ENCHANCEMENT OF WETTABILITY, BIOCOMPATIBILITY AND MECHANICAL PROPERTIES OF TiO2 NANOTUBES ARRAYS GROWN IN SBF-BASED ELECTROLYTE

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ABSTRACT

Titanium is widely used as a dental and orthopedic implants. In order to reduce the corrosion process in biological environment, the metal undergoes the surface oxidation process. In this sense, several techniques of surface modification of its oxide are investigated with the aim of improving properties such as wettability, biocompatibility and mechanical properties. In the present work, modified Titania Nanotubes (TiO2NTs) coatings were electrochemical produced from commercial titanium by anodizing in medium containing simulated body fluid (SBF). The addition of SBF in the specific formulation of 1% v\v to the electrolyte does not modify films morphology (no pit corrosion by chloride was observed) nor the structural characteristics after the heat treatment. However, coatings synthesized in SBF-based solution presented an improvement in wettability and also in the deposition of HA, which indicates that the incorporation of ions from the electrolyte promotes the formation of coatings with higher bioactivity.

KEYWORDS: TiO2NT, SBF-based electrolyte, Ti implants

1 **INTRODUCTION**

Anodic self-ordered nanotubes arrays have been intensively investigated in recent years for its wide potential applications in fields as solar cells [1], photocatalytic degradation [2,3], gas sensors [4] and also biomaterials [5–8]. The specificity required in this applications boosted the tailoring of properties throw nanotechnology, like the growth of TiO2NTs coatings and in the combating of failures of several materials used before. TiO2NTs has proven to be the most suitable compound by showing biological and chemical inertness, cost-effectiveness and long-term stability against corrosion [9]. Thus, the surface modification of metallic implants based on titanium and its alloys presents itself as an advantageous branch of biomaterial research.

TiO2NTs doping with ions, such as Ca^{2+} and PO_4^{3-} , was extensively investigated over the years as a strategy to improve biocompatibility and oxide adhesion. In this work it is presented a new methodology of superficial modification, where the nanotubular structure is grown in a solution containing simulated body fluid (SBF).

Despite SBF being corrosive to titanium because of the chloride ions containing [10], the TiO2NTs synthesis using an electrolyte containing low amounts of SBF can modify the oxide during its growth, promoting the incorporation of species present on human blood plasma increasing biocompatibility and osseointegration of the modified implants.

2 MATERIAL AND METHODS

2.1 TiO2NTs coatings preparation

Anodic TiO2NTs were produced from commercial titanium (T) and Titanium alloy Ti_6Al_4V (A). Titanium sheets (10 mm×15 mm) were polished (1200 mesh) and nanostructured coatings were grown by potentiostatic anodization applying 25 V for 90 minutes, using a pair of platinum sheet as a counter electrode. The electrolyte was constituted by 0.75% w/w of ammonium fluoride, (10-x)% v/v water, where x = 0% and 1% v/v of SBF in ethylene glycol. As-formed TiO2NTs were annealed (450 °C, 2 h, 2ºC/min). Samples were named as T and A (for 0% substitution) and TSBF and ASBF (for 1% v/v substitution). Electrochemical behavior analyses was done extracting curve parameters [11]. TiO2NTs morphology was characterized by FE-SEM (Zeiss, Supra 35) by ImageJ software processing. Structural analysis was carried out by X-ray Diffraction (Higaku 600 Benchtop). Wettability analysis was performed in a hamé-hart Goniometer/Tensiometer Model 250.

2.2 Bioactivity Assay

After synthesis and annealing, samples were soaked in 50 mL SBF at 37 °C to evaluate their bioactivity according to methodology proposed by Kokubo [12]. According to the authors [12], ionic concentrations in SBF are nearly equal to those in human blood plasma. SBF solution was prepared using analytical-reagent grade chemicals, buffered at pH 7.4 at 36.5 °C with 50 mM tris ($(CH_2OH)_3C(NH_2)$) and HCl. After soaking for 14 days, the growth of bone-like apatite on coating surfaces was evaluated by XRD and results are showed as % of HA [13].

2.4 Nanoindentation

Hardness (H) and elastic modulus (E) were measured by instrumented nanoindentation technique using a ZHN Nanoindenter, following the QCSM (Quasi-Continuous Stiffness Method) with a Spherical diamond tip (radius = 5µm). It was applied by nanoindenter 100 mN as maximum force, and 7 x 5 indentations matrix were obtained.

