Formation and characterisation of nanoporous TiO_2 layers on microroughened titanium surfaces by electrochemical anodisation

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Nanoporous titanium dioxide (TiO_2) layers were successfully formed by an electrochemical anodisation method on microroughened titanium (Ti) surfaces in fluoride containing aqueous electrolyte. Microroughened Ti surfaces were produced by sandblasting with Al_2O_3 particles of 50 μm in diameter and acid-etching in a blend of HCl/H_2SO_4 solution. The surface morphology, topography and chemical composition of the specimens were analysed by scanning electron microscopy, atomic force microscopy and X-ray photoelectron spectroscopy. The surface roughness and the wettability of treated Ti surfaces were measured using profilometry and a contact angle measurement system, respectively. With anodising of sandblasted-/acid etched surfaces, micrometre- and nanometre-scale textures on titanium specimens were created. Results showed that these developed nanoporous-microroughened surfaces exhibited lower contact angle values than the other treated Ti surfaces. The sandblasted/acid-etched/anodised Ti specimen had a surface morphology with distinctively formed hills and valleys and higher surface roughness than the other anodised specimens. This study indicated that nanoporous TiO_2 structures fabricated on microroughened Ti can be an effective way to modify the titanium surfaces for the future development of implant applications.

1. Introduction: Titanium and its alloys are widely used in dental and orthopedic implants because of their superior mechanical properties, excellent corrosion resistance and biocompatibility [1–3]. The success of dental implants depends on their early osseointegration [4]. Various surface modifications have been carried out to improve their osseointegration and to obtain the most biocompatible implant surface. Some of these surface modification techniques include sandblasting, acid etching, micro-arc oxidation, ion implantation etc. [5–8]. The rate and quality of osseointegration in titanium implants is related to some of their properties such as surface topography, roughness, wettability and chemical composition. These properties affect biological interactions between the implant surface and the bone [9].

For the last 20 years, studies have been conducted on implant surfaces having micrometre-scale morphology. However, recently, researchers have been focusing on implant surface modifications producing nanoscale features that are considered to interact with some proteins more effectively than conventional materials. Therefore, nanostructured surfaces are one of the hottest topics for biomedical applications.

Titanium dioxide (TiO_2) surfaces can be obtained by anodisation of titanium in a suitable electrolyte. The anodic oxidation process changes the characteristic of the oxide layer and renders it more biocompatible [10]. In addition, anodic oxide layers with different thicknesses (ranging from tens of nanometres to tens of micrometres) can be produced at appropriate anodisation conditions [11].

Many studies have shown that the surface roughness of titanium implants significantly influences the rate of osseointegration and biomechanical fixation [12, 13]. Sandblasting results in surface roughness whereas acid etching leads to a microtexture and removal of the surface contaminants [14]. Today, the sandblasting method is widely used by many implant manufacturers to roughen titanium implant surfaces. The surface composition of titanium implants affects the hydrophilicity of the surface. Hydrophilic surfaces seem to favour the interactions with biological fluids, cells and tissues compared to hydrophobic surfaces [15, 16]. These interactions directly determine the cellular processes of adhesion, proliferation and differentiation [17]. In the present study, nanostructured TiO_2 layers were formed on the surfaces of sandblasted and sandblasted/acid etched commercially pure titanium specimens in an aqueous HF acid solution. Moreover, we compared the morphologies, topographies, roughnesses and wettabilities of the differently treated titanium surfaces.

2. Experimental details: Commercially pure titanium of ASTM Grade 2 was used as substrate material. A titanium bar having a diameter of 25 mm was cut into disks with thicknesses of 5 mm, followed by degreasing with acetone and water. All of the disks were mechanically polished with gradually finer SiC papers up to #1200 and then ultrasonically cleansed in ethanol and distilled water. Prior to the experiments, acid activation was performed in a mixture of nitric acid (HNO_3) and hydrofluoric acid (HF) to remove the naturally occurring oxide layer.

Surface treatment details are given in Table 1. Alumina particles (Al_2O_3) with 50 μm particle size were used for sandblasting. The etching process was performed in a mixture of H_2SO_4/HCl acids. Anodisation was carried out at a constant potential of 20 V in a

Table 1 Details of surface treatments

Specimens	Treatments details
sandblasted	the surface was treated with Al_2O_3 sandblasting
acid-etched	the surface was etched with H_2SO_4/HCl
sandblasted/acid-etched	the surface was first sandblasted followed by acid etching
anodised	the surface was anodised in an HF solution
sandblasted/anodised	the surface was first sandblasted followed by anodising
sandblasted/acid-etched/anodised	the surface was sandblasted, acid-etched and anodised, respectively

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1 wt% HF solution using a DC power supply (CRS Power, Istanbul, Turkey) at room temperature. The distance between the anode and the cathode was 30 mm.

The morphologies of the specimens were analysed using a scanning electron microscope (JEOL-JSM 6060 SEM). An atomic force microscope (Nanosurf Easyscan 2 AFM) was used to further examine the surface topographies. The surface chemical compositions were analysed using X-ray photoelectron spectroscopy (XPS, Thermo Scientific, UK) with monochromatic Al Kα (1486.6 eV).

Chamber pressure during XPS analyses was kept lower than 10^{-6} Pa. Survey scans were performed using an analyser pass energy of 150 eV and a step size of 1 eV. The atomic concentrations and binding energies were determined using the curve fitting method implemented in the Advantage software program provided by the device manufacturer (Thermo Scientific, UK). The spectrometer was calibrated against the Au 4f peak of pure gold.

Surface roughnesses of the specimens were analysed using a surface roughness tester (Ambios XP-2 Surface Profilometer). Three measurements were performed for each specimen. The measured roughness parameters were Ra (arithmetic average roughness), Rq (root-mean-square) and Rt (maximum height of the profile).

Wettability studies were conducted by using a video-based contact angle measurement system and the results were analysed with the TT software (Tekno-Tip, Turkey). Deionised water was used as the testing liquid. Samples from each group were prepared, and three measurements were performed for each specimen to evaluate the average contact angle.

3. Results and discussion: SEM images of the differently treated titanium surfaces at low and high magnifications are presented in Figs. 1 and 2. The sandblasted surface had complex distributed morphologic features (Fig. 1*a*). A dense, microporous structure lying along different directions was formed via the acid-etching process performed after polishing (Fig. 1*b*). The acid-etching process yielded more uniform micropits (0 and 5–3 µm in diameter) on the sandblasted surface, as seen in the SEM images of these titanium surfaces in Fig. 1*c*.

The formation and growth mechanism of the nanoporous TiO_2 layer is closely related to the anodisation conditions and it can be explained in terms of a competition between electrochemical and chemical reactions [18]. Anodic oxidation of microroughened titanium in a fluoride containing electrolyte led to the formation of a compact layer of TiO_2 with a uniform and regular array of surface structures. In this study, TiO_2 nanopores of about 250 nm long, with 10–20 nm vessel wall thickness and inner diameters ranging from 40 to 70 nm were obtained (Figs. 2*a*–*c*). The sandblasted/anodised titanium specimen's surface exhibited nano/microtextures and had rougher surface features compared to the anodised specimen (Fig. 2*b*). However, the sandblasted/acid etched/anodised specimen surface had shown deeper craters than the sandblasted/anodised surface.

The surface topography of sandblasted/acid etched/anodised surface was characterised by small craters with holes at the centre, resembling a volcano (Fig. 2*c*). It was reported that surfaces modified at nanostructure levels can enhance osteoblast adhesion and biomechanical stability of the implants in bone tissue, improving cell adhesion, tissue biocompatibility responses and greatly enhance ossointegration [19–23]. The micro and nanostructured titanium surface fabricated throughout our study bears a novel surface morphology for future research efforts.

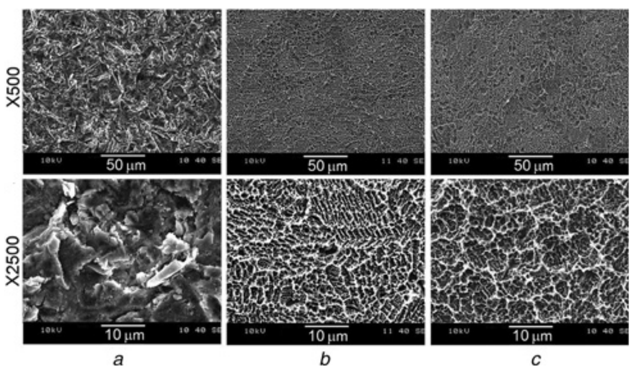

Figure 1 *SEM images after different treatments with high magnification are for observation of nanoscale morphologies*
a Sandblasted surface
b Acid-etched surface
c Sandblasted/acid-etched surface

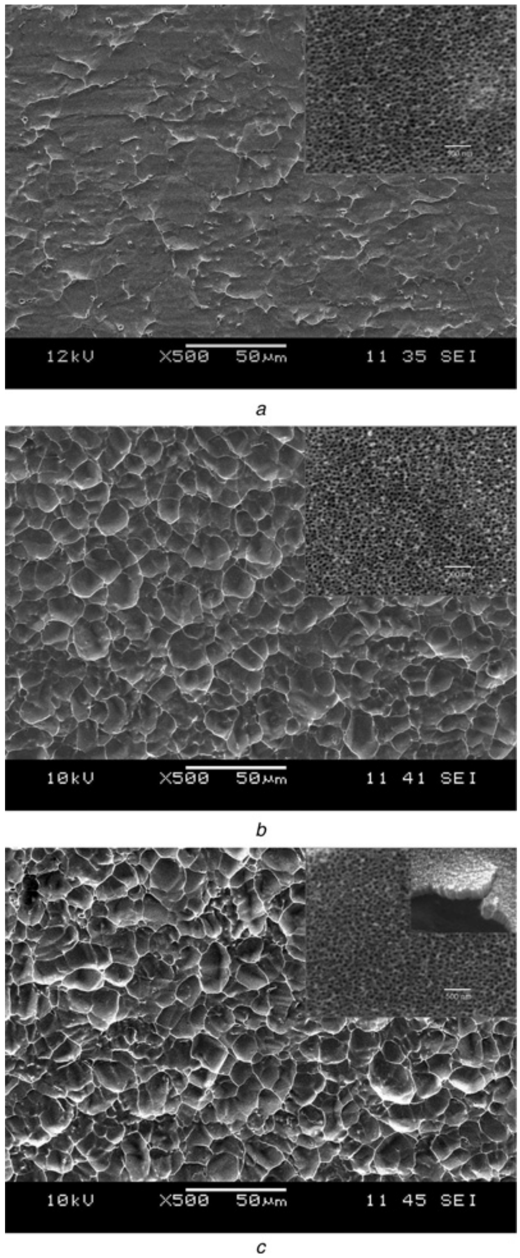

Figure 2 *SEM images after different treatments*
a Anodised surface
b Sandblasted/anodised surface
c Sandblasted/acid-etched/anodised surface
The inset pictures with high magnification are for observation of nanoscale morphologies

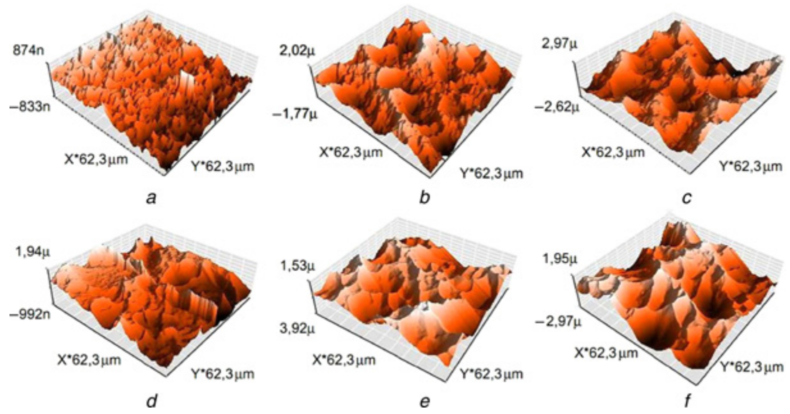

Figure 3 *AFM images after different treatments*
a Sandblasted surface
b Acid-etched surface
c Sandblasted/acid-etched surface
d Anodised surface
e Sandblasted/anodised surface
f Sandblasted/acid-etched/anodised surface

