Surface Characterizations of Variously Treated Titanium Materials

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The attachment of cells to titanium surfaces is an important phenomenon in the area of clinical implant dentistry. A major consideration in designing implants has been to produce surfaces that promote desirable responses in the cells and tissues. To achieve these requirements, the titanium implant surface can be modified in various ways. This research was designed to elucidate the relationship between surface roughness (R_a) and contact angle (θ) of various engineered titanium surfaces of commercially pure titanium, titanium-aluminum-vanadium alloy (Ti-6Al-4V), and titanium-nickel (TiNi) alloy. The contact angle was measured using distilled water, 1% sodium chloride solution, human neutrophils, and osteoblast-like cells. Surface oxide crystallography was identified by transmission electron diffraction. It was found that: (1) there were no significant differences in contact angles among the 4 media; (2) for commercially pure titanium, a combined treatment (hydrofluoric acid/nitric acid/water $\rightarrow$ sodium hydroxide $\rightarrow$ oxidation) showed the lowest θ (10.51 degrees in water), while the surface treated with sulfuric acid showed the highest value (72.99 degrees in water); (3) for all commercially pure titanium samples, when θ is greater than 45 degrees, the contact angle increases linearly with R_a (hydrophobic nature) and the surface is covered with rutile-type oxide only, while the contact angle decreases linearly with R_a when θ is less than 45 degrees (hydrophilic nature) and the surface is covered with a mixture of rutile and anatase oxides; and (4) a similar trend was found on Ti-6Al-4V and TiNi surfaces. (INT J ORAL MAXILLOFAC IMPLANTS 2001;16:333–342)

Key words: *oxides, surface properties, titanium, titanium alloy (Ti-6Al-4V), titanium nitride (TiNi), wettability*

A major consideration in designing implants has been to engineer surfaces that promote desirable responses in the cells and tissues contacting the implants. Cellular behaviors such as adhesion, morphologic change, functional alteration, and proliferation are greatly affected by surface properties, including hydrophilicity, roughness, texture, and morphology.[1,2] Surface modification of titanium materials has been shown to improve bony apposition, tissue adhesion, and migration.[3]

It has been shown that methods of implant surface preparation can significantly affect the resultant properties of the surface and subsequently the biologic responses and rates of cellular attachment that occur at the surface.[4,5] Recent efforts have shown that the success or failure of dental implants can be related not only to the chemical properties of the implant surface, but also to its micromorphologic nature.[6,7] The response of cells and tissues at implant interfaces can be affected by surface topography on a macroscopic basis,[7,8] as well as by surface morphology or roughness on a microscopic level.[6,9,10]

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SURFACE ROUGHENING

There are basically 2 ways to modify the surface layer, ie, creation of a convex texture or a concave texture. Additive treatments such as plasma spray-coating of hydroxyapatite particles or titanium beads, or physical or chemical vapor deposition, are performed to create convex surface morphology. It is possible that deposited particles can fracture from the convex surface. In contrast, mechanical treatment such as sandblasting or chemical treatment with acid or alkaline can create a concave surface texture. This research project dealt only with concave surface modifications.

Michaels and coworkers[11] determined that a higher percentage of osteoblast-like cells attached to roughened commercially pure titanium (cpTi) surfaces produced by sandblasting than to smoother surfaces, which were polished with 1-µm diamond paste. Keller and colleagues[12] later confirmed this. Thomas and associates[13] found that roughened surfaces had an increased implant surface area that resulted in greater surface coverage by bone, as compared with smooth polished surfaces. Buser and coworkers[7] reported that increased surface area positively correlated with increased bone-implant contact. In their study, the highest extent of bone-implant interface was observed on acid-treated surfaces (hydrochloric acid/sulfuric acid [HCl/H_2SO_4]) and hydroxyapatite-coated implants. There have been several studies on the effect of acid treatment, such as a mixture of HCl/H_2SO_4[14,15] or hydrofluoric acid/nitric acid (HF/HNO_3).[14] The results suggest that chemical etching of the titanium implant surface significantly increases the strength of osseointegration. Another approach that has been recently developed is an alkali and heat treatment.[16–20] In conclusions from these works, both alkali and heat treatment are deemed essential in the preparation of bioactive surfaces to promote osseointegration.

WETTABILITY

Measurