Investigation of the mechanical and chemical characteristics of nanotubular and nano-pitted anodic films on grade 2 titanium dental implant materials

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ABSTRACT

Objective: The objective of this study was to investigate the reproducibility, mechanical integrity, surface characteristics and corrosion behavior of nanotubular (NT) titanium oxide arrays in comparison with a novel nano-pitted (NP) anodic film.

Methods: Surface treatment processes were developed to grow homogenous NT and NP anodic films on the surface of grade 2 titanium discs and dental implants. The effect of process parameters on the surface characteristics and reproducibility of the anodic films was investigated and optimized. The mechanical integrity of the NT and NP anodic films were investigated by scanning electron microscopy, surface roughness measurement, scratch resistance and screwing tests, while the chemical and physicochemical properties were investigated in corrosion tests, contact angle measurement and X-ray photoelectron spectroscopy (XPS).

Results and discussion: The growth of NT anodic films was highly affected by process parameters, especially by temperature, and they were apt to corrosion and exfoliation. In contrast, the anodic growth of NP film showed high reproducibility even on the surface of 3-dimensional screw dental implants and they did not show signs of corrosion and exfoliation. The underlying reason of the difference in the tendency for exfoliation of the NT and NP anodic films is unclear; however the XPS analysis revealed fluorine dopants in a magnitude larger concentration on NT anodic film than on NP surface, which was identified as a possible causative. Concerning other surface characteristics that are supposed to affect the biological behavior of titanium implants, surface roughness values were found to be similar, whereas considerable differences were revealed in the wettability of the NT and NP anodic films.

Conclusion: Our findings suggest that the applicability of NT anodic films on the surface of titanium bone implants may be limited because of mechanical considerations. In contrast, it is worth to consider the applicability of nano-pitted anodic films over nanotubular arrays for the enhancement of the biological properties of titanium implants.

Key words: anodization, nanotubes, nano-pitted, mechanical integrity, titanium-oxide film

1. INTRODRUCTION

Self-ordered, vertically oriented nanotubular titanium-dioxide (TiO_2) arrays have the capability of attenuating the attachment of biofilm forming pathogenic bacteria on the surface of medical grade titanium substrates, while mesenchymal stem cells show improved osteogenic differentiation on such surfaces [1-2]. This phenomenon has made nanotubular TiO_2 arrays promising candidates to enhance the biological performance of titanium bone substitutes, such as dental and orthopedic implants [3]. The growing demand for a higher quality of life after joint and tooth replacement has become an essential requirement from the patients side, whereas the survival of those bone implants is compromised by the increasing incidence of implant-associated infections, recently [4-5]. The spread of antibiotic resistance among the biofilm forming bacteria exacerbates the problem that may reduce the success rate of implants and implant revisions or even result in life-threatening complications [6,7,8]. Therefore, the development of alternative antibacterial strategies that do not contribute to the spreading of antibiotic resistance, but rather prevent the occurrence of implant-associated infections has become an urgent issue not just for implant manufacturers but also for the world's health care system [9].

It has been suggested that the anodic growth of nanotubular TiO_2 arrays may offer a cost-effective and reliable method for the surface treatment of titanium implants so as to enhance their resistance against infections [10-11]. This idea is driven by the fact that through the precise control of the electrochemical process parameters homogenous nanotubular TiO_2 arrays can be grown on titanium substrates, for instance on titanium foils [12]. The anodic growth of nanotubular TiO_2 arrays can be a reliable method, provided that the process parameters are set in a suitable range that allows the production of uniform surfaces, which can be easily investigated in *in vitro* experimental settings, e.g. in biocompatibility and microbiology studies [13]. The response of various mammalian and bacterial cells was investigated on nanotubular TiO_2 arrays and correlations have been demonstrated between the survival rate of the cells and the physical, chemical and physicochemical properties of the nanotubes [14-15]. These findings demonstrated the superiority of the anodic nanotubular TiO_2 arrays in terms of osteogenic differentiation rate of mesenchymal stem cells and antibacterial property over micro-rough implant surfaces that were created by etching, sandblasting or by the combinations thereof.

On the other hand, little is known about the mechanical resistance and the reproducibility of the anodic nanotubular TiO_2 arrays on the surface of bulk titanium substrates in comparison to the conventional surface treatment methods, e.g. etching, sandblasting [16-17] or spark anodization [18]. Furthermore, there is limited information in the literature concerning the mechanical performance of the anodic nanotubular TiO_2 arrays on the surface of 3-dimensional implant geometries. As yet, the comparison of the mechanical integrity of the anodic nanotubular TiO_2 arrays from practical point of view with other types of anodic films that exhibit different nanosurfaces has also been a lacking chapter in the art.

The objective of our study was to investigate the characteristics and reproducibility of the anodic growth of homogenous, self-ordered, vertically oriented nanotubular TiO_2 arrays on the surface of bulk titanium substrates, such as discs and dental implants in comparison to a nano-pitted anodic films, which were obtained in a novel two-stage anodizing process. We also investigated the durability of anodic films under mechanical stress that may emerge when a dental implant is driven into the jawbone.

2. MATERIALS AND METHODS

2.1 Surface treatment process

Various electrochemical treatment parameters were applied to Grade 2 titanium (Createch, France) discs (h = 2 mm, ∅14 mm) and screw dental implants (Sanatmetal, Hungary) so as to create homogenous anodic films that exhibit either nanotubular (NT) or nano-pitted (NP) TiO_2 features on the surface. Table 1 gives an overview on the sequence of the surface treatment steps and the range of parameters that were systematically optimized. The discs were subjected to a three-sequence surface treatment process. In the first step, the machining marks were removed by electrochemical polishing. In the second step, the polished discs were subjected to acid etching in order to initiate the formation of hydroxide islands on the surface to catalyze nanopore formation [19]. In the third step, nanotubular and nano-pitted anodic films were grown on the surface of the discs. In the following the final parameter and experimental set-ups are further detailed that had been applied for the production of the test samples.