2.5 Statistical analysis

Statistical significance was analyzed by single-factor analysis of variance (ANOVA) using a confidence level of 95%, where the effect of the experimental conditions on several properties of the system was observed. The results data were expressed as the mean ± standard deviation (SD).

3 RESULTS AND DISCUSSION

Fig. 1a and 1b show the current density (CD)–transient curves of TiO2NTs coatings grown with and without SBF. The evolution of current behavior can be separate by three different stages of pore formation process, denoted by I, II and III. In the early stage I, oxide growing leads to an exponential current decrease with the increasing oxide electrical resistivity. Current reaches a Minimum Current Density (MCD) value, related to the formation of on average 50 nm thick barrier oxide at 20–25 V [16]. Because of the electrical field ions such as, O_2^{2-}, OH^-, Ti^{4+} and F^- moves, creating and dissolving the oxide layer [17]. Table 1 shows electrochemical parameters extract from Fig. 1a and 1b. MCD values do not present statistical significance between the studied conditions. As described above ions move to barrier layer inducing chemical reactions as oxide formation ($Ti^{2+} + 2O^{2-} \Leftrightarrow TiO_2 + 2e^-$) and oxide dissolution $TiO_2 + 6F^- + 4H^+ \rightarrow [TiF_6]^{2-} + 2H_2O$), creating pores. This pores are metastable and evolves to nanotubes due to the increase of paths for ionic attack [17], increasing current densities to a Maximum Current Density (MACD). The analysis of this parameter indicates that the SBF also does not influence the MACD parameters as well as the slope from MCD to MACD.

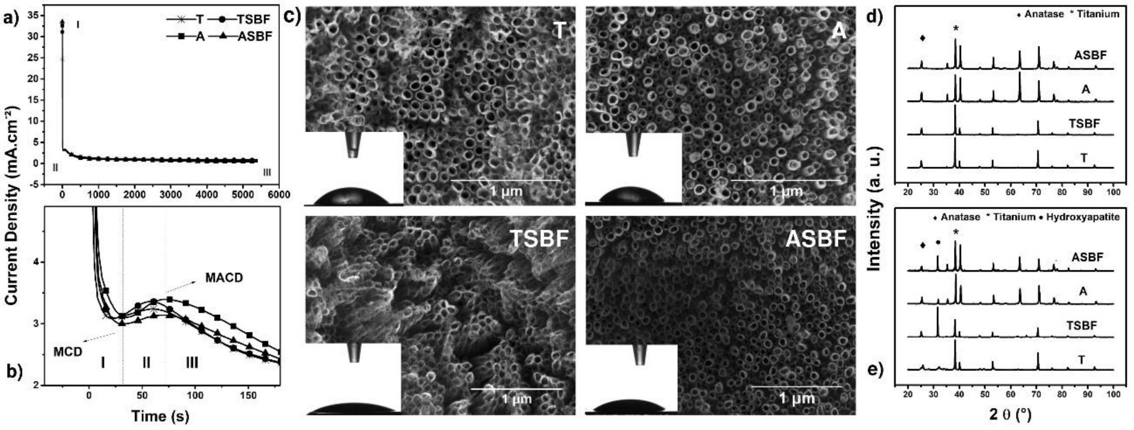

Figure 1 a) CD–transient curves; b) Magnification of a); c) 100 kx magnification of TiO2NTs samples; d) XRD characterization of samples after annealing and d) after 2 weeks immersed on SBF.

Besides being important in the dissolution of the oxide, fluoride anions can be incorporated and even infiltrates into metal-oxide interface creating a fluoride rich layer, due to their high ionic mobility and radius [18], decreasing oxide-substrate adhesion [19]. In this sense, low radius anions present in the SBF modified electrolyte could compete with fluoride ions and be incorporated as well [20]. Biologically important inorganic species, such as phosphate ions [21], can lead to an improvement of hydroxyapatite (HA) deposition on TiO_2NT surface [22]. Cations could be also incorporated by a non-uniform flow phenomena by a local cracking caused by O_2 evolution [23,24].

