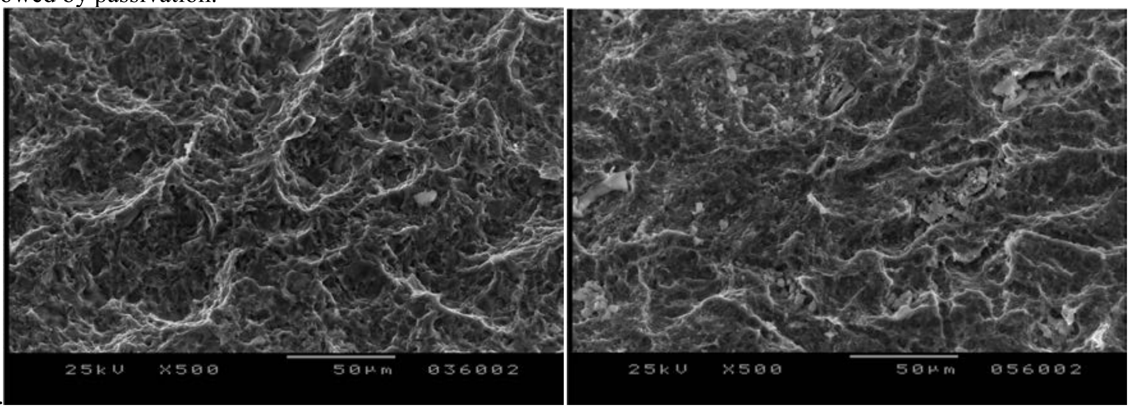
a.  b.

Fig. 4. a. SEM micrograph of sample 36 – sand blasted and acid etched in a mixture of (H_2SO_4+HCl) (1:1) at 60°C, for 24 hours, showing an enhanced moderately rough surface topography; b. SEM micrograph of sample 56 – sand blasted, acid etched in a mixture of (H_2SO_4+HCl) (1:1)at 100°C, for 1 hour, and passivated in 30% HNO_3 at room temperature for 15 min , showing a moderately rough surface topography; (secondary electrons images, magnification 500X, tilt angle 25°)

Both samples present a moderately rough topography, on sample 36 there are more micropores opened in the surface. Even the roughness is lower in the case of sample 56 (R_a = 1.92) compared with sample 36 (R_a = 2.29), its wettability is much lower (θ_c = 81.44° compared with θ_c = 46.22°). In these conditions seems that the main influence on surface hydrophilic properties doesn't come from roughness and surface topography, but from its chemistry, surface energy and residual stresses. Based on our current results on titanium based modified surfaces for medical implants future researches on preparation process and complex in vitro and in vivo characterization of sand blasted and acid etched active type of surfaces (SLA active) are planned.

4. Conclusions

We found that preparation process of medical implants influences not only the morphology of the surface, but induces changes in wettability. The influence of sand blasting of medical implants prior to acid etching in a dual bath of H_2SO_4 1n and HCl 1n (1:1) at low temperature (60°C) on contact angle was found to be beneficial when using long acid etching times (12 h and 24 h). Increasing the process time for acid etching leads to a decrease in contact angle which is a desired result. Increasing the temperature of acid etching bath leads to an increase in contact angle, thus decreasing the wettability. The passivation treatment of medical implants performed in 30% HNO_3 at room temperature for 15 min, leads to an increase of contact angle on sand blasted surfaces, lowering the hydrophilic properties of surfaces, and does not influence significantly the contact angle on surface without sand blasting treatment. The best preparation procedure, in terms of wettability measured by contact angle, was found to be sand blasting, followed by acid etching in above mentioned dual acid bath at 60°C for 24 hours. In this case we measured a contact angle of 46° showing good hydrophilic properties. The samples prepared by using this procedure presents a proper microrough surface topography with less than 10 μm micropores opened on the surface, required by a good osseointegration.

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