Atomic force microscopy (AFM) images of the treated titanium surfaces are presented in Fig. 3. The AFM results can be used implicitly to evaluate the area of the treated implant surfaces that will effectively be in contact with the biofluid during bone integration [24]. Three-dimensional AFM analyses indicated that all of the treated titanium surfaces had rough surface topographies. The sandblasted surface had a topography with more compact features than the other treated specimen surfaces (Fig. 3*a*).

It can be seen in Fig. 3*b* that the surface of the acid-etched specimen has a topography with denser indentations and protrusions than others. In Fig. 3*c*, for the sandblasted/acid-etched specimen, the hills and valleys were formed more distinctively.

The lateral surfaces of the sandblasted/anodised (Fig. 3*e*) and sandblasted/acid-etched/anodised (Fig. 3*f*) specimens were smoother compared to the lateral surface of the anodised (Fig. 2*d*) specimen. In addition, the valleys on the surfaces of the sandblasted/anodised (Fig. 3*e*) and sandblasted/acid-etched/anodised (Fig. 3*f*) specimens were larger and deeper than others.

Surface compositions of the differently treated specimens determined by XPS analyses are shown in Table 2 and Fig. 4. The majority of elements on all surfaces were titanium, oxygen and carbon. The anodised, sandblasted/anodised and sandblasted/acid-etched/anodised specimens also contained trace amounts of fluorine. Ti 2p and O1s peaks were indicative of the formed titanium dioxide (TiO_2) layer. Aluminium was detected on the sandblasted specimen's surface, and so the Al2p peak was associated with residual alumina particles on the surface, resulting from the sandblasting treatments with Al_2O_3. These particles might be released into the vicinity of tissues and cause deleterious effects on the implant osseointegration [8]. However, acid etching and anodising treatments after sandblasting effectively eliminated residual alumina contamination, minimising this risk.

Values of roughness parameters for the differently treated titanium surfaces are shown in Table 3. Surface roughness measurements exhibited statistical differences between the roughness values of the specimens after the treatments. Surface roughness value of the only sandblasted specimen was nearly double that of the only acid-etched one.

Surface roughness of the sandblasted specimen increased after the acid etching treatment. Surface roughness parameters for the sandblasted/acid-etched titanium sample were found to be higher than the other specimens. In contrast, the lowest surface roughness values were found for the anodised specimens. The anodising process caused a slight reduction in the surface roughness parameters of the sandblasted and sandblasted/acid-etched titanium surfaces. There are numerous reports indicating that rough surfaces have better biomolecular adsorption, improve extracellular matrix production and promote differentiation of the mesenchymal cells towards an osteoblastic phenotype [25–30]. The surface roughness values of the specimens except the only-acid-etched and

Table 2 Surface elemental composition of treated Ti specimens

Specimens	Surface concentration (at.%)				
	Ti	O	C	F	Al
sandblasted	4.99	48.84	21.46	—	24.71
acid-etched	9.17	41.62	48.45	—	—
sandblasted/acid-etched	10.43	48.36	41.21	—	—
anodised	16.26	53.45	25.10	5.19	—
sandblasted/anodised	14.91	52.67	27.75	4.67	—
sandblasted/acid-etched/ anodised	16.11	52.61	27.44	3.84	—