Morphological and structural analysis of the coatings are presented in Figure 1c and 1d. FE-SEM analysis shows that is possible to obtain a well-defined nanotubular structure for all synthesis conditions. Pore inner diameter and porosity of the coatings, presented on Table 1, are not influenced by the composition of the electrolyte.

For both substrates, there is a significant decrease in contact angle, also show in Table 1, indicating the increase in wettability of the coatings as SBF is added to the electrolyte. As highlighted by Saha, et al. [25], TiO_2 surface hydrophilicity is related to the density of surface hydroxyl groups, which interacts with water molecules through hydrogen bonds. Macak, et al. [26] showed that homogeneous nanoporous structure can significantly increase the surface area, being beneficial on the improvement of the material-fluid contact [26,27] as well. Fig. 1c and Table 1 suggest that in face of results, SBF electrolyte addition could transform surface parameters, altering significantly its surface chemistry.

The XRD pattern analysis of TiO_2NT samples (Fig 1d) shows that anatase crystal phase is present after the annealing process and there is no other phase as salts used in SBF preparation. Bioactivity assay (Fig 1d) was performed and samples were analyzed by HA percent by XRD quantification (Table 1).

Table 1. Electrochemical, morphological, structural, wettability behavior and mechanical parameters for anodized samples.

	MCD (mA/cm^2)	**MACD (mA/cm^2)**	$\left(\frac{di}{dt}\right)_{II}$	**Inner diameter (nm)**	**Contact angle (°)**	**HA (%)**	**H (GPa)**	**E (GPa)**
T	3.07 ± 0.18^{a}	3.11 ± 0.10^{b}	0.007 ± 0.002^{a,b}	65,32 ± 2,14^{a}	51.20 ± 0.10 ^{a}	20.98	0.02	11.45
TSBF	3.35 ± 0.26^{a}	3.89 ± 0.27^{a}	0.012 ± 0.001^{a}	64,88 ± 2,32^{a}	30.75 ± 1.95^{c}	23.59	0.10	20.37
A	2.94 ± 0.09^{a}	3.20 ± 0.10^{a,b}	0.010 ± 0.001^{b}	62,47 ± 1,94^{a}	51.60 ± 1.40^{a}	5.18	0.05	17.55
ASBF	2.98 ± 0.02^{a}	3.14 ± 0.06^{b}	0.006 ± 0.001^{b}	66,75 ± 2,60^{a}	42.10 ± 0.30^{b}	13.29	0.05	14.73

* Data that share the same letter do not present statistical significant.

The literature indicates that anatase have greater ability to induce apatite formation. Apatite layers can deposit faster in this than in amorphous coatings [29], due the presence of hydroxyl groups [29,30]. Higher

HA deposition could be related to higher wettability of samples prepared with SBF-based electrolyte, showing that SBF play an important role in samples bioactivity. Wei et al. [44] have shown the wettability increases the protein adsorption and also the initial attachment of osteoblastic cells.

The study of TiO2NTs mechanical properties is important when considering implant long-term stability. The incompatibility of elastic modulus, for example, could cause bone loss and eventually failures [32,33]. The measured properties of the nanotubes depends on tip geometry [34]. For this study, the spherical diamond tip was chosen due to nanotube geometric characteristic. When a sharp tip is used, such as Berkovich, some nanotubes are concentrated in the tip edges and the most suffers shear forces causing flexing of the nanotubes in contact of the side of the tip. This effect is decreased by using the spherical tip. Fig. 3 shows the investigation of H and E modulus by nanoindentation.