Table 1 shows the sequence of the surface treatment process and the range of tested parameters

Electrochemical polishing	
Anode-cathode configuration	Anode-cathode distance: 5 mm Cathode geometry: planar, cylindrical mesh
Material of the cathode	316L stainless steel
Electrolytes	Struers AII solution, and NANOTI EP Electrolyte
Voltage	10V – 80V
Temperature	− 40°C – (+ 40°C)
Agitation	Stirring: 100 – 1000 rpm, or Laminar flow: 0.1 – 0.5 l/min
Time	10 sec – 1200 sec
Etching	
Etchants	HCl, H_3PO_4, $(COOH)_2 \times 2H_2O + H_2O_2$, $HF + H_3PO_4 + dH_2O$
Temperature	20°C – 60°C
Agitation	Ultrasonic
Time	30 sec – 1200 sec
Anodization	
Anode-cathode configuration	Anode-cathode distance: 3 – 65 mm Cathode geometry: planar, cylindrical mesh

Material of the cathode	316L stainless steel,
Electrolyte	HF, HCl, H_3PO_4, $NH_4F + H_2O + C_2H_4(OH)_2$, $NH_4F + H_2O + C_3H_8O_2$ NANOTI AN I & NANOTI AN II Electrolytes
Voltage	20V – 100V
Temperature	− 40°C – (+ 50°C)
Agitation	Stirring 100 – 1000 rpm, laminar flow 0.1 – 0.5 l/min, no agitation
Time	200 sec – 3600 sec

2.2 Electrochemical polishing

The electrochemical polishing was carried out in a two-electrode setup (anode-cathode distance was 5 mm) by using a DC power source (Elektro-Automatik, EA-PS8080-40) in a steady electrolyte flow with 0.1l/min velocity using a thermoplastic mag drive centrifugal pump (HTM6 PP, GemmeCotti), while the temperature of the electrolyte was kept at 15 °C. As electropolishing compound NANOTI EP Electrolyte (NANOTI Ltd, UK) was used (Table 2).

2.3 Chemical etching

The chemical etching of the electropolished workpieces was carried out in the compound of 0.1 wt% HF, 1 wt% H_3PO_4 and distilled water (Molar Chemicals, Hungary) in an ultrasonic bath for 3 min at room temperature. After etching the workpieces were rinsed in distilled water for 4 min in an ultrasonic bath in order to remove residual acid from the surface. After rinsing the workpieces were further cleaned in absolute acetone and absolute ethanol for 5 - 5 minutes in ultrasonic cleaner (Table 2).

2.4 Anodization

The anodization of the electrochemically polished and etched workpieces was carried out in a two-electrode electrochemical reactor using a continuous direct power supply (Elektro-Automatik, EA-PS 8360-15 2U). The anodizing parameters of NT and NP samples are given in Table 2 and Table 3 respectively. Various anodizing parameters have been tested to grow homogenous nanotubular arrays on the surface of titanium discs in order to investigate the influence of process parameters on the mechanical stability of the NT anodic films. The final treatment parameters and experimental groups are given in Table 3.

Table 2 shows the parameter sets that were suitable to create homogenous NT anodic films

		NT-1	NT-2	NT-3	NT-4
Electrochemical polishing	Voltage	30 V			
	Electrode	316L stainless steel			
	Duration	35 sec			
	Electrolyte	NANOTI EP Electrolyte			
	Cleaning	Ultrasonic cleaner: 5 min in absolute acetone and 5 min in absolute ethanol			
Chemical etching	Etchant	0.1 wt% HF + 1 wt% H_3PO4 + (distilled) H_2O			
	Duration	3 min (in an ultrasonic bath)			
	Cleaning	4 min (in an ultrasonic cleaner)			
Anodization	Voltage	80 V			100 V
	Electrode	316L stainless steel			
	Duration	600 sec	900 sec	300 sec	300 sec
	Electrolyte	0.6 wt% NH_4F + 3 wt% H_2O + $C_2H_4(OH)_2$			0.3 wt% NH_4F + 3 wt% H_2O + $C_2H_4(OH)_2$
	Heat treatment	400 °C for 2h	–		

Table 3 shows the experimental groups and the range of final treatment parameters

		EP	NT-2	NP	SBAE
Electrochemical polishing	Voltage	30 V			–
	Electrode	316L stainless steel			–
	Duration	35 sec			
	Electrolyte	NANOTI EP Electrolyte			–
	Cleaning	Ultrasonic cleaner: 5 min in absolute acetone and 5 min in absolute ethanol			–
Chemical etching	Etchant	–	0.1 wt% HF + 1 wt% H_3PO_4 + dH_2O		H_2SO_4 + HCl
	Duration	–	3 min (in an ultrasonic bath)		5 min
	Cleaning	–	4 min (in an ultrasonic cleaner)		–
Anodization stage I	Voltage	–	80 V	20 V	–
	Electrode	316L stainless steel			
	Duration	–	900 sec	180 sec	
	Electrolyte	–	0.6 wt% NH_4F + 3 wt% H_2O + $C_2H_4(OH)_2$	NANOTI AN I Electrolyte*	
Anodization stage II	Voltage	–	–	15V	–
	Electrode	316L stainless steel			–
	Duration	–	–	60 sec	
	Electrolyte	–	–	NANOTI AN II Electrolyte	–

**The fluoride content of NANOTI AN I Electrolyte is below 0.3 wt%.*

2.5 Experimental design

One optimal NT forming and one optimal NP forming protocol were selected for further investigations (Table 3). The mechanical characteristics of the NT and the NP anodic films were assessed first on the surface of discs and on dental implants in a later experimental phase. The selected types of the anodic films on the surface of discs were further assessed by X-ray photoelectron spectroscopy. The anodic films were subjected to scratch resistance and corrosion tests on the surface of discs, while they were investigated in screwing tests on the surface of dental implants. The uniformity of anodic films on the surface of 2-dimensional disc and 3-dimensional screws was assessed by contact angle measurement. Concerning discs, the NT and NP surfaces were compared to electrochemically polished (EP) and a sandblasted/acid etched (SBAE) reference surfaces (Table 3). The sandblasting of the discs was performed by KLS Martin GmbH (Freiburg, Germany), while the acid etching was carried out in a subsequent step according to our own protocol (Table 3). Concerning implants, the NT and NP surfaces were compared to each other in screwing tests without external reference surface. In the following the investigation methods are detailed.

2.6 Microscopy

Stereomicroscopic (Olympus SZX16, Pennsylvania, United States) and scanning electron microscopic (Philips XL 30, Zagreb, Croatia) images were taken of the titanium discs and dental screw implants in order to investigate the titanium-oxide films. The diameter of nanotubes (NT) and the area of pits (NP) were determined by image processing.

2.7 Scratch resistance test

The mechanical integrity and failure mode of NP and NT anodic films were investigated on the surface of discs in scratch resistance test. A tensile testing machine (INSTRON 5965 (5 kN) with a high-performance pneumatic wedge grip with 2 kN lateral force capacity) was used to perform the tests by making 5 scratches on the surface of 3 titanium discs in each experimental group. A custom-made martensitic stainless steel stylus was produced by the 90° bending of a commercially available tweezers (VetusTweezers). The quantitative set of the normal load was not possible in this setting; however, the achievement of identical normal loads was attempted by the fixation of the disc and stylus in the same position relative to each other in all measurements through the lateral adjustment of the lower and the upper wedge grips of the tensile testing machine. The stylus had a tapered head with 10 μm radius. The displacement rate of the stylus was 100 μm/sec. During the measurements the lateral load-displacement diagram was recorded by BlueHill

3 software (Materials Testing Software, Instron, Norwood, MA, USA). As reference, electrochemically polished discs were prepared as it was described in section 2.3.

2.8 Screwing test

The mechanical integrity and failure mode of NP and NT anodic films were investigated on the surface of 3-3 dental implants in screwing test. Solid rigid polyurethane foam blocks (Sawbones®) were used to simulate trabecular and cortical bone density types. According to the instructions of the implant manufacturer cylindrical holes were drilled in advance into the foam blocks in order to reduce the mechanical stress that occurs during the driving of the screw. Scanning electron microscopic (SEM) and stereomicroscopic images of the implant surfaces were taken before and after the screwing test.

2.9 Surface roughness measurement

Optical profilometry (ALICONA Infinite Focus) was used to determine the surface roughness of NP, NT and electrochemically polished surfaces on discs. The measurements were performed on surface areas where obvious surface flaws did not appear (n = 3). The measurements were performed alongside the full diameter of the discs.

2.10 Contact angle measurement

Distilled water and diiodo-methane were used as test fluids for contact angle measurements on the surface of titanium discs and 3-dimensional screw dental implants using a drop shape analyzer (Krűss, DSA25). The measurement was performed on surfaces that had been produced 3 months before the experiment and were stored in non-sealing containers under ambient atmosphere. The surfaces were not subjected to UV irradiation or any other manipulation before contact angle measurement in order to allow the investigation the real performance of the surfaces as they were on off-the-shelf products. The measurement started after 3 sec of dropping, the drop volume was $0.3 \pm 0.05 \mu l$. Conic section method was used to determine the contact angles of the drops, while surface free energy, polar part and disperse part were calculated (ADVANCED Software, Krűss). Concerning discs sandblasted/etched, turned, electrochemically polished, NP and NT surfaces were investigated. The drops were placed both on the external area and the central (middle) regions of the discs and 3 consecutive measurements were performed in each region. Ten (n = 10) samples were investigated within each experimental subgroup (surface type) for each test fluid. Concerning screw dental implants electrochemically polished, NT and NP surfaces were investigated. Six (n = 6) screws were subjected to contact angle measurement within each experimental subgroup (surface type) for both test fluids, while 3 consecutive measurements were performed in the neck and middle (threaded) regions of the implants on identical surface areas.

2.11 X-ray photoelectron spectroscopy

Surface chemical analysis of the surface types was performed on discs by X-ray photoelectron spectroscopy (XPS) with an Amicus spectrometer (Kratos Analytical, UK) using non-monochromatic Al-Ka X-ray source operated at 240 W with 8 kV. High-resolution spectra were acquired at 75 eV pass energy at an electron take-off angle of 0° between surface normal and the electron-optical axis of the analyzer. Peak positions on the binding energy scale were corrected with respect to the Ti 2p3/2 peak for TiO_2 (458.8 eV). The spectra were fitted with the CasaXPS software (version 2.2.67). Quantitative elemental compositions were calculated from peak areas using experimentally determined sensitivity factors tabulated in CasaXPS and the spectrometer transmission function.

2.12 Corrosion test

The corrosion test of the NT-2 and NP anodic films on the surface of discs was performed in 10 parallel measurements by the static immersion method in accord with ISO 10271:2011 “Corrosion test methods for metallic materials”. Briefly, in the first step, the surface of the discs was cleaned ultrasonically in ethanol for 5 minutes then dried with compressed air. Concerning both experimental groups 10 individual specimens were investigated in 10 separate containers that were filled with the aqueous solution of 0.1 mol/l sodium chloride and 0.1 mol/l lactic acid, resulting in a pH value of 2.29. The containers were sealed and kept at 37°C for 7 days. The pH of the residual and reference solutions was recorded after the 7 days incubation period by a pH meter (Voltcraft PHT-02 ATC). The calculated disc area was 3.96 cm^2, which was related to the electrochemically polished surface and did not implicate the surface augmentation owing to nano-, and micropores. As reference, sandblasted/etched (SBAE) and electrochemically polished (EP) discs were used.

2.13 Element analysis

Inductively coupled plasma optical emission spectrometer (ICP-OES, Plasmalab) with 40-channel analyzer (Labtest) was applied to measure the concentration of the dissolved titanium ions in the aqueous chloride containing medium that was used for corrosion testing. After the 7 days incubation period samples were taken from the corrosion test liquid and the liquid was subjected to analysis. Three (3) liquid samples were taken from each container for spectrophotometric analysis in three consecutive measurements. The means of the measured concentrations were calculated along with standard deviations. As reference, the titanium ion content of a blank solution was determined in three measurements and the mean ($2.4 \times 10^{-3} \pm 3.4 \times 10^{-4}$ mg/l) was calculated, which was deducted from the means of the test solutions.

2.14 Statistical analysis

One-way ANOVA analysis was performed (Tukey's post hoc test) in order to investigate the difference in the concentrations of dissolved Ti and alloying ions that were measured in element analysis concerning the NT and NP anodic films and the reference surfaces. A p value < 0.05 was considered significant.

3. RESULTS

Homogenous nanotubular arrays appeared on the surface of discs that were anodized according to NT-1, NT-2 and NT-3 parameters, whereas the nanotubes slightly deformed when NT-4 parameters were applied (Figure 1).

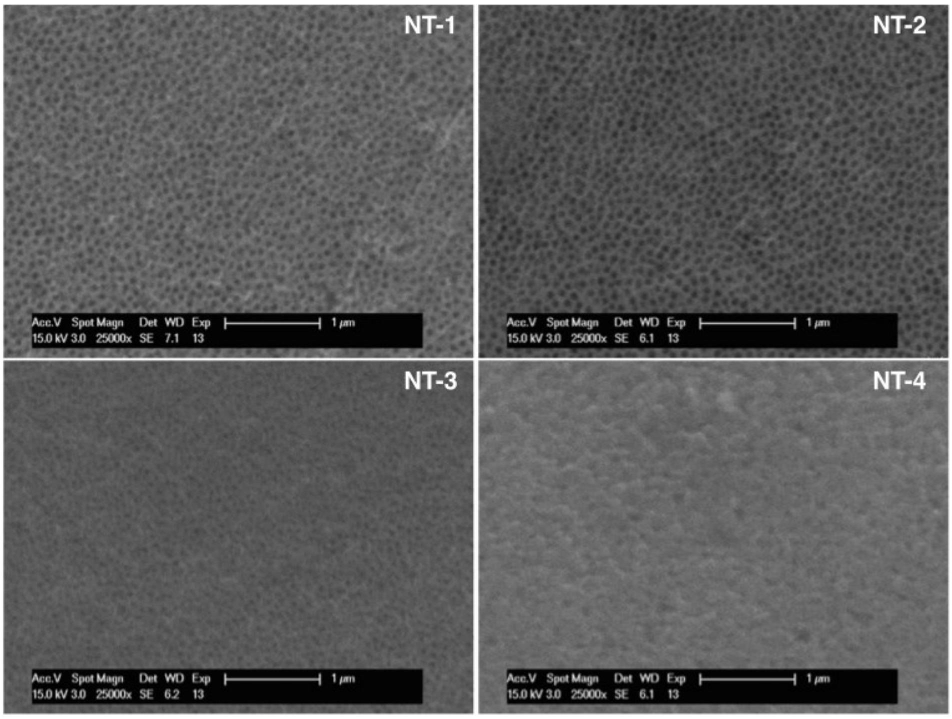

Figure 1 Scanning electron microscopic images of titanium discs that were subjected to anodization according to NT-1, NT-2, NT-3 and NT-4 process parameters. The nanotubular arrays clearly appeared when NT-1, NT-2 and NT-3 parameters were applied, whereas the orifice of the tubes might have been closed and exhibited grain-like features in case of NT-4.

The reproducibility of the various NT anodic films showed considerable variance that often detrimentally affected of the surface quality, which was manifested either in spontaneous peeling or in the emergence of crevices (Figure 2).

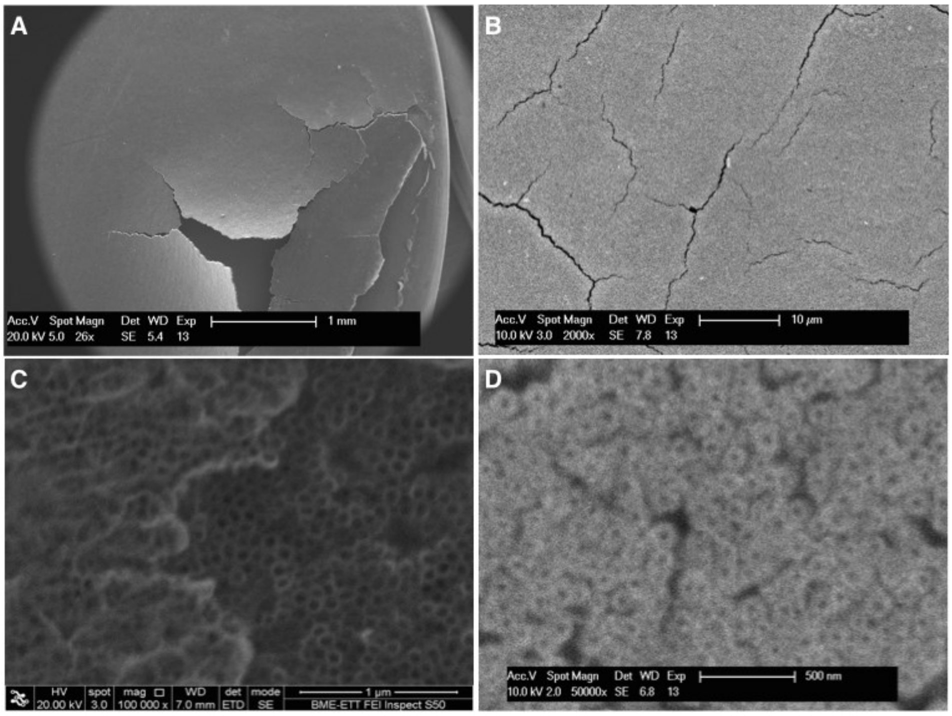

Figure 2 Typical surface flaws of nanotubular anodic films on titanium discs. Panel A shows the macroscopic image of the spontaneous peeling of a nanotubular anodic film. Panel B shows crevices in a nanotubular anodic film, on which spontaneous peeling was eliminated by the reduced concentration of the etchant in the preceding step. Panel C shows the inhomogeneity of the nanotubular film that is caused by long etching time (5 min). Panel D shows nano-scale cracks between the tubes after heat treatment. Heat treatment was effective to eliminate the spontaneous peeling and micro-scale cracks of the nanotubular anodic film (NT-1) but nano-scale homogeneity was never achieved.

The growth of nanotubular anodic film according to NT-2 process parameters showed the highest reliability compared to other NT anodizing parameters, thus the NT-2 surface was selected for further investigations as a representative nanotubular anodic film (Table 2). On the other hand, the growth of NP anodic film through two-stage anodization showed high reliability that allowed reproducing essentially similar NP surfaces (Figure 3).

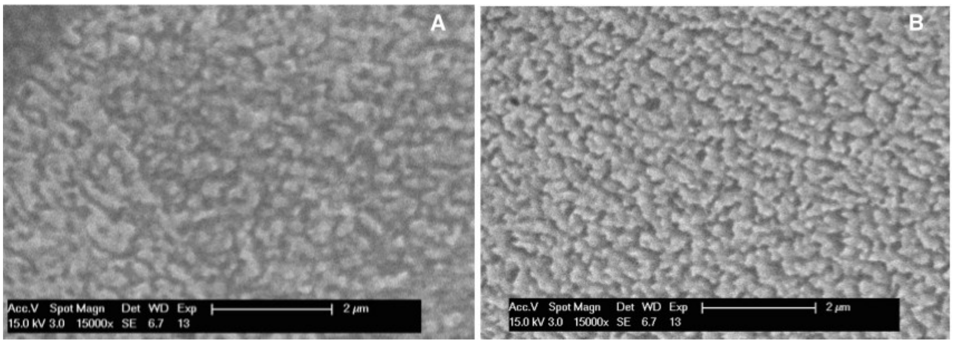

Figure 3 Representative electron scanning microscopic images of nano-pitted anodic films that were created by two-stage anodizing on panel A and B.

The number of pits (NP) was 93 ± 19 in a 2 x 2 µm area on the surface of the discs. The pit area was 3.16 ± 0.52 μm^2. The number of nanotubes (NT-2) was 251 ± 24 in a 2 x 2 µm area. The diameter of the nanotubes was 79 ± 22 nm. The surface roughness of the NP anodic films was (Ra) 0.74 ± 0.1µm and (Rz) 3.92 ± 0.58 µm, for NT anodic films (Ra) 0.75 ± 0.11µm and (Rz) 3.58 ± 0.29µm, while for electrochemically polished surface (Ra) 0.15 ± 0.05µm and (Rz) 0.7 ± 0.23µm.

The XPS analysis showed various dopants on the four titanium-oxide surface types (Figure 4). Carbon and nitrogen always appeared in characteristic concentrations on each surface. Fluorine was detectable only on anodic surfaces but it appeared in a magnitude larger concentration on the surface of NT-2 (4.7%) than on NP (0.6%). The NP surface had the largest number of dopants, however the concentration of those was considerably lower compared to the other three surfaces, especially SBAE and NT-2.

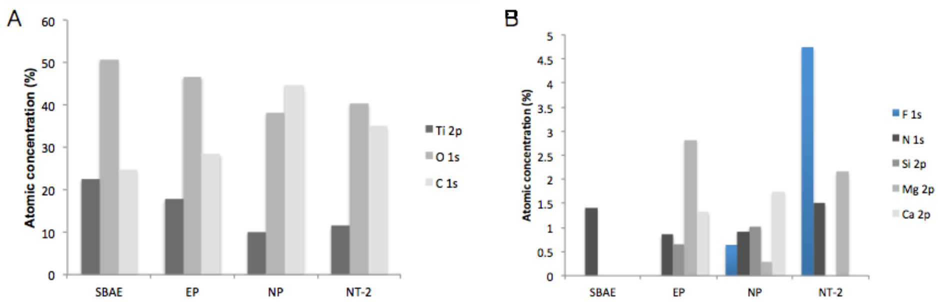

Figure 4 Chemical composition of the four surface types. Panel A shows the main proportion of the elements, while panel B shows the dopants of the surface. The blue bars indicate the fluorine content on the NP and NT-2 anodic films. The origin of carbon and nitrogen are organic pollutants and nitrous gases of the ambient air that bind to the titanium-

oxide surface in complex redox-reactions [20].The origin of other dopants, such as magnesium, calcium and silicon on the surface of EP, NT-2 and NP is unknown.

The NT-2 anodic film showed the highest wettability and surface free energy on the discs (Table 4). In contrast, NP anodic film showed the closest contact angle values and surface free energy to EP surface but they were remarkably different compared to NT-2 and SBAE surfaces. On the other hand, the magnitude of difference in terms of contact angle and surface energy values decreased on implants compared to measured values on discs, in general. It can be discerned that the NT-2 anodic film showed remarkably higher wettability and surface free energy than NP surface. However, the measured values showed considerably lower standard deviation in case of NP anodic film compared to NT-2. Interestingly, the sandblasted/etched surface showed explicit hydrophobic property.

Table 4 shows the results of contact angle measurements

	NP	NT-2	SBAE	EP
Contact angle of water on discs (°)	77.1 ± 3.5	41.8 ± 15.5	118.1 ± 16.1	65.9 ± 4.6
Contact angle of water on implant (°)	75.9 ± 7.1	67.2 ± 18.2	–	83.7 ± 12.3
Contact angle of diiodo-methane on disc (°)	57.1 ± 2.0	38.5 ± 16.2	87.1 ± 10.2	52.7 ± 3.4
Contact angle of diiodo-methane on implants (°)	66.5 ± 5.3	46.3 ± 21.1	–	62.4 ± 4.5
Surface free energy on discs (mN/m)	37.2 ± 2.8	62.8 ± 17.5	14.1 ± 5.4	44.6 ± 4.6
Surface free energy on implants (mN/m)	34.3 ± 7.0	46.1 ± 21.5	–	32.2 ± 7.5

Considerable difference was revealed in the mechanical durability of NT-2 and NP anodic films in the screwing test. The NT-2 arrays lost their homogeneity and often exfoliated from the surface of the screw dental implants when they were introduced either into trabecular or cortical bone density foam blocks. In contrast, the NP anodic film remained intact on the surface of the dental implants even after screwing in cortical bone density foam block (Figure 5).

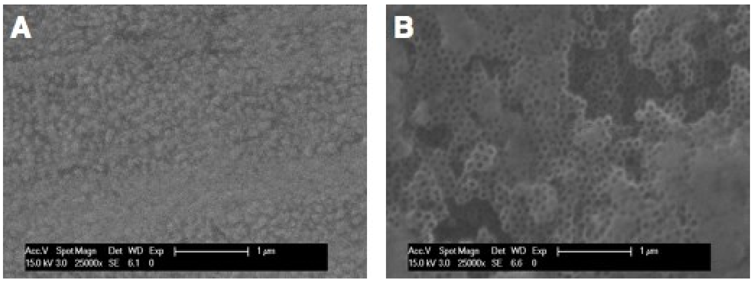

Figure 5 Representative scanning electron microscopic images of the surface of NT-2 and NP after driving in and out from cortical bone density type polyurethane foam blocks. Panel A shows that the NP surface remained intact, whereas Panel B shows that vertical irregularities appeared on NT-2 surface after screwing stress.

The scratch resistance tests supported the tendency of NT-2 anodic film to flake off when ~ 2 N lateral force was applied to the surfaces of the discs. Contrarily, the NP anodic film did not exfoliate when the same lateral force was applied to them but moderate sideward and terminal material extrusion appeared on the track of the stylus (Figure 5). The load-displacement diagrams did not show considerable difference between the experimental groups (data not shown).

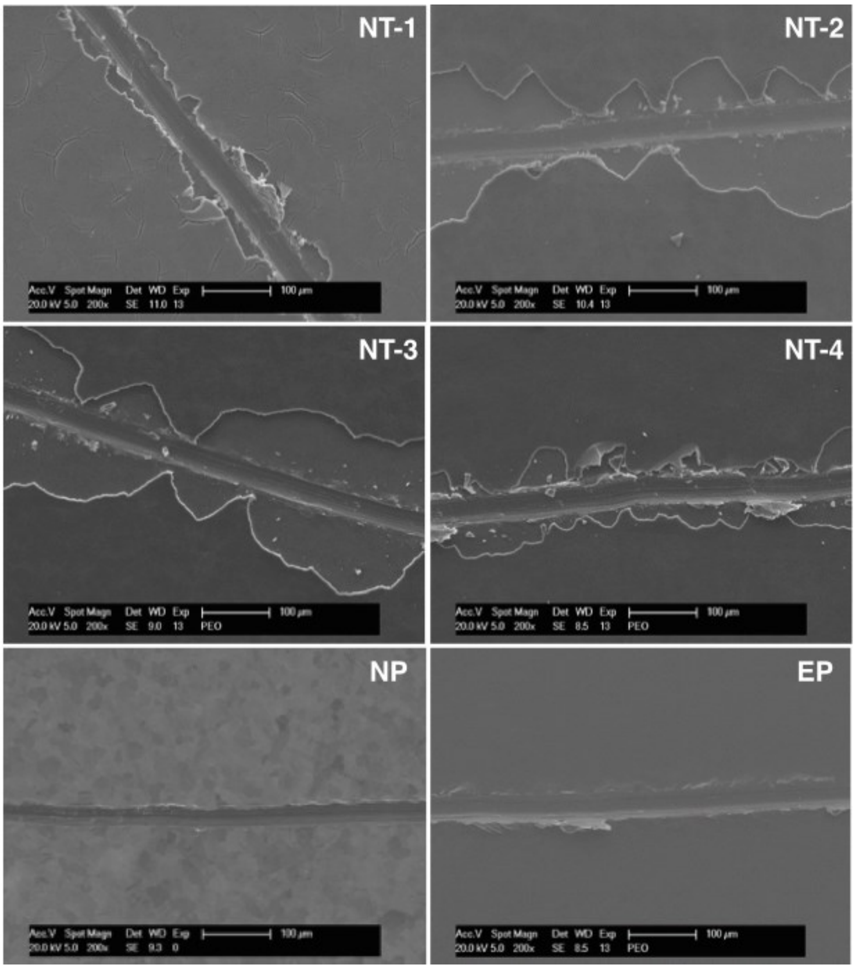

Figure 6 Representative scanning electron microscopic images of the surface of NT, NP and EP titanium discs after scratch resistance test. The NT anodic films exfoliated when lateral force was applied to their surfaces, whereas the NP anodic film and EP surface did not show the same tendency but sideward and terminal material extrusion appeared on the track of the stylus.

The corrosion resistance of NT-2 anodic films was significantly lower (concentration of dissolved Ti ions: 23.89 ± 6.7 mg/ml; $p^* < 0.001$) than that of NP anodic films (concentration of dissolved Ti ions: 1.11 ± 0.77 mg/ml) and reference surfaces. After corrosion test slight recesses appeared on the surface of NT-2 that disintegrated the homogeneity of the nanotubular arrays (Figure 6).

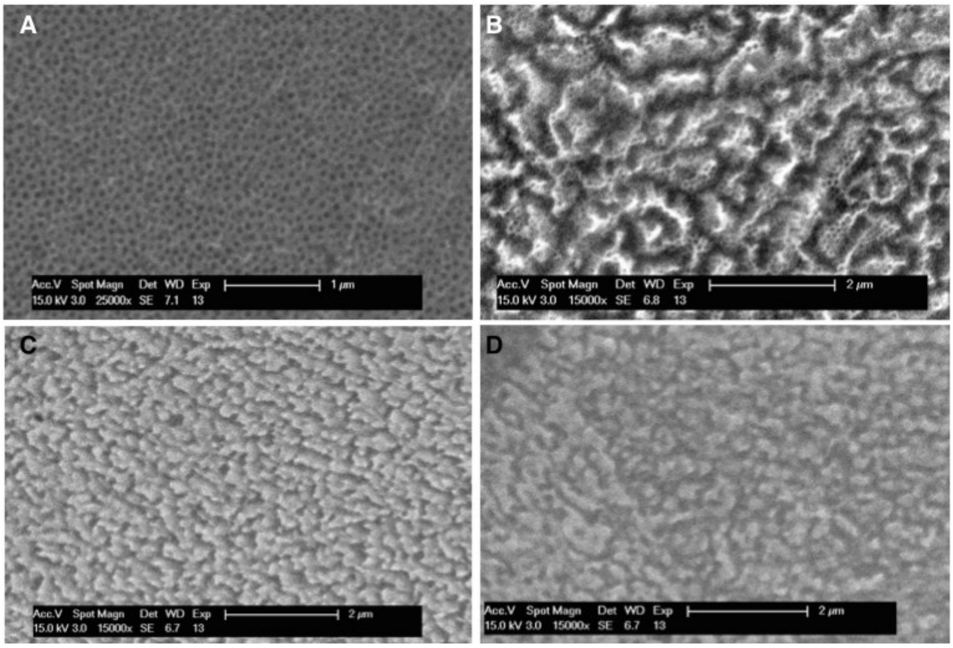

Figure 7 Representative scanning electron microscopic images of NT-2 and NP anodic films before (A and C) and after corrosion test (B and D), respectively. Panel A shows homogenous nanotubular film before corrosion test, while panel B shows valley-like structures that appeared on the surface of NT-2 anodic film and disintegrated the vertical homogeneity of the nanotubular array. Panel C shows the NP anodic film before corrosion test, while panel D shows that the corrosive environment did not affect the surface features of the NP anodic film substantially.