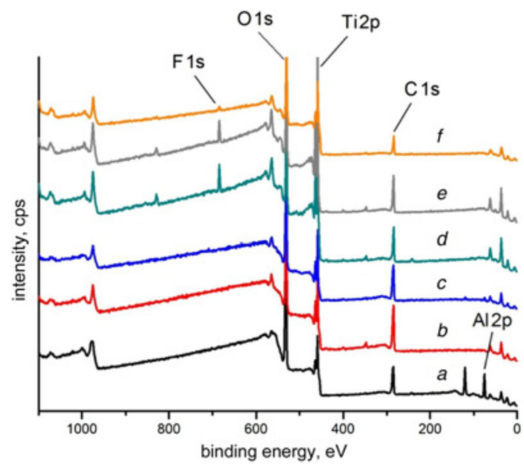

Figure 4 *XPS spectra after different treatments*
a Sandblasted surface; *b* acid-etched surface; *c* sandblasted/acid-etched surface; *d* anodised surface; *e* sandblasted/anodised surface; *f* sandblasted/acid-etched/anodised surface

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Table 3 Surface properties of treated Ti specimens

Specimens	Surface roughness parameters			Contact angle, deg
	Ra, μm	Rq, μm	Rt, μm	
sandblasted	0.88 ± 0.07	1.10 ± 0.08	7.35 ± 1.31	36.92 ± 4.26
acid-etched	0.47 ± 0.04	0.57 ± 0.04	3.27 ± 0.51	9.70 ± 1.62
sandblasted/ acid-etched	1.01 ± 0.06	1.28 ± 0.07	9.72 ± 0.79	8.41 ± 0.75
anodised	0.34 ± 0.02	0.43 ± 0.04	3.24 ± 0.51	20.61 ± 2.72
sandblasted/ anodised	0.81 ± 0.07	1.03 ± 0.10	7.75 ± 1.64	<4
sandblasted/ acid-etched/ anodised	0.94 ± 0.05	1.09 ± 0.07	6.92 ± 0.32	5.58 ± 0.48

only-anodised ones are very close to the SLA (sandblasted, large-grit and acid-etched) surfaces [15].

Contact angles of the titanium surfaces modified with different treatments are given in Table 3. Measurements were carried out through the sessile drop method with a microliter syringe. The results showed that all of the treated titanium surfaces are hydrophilic. As shown in Table 3, the highest contact angle was observed on the sandblasted surface. The sandblasted/anodised surface presented a lower contact angle (<4°). The sandblasted/acid-etched/ anodised titanium surface also yielded a substantially low contact angle. The changes in wettabilities can be correlated with the formation of micro/nanostructured features on the titanium surfaces. The contact angle values of the sandblasted and sandblasted/acid-etched titanium surfaces decreased after anodisation, which means that the hydrophilicity of titanium surfaces increased because of the formation of the TiO_2 layer grown in HF electrolyte medium. The nanostructured TiO_2 layer provides the liquid to penetrate into the nanopores and thus lower the contact angle, which creates a more hydrophilic surface [31].

According to the literature, a hydrophilic surface is better suited for blood coagulation than a hydrophobic one and wettability increases the adsorption of proteins and adhesion of osteoblasts on the implant surface [31]. As a consequence of these facts, dental implant manufacturers attempt to develop microroughened and highly hydrophilic surfaces in order to ensure a higher osseointegration rate and quality for their products.

4. Conclusion: In summary, the morphology, topography, chemical composition, roughness and wettability of nanoporous TiO_2 layers formed on microroughened titanium substrates were investigated. Nanoporous-microroughened surfaces were successfully prepared on titanium by combinations of sandblasting/acid-etching/ anodising. The properties of these developed surfaces were compared with the other treated titanium surfaces. SEM analyses showed that different surface treatments altered the surface morphologies. This study revealed that titanium surfaces anodised after the sandblasting and sandblasting/acid-etching treatments had micro and nanostructured surface features. Titanium surfaces modified by different surface treatment methods yielded substantially low contact angle values, thus rendering them superhydrophilic. The sandblasted/anodised surface also showed superhydrophilic behaviour which is an important factor for interactions between the implant and bone tissue. Finally, the sandblasted/acid-etched/anodised surface, eliminating any residual alumina particles, having deeper and denser microvoids, higher surface area thanks to TiO_2 nanopores, ideal roughness and low contact angle values, is a promising alternative in dental implant applications. This research can be taken as a reference in developing new kinds of implant surfaces.

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