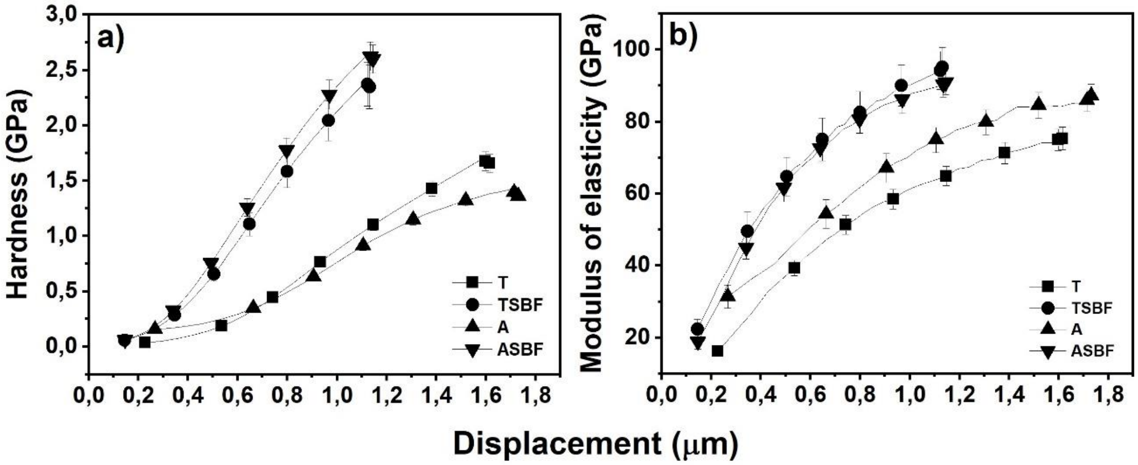

Fig. 2. a) Mean values of hardness (GPa); b) Modulus of elasticity (GPa) as a function of surface displacement (μm) by nanoindentation.

The Hardness and elastic moduli presented by TiO_2 samples (Table 1) are very similar to those found in human femoral bone, wherein the values are for elastic moduli 19.1 ± 5.4 GPa (osteonal), 21.2 ± 5.3 GPa (interstitial lamellae) and the average hardness ranging from 0.234 to 0.760 GPa [35,36]. Fig. 2a and 2b show the apparent elastic modulus E and hardness H, respectively, obtained from the quasi-continuous stiffness measurement (QCSM) method. Both values of H and E modulus tend to increase in depth. This result is related to the deformation of TiO2NTs under spherical indentation [34] and substrate mechanical property contribution [32]. On this mechanism, TiO2 nanotubes deform elastically in small indentation depths. As depth is increased, this nano-geometries fracture in border regions and the center becomes compacted as a response of nanoindenter tip. The final increases in depth result in densification, where mechanical characteristics change becoming a combination of substrate and dense coating mechanical properties.

Similar to E-modulus, Hardness values increase as well when depth approximates to substrate under the same mechanism previously commented. However, the hardness curves show smaller gradients when compared with E- modulus, due to the plastic fields under the indenter reach minor distances than the elastic fields [36]. There are reported relationship between porosity with hardness and elastic modulus. Normally, when the porosity increases the H and E decrease [37–39]. However, in this study, the sample that presents the biggest porosity also have the biggest hardness and highest E-modulus compared to other conditions. This phenomenon can be explained by pore geometry and the influence of mechanical properties of the substrate [36,40]

The metallic substrate and the nanotube coating will work together. As mentioned previously, to prevent loss mass of the bone surrounding the implant, the elastic modulus of implant surface needs to be close to the osseous tissue [32,33]. All the coatings present its elastic moduli lower than the substrate, which means that exists a zone of mechanical accommodation between the bone and the implant. Minimizing substrate contribution, H values were extracted in nanoindentation displacement at 10% of the thickness of the nanotube layer [41] (shown in Table 1). E-modulus data, obtained for all samples, are in agreement with E-modulus values obtained by nanoindentation reported by Crawford et al. [32], Shokuhfar et al. [42] and Alves et al. [43]. Contrasting the data obtained for samples prepared from Titanium with and without SBF is possible to observe an increase in almost 76% in E modulus. In Fig. 2a and 2b, it is also possible to notice a significant change in E modulus and H behavior in samples prepared in SBF-based electrolyte. This result confirms that the incorporation of SBF species could improve the implant mechanical properties.

4 CONCLUSIONS

The aim of this study was the fabrication of bioactive nanotube arrays using SBF-based electrolyte. The SBF addition promotes no significant changes on morphological and microstructural parameters, however there is a significant increase in wettability. This characteristic promotes higher amount of HA deposited on sample surfaces, indicating there is an improvement on the samples bioactivity with SBF addition. Higher hydrophilic behavior tends to enhance hydrophilic cells adhesion, like osteoblasts. Mechanical properties were also enhanced by SBF electrolyte addition. In summary, the modification of the electrolyte with SBF is an interesting methodology to enhance mechanical and biological properties of titanium implants.

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