On the other hand, the corrosion behavior of NP anodic films was essentially similar to that of sandblasted/acid etched TiO_2 surfaces that exhibit micro-rough features. Interestingly, there was not credible difference between the concentrations of dissolved titanium ions concerning the electrochemically polished (concentration of dissolved Ti ions: 0.343 ± 0.009 mg/ml) and sandblasted/acid etched surfaces (concentration of dissolved Ti ions: 0.589 ± 0.463 mg/ml), and the NP anodic films (Figure 7).

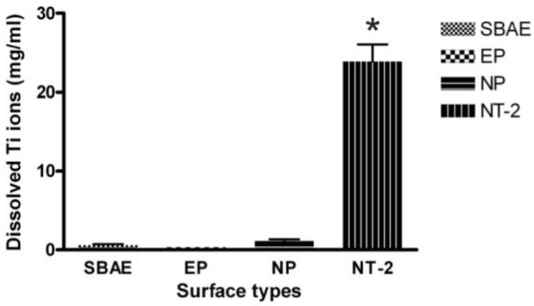

Figure 8 Concentration of Ti ions that were measured in element analysis (n = 10). Significantly higher amount of Ti ion dissolved from the surface of NT-2 anodic films ($p^* < 0.001$) than from NP, EP and SBAE. However, there was not statistical difference between the dissolved Ti ion concentrations concerning the NP, EP and SBAE surfaces.

4. DISCUSSION

The aim of our study was to investigate the mechanical and chemical characteristics of NT and NP anodic films on the surface of titanium discs and dental implants. Our results show that the growth of homogenous, self-ordered, vertically oriented nanotubular TiO_2 anodic films on the surface of bulk titanium substrates is particularly sensitive to the anodizing process parameters that detrimentally affects their reproducibility. The low reproducibility of the nanotubular anodic films was manifested in their weak mechanical durability that resulted either in spontaneous peeling or in exfoliation when moderate lateral force was applied to the surfaces. Contrarily, the NP anodic film was highly reproducible in a two-stage anodizing process. Both in its mechanical integrity and corrosion resistance the NP anodic film outperformed the NT-2 film. The surface roughness values were similar concerning NP and NT anodic films, whereas considerable differences were revealed in the contact angle values of the NT and NP anodic films and the latter showed low standard deviation. These findings suggest that the applicability of nanotubular anodic films on the surface of titanium bone substitutes may be limited because of mechanical considerations. On the other hand, it is worth to consider the applicability of nano-pitted anodic films over nanotubular surfaces for the purpose of the enhancement of bone substitutes, such as dental implants.

The low standard deviation of the contact angle values suggests that homogenous NP anodic film can be grown in the neck region and on the threaded part of a dental implant. Albeit, the NT-2 anodic film showed the better wettability but the relatively high standard deviation suggest the lower homogeneity of such surface on 3-dimensional screw implants compared to NP anodic films. This might be caused by the emergence of surface flaws (Figure 2) that could detrimentally affect the physicochemical and mechanical properties of NT-2 anodic film.

The mechanical integrity of the surface of a titanium bone substitute is essential so as to achieve its intended biological performance [21 -]. However, it is very difficult to grow a nanotubular anodic film that shows homogenous microscopic appearance and mechanical integrity even through the systematic optimization of the anodizing parameters [22]. Among the various anodizing parameters the temperature was found to be the most critical in our setup that affected the practical adhesion of the nanotubular anodic films to the bulk titanium substrates. Interestingly, the local temperature had to be kept between 5-8°C in the close proximity of the anodic workpiece when NT-2 surfaces were grown, otherwise spontaneous peeling of the anodic films occurred. However, in spite of the deliberate control of the process parameters of anodizing the NT-2 anodic film easily exfoliated even when moderate forces were applied to the surface. In this study we did not investigate the underlying reason of the low mechanical resistance of nanotubular anodic films but it has been reported by other authors that the detachment of such anodic films may be associated with the formation of a titanium fluoride layer between the oxide film and the metal substrate. According to Habasaki and his co-workers the development of the titanium fluoride layer might be the result of the fast inward migration of fluoride ions during anodic film growth under high electric field [23]. Our findings might be supported by the hypothesis of Habasaki, if we consider the anodizing parameters under the NP and NT-2 films were grown. In the first sequence of the anodic growth of NP films considerably lower fluoride ion concentration and field force was applied for shorter time than in the case of the NT-2 films, which was supported by the different concentrations of fluorine dopant on those surfaces (Figure 4). The lower exposure to fluoride ions may have reduced the rate of the development of the titanium fluoride layer between the NP anodic film and the titanium substrate, which may have prevented the detachment of the NP films. However, this assumption needs to be confirmed by further investigations in the future.

On the other hand, the development of a titanium-fluoride layer between the anodic film and the titanium substrate may not explain alone the significant differences in the corrosion behavior of the NP and NT-2 films. Presumably, the difference in the electric properties of the NP and NT-2 anodic films is responsible for the different corrosion resistance, however this hypothesis should be confirmed in further experiments in the future. The significant Ti ion dissolution and the microscopic appearance of NT-2 anodic films after the corrosion test suggested intense initial corrosion. The tendency for intense corrosion could be a risk factor of corrosion fatigue as well as attraction of pro-inflammatory cells [24] or of prolonged inflammatory reactions induced by production of reactive oxygen species [25] caused by the released titanium ions. Further, the exfoliation of the anodic film would interfere with the biological performance due to particle-based activation of immune cells such as neutrophils [26]. This supports the findings of other authors who demonstrated that nanophase topographies might significantly influence the biological property of the various titanium-oxide films, such as the response of bacteria [27].

The high sensitivity of the surface quality of the nanotubular anodic films to the variance of the anodizing parameters may extremely increase the complexity of the process validation, which may cause undue costs for an implant manufacturer. The validation of critical processes in the production line is a general requirement for the European and American implant manufacturers and the enforcement of this requirement has become more imperative, recently [28]. However, our findings suggest that the reproducibility and the mechanical integrity of the nanotubular anodic films on 3-dimensional implant geometries is low, if the conventional anodizing parameters are applied, e.g. continuous direct current is applied in aqueous fluoride containing media for minutes or hours. On the other hand, our results do not imply any manner the non-existence of a parameter set that is suitable to create nanotubular anodic film on the surface of bulk titanium substrates.

Nevertheless, the improvement of anodizing processes may lead to new nanosurfaces with physical characteristics that are more suitable to enhance the biological performance of titanium bone substitutes than the currently known nanotubular anodic films. Our novel two-stage anodizing process supports this hypothesis, given that it is a robust method that yields reproducible nano-pitted anodic films with good mechanical integrity and corrosion resistance.

5. CONCLUSION

Our findings suggest that the applicability of nanotubular anodic films on the surface of titanium bone implants may be limited because of mechanical considerations. In contrast, it is worth to consider the applicability of nano-pitted anodic film over nanotubular arrays for the enhancement of the biological properties of titanium implants.

6. ACKNOWLEDGEMENT

The present work was funded by the REA in the 7th Framework Program within the funding scheme of SME Instrument under the scope of the NANOTI Project (FP7-SME-2013-606624) and Hungarian National Research, Development and Innovation Fund VKSZ_14-1-2015-0151.

7. DISCLOSURE

Miklós Weszl and Krisztián Tóth László are the shareholders of NANOTI Limited. Nihal Engin Vrana is a full time employee of Protip Medical and holds stock options of Protip Medical. There are pending patent applications that cover the subject matter of the anodic surface treatment of titanium implants (PCT/IB2016/050464) and (P1600046).

8. VITAE

Miklós Weszl is the co-founder of NANOTI Limited and a PhD candidate in the field of regenerative medicine. He obtained his Pharm.D. degree at Semmelweis Univesity in 2008, where he is an active researcher in the Department of Biophysics and Radiation Biology. His major research interests are bone substitutes, surface chemistry and biology, nuclear medicine (imaging) and clinical development. Miklós has published 7 papers in peer-reviewed journals (citations: 50, h index: 5), the inventor of 1 granted patent, and 1 other applications is in PCT pahse.

Krisztián László Tóth has MSc in civil engineering, BA in economics, and the co-founder of NANOTI LIMITED. His research interest includes statistical science, mathematical modeling and data driven decision-making. Krisztián is working on his PhD and he has 1 publication in a peer-reviewed journal in the field of biomaterial sciences.

Imre Kientzl has an MSc in mechanical engineering, a degree in welding and a PhD in materials science. He has +15 years experience in metal working, metallic alloys and composites. His specialty is laser cutting, welding and materials science. He has published 3 articles in peer-reviewed academic journals and holds 1 European patent.

Peter Nagy has an MSc in mechanical engineering, a degree in welding and a PhD in materials science. He has +10 years experience metal working and metallic alloys. His specialty is precision metal working and welding. He has published 6 articles in peer-reviewed academic journals (over 10 citations) and holds 1 European patent.

David Pammer is an assistant lecturer at the Budapest University of Technology and Economics, Department of Materials Science and Engineering. David is a PhD student, his researches are focusing on the measurement of the primary and secondary stability of dental implants, and developing new dental implant geometries. His further research interest is metal additive manufacturing (3D printing) in medical and other industry areas.

Liza Pelyhe received her M.S. degree in Biology, specialization Molecular Biology from Eötvös Loránd University Faculty of Science, Budapest, Hungary in 2008 and in Biomedical Engineering from Budapest University of Technology and Economics, Hungary, Faculty of Electrical Engineering and Informatics in 2011. Her Ph.D. obtained with the same university, Faculty of Mechanical Engineering, Department of Materials Science and Engineering is focused on the measurement methods for the determination of visibility and material properties of angioplasty devices. Her research interests include medical devices and their material properties.

Nihal Engin Vrana is Vice President (Scientific affairs) of Protip Medical. He obtained his PhD in 2009 at Dublin City University as a EU FP 7 Marie Curie fellow. His major research interests are titanium implants, tissue engineering, cell encapsulation immunomodulation, real-time monitoring of implants and cell biomaterials interactions. He has published 43 articles in peer-reviewed academic journals (over 800 citations, h index: 17) 5 book chapters and holds 3 European patents (1 more in progress).

Cornelia Wolf-Brandstetter is senior scientist at Max Bergmann Centre of Biomaterials (Technische Universität Dresden). She obtained her PhD 2004 at the Technische Universität Dresden studying protein adsorption onto titanium oxide surfaces. She continued to work at this institution in the field of pro-osteogenic as well as pro-angiogenic functionalization of titanium implant surfaces including characterization with physicochemical and in vitro cell culture methods. Her further research interest is the antibacterial surface modification and respective analysis. She has published 18 articles (as C. Wolf-Brandstetter and C. Wolf) in peer-reviewed journals (over 700 citations, h: index 11), 5 book chapters and holds 1 European patent.

Prof. Scharnweber is head of the group „Biomaterial-Development„ at the Max Bergmann Center of Biomaterials at TU Dresden. His major scientific interests are on (i) properties of oxide layers on titanium-based materials, (ii) techniques for surface-engineering of biomaterials, (iii) matrix-engineering and (iv) the design of defined biochemical and physical cellular microenvironments. Besides being editor of a book and author of numerous book chapters he has published more than 150 publications (WEB of Science) resulting in a h-factor of 37. He is applicant of more than 20 patents and patent applications.

Dr. Joób-Fancsaly Árpád is an associate Professor at the Department of Oro-Maxillofacial Surgery (Semmelweis University, Budapest). He obtained his PhD 2004 at Semmelweis University studying surface characteristics of dental implants. He takes seminars and lectures in oral surgery and implantology for graduate and postgraduate students, dentists. He is the President of Hungarian Society of Implant Dentistry. He has published 33 articles in peer reviewed journals (over 141 citations,, h:index:5) 1 book, and 7 book chapters.

Eszter Bognár has MSc degrees from mechanical engineering, biomedical engineering, economics and PhD in materials science. She has +15 years experience in the field of medical devices. Her specialty is material science, medical materials. She has published 12 articles in peer-reviewed academic journals (over 50 citations, h index: 3) and holds 1 European patent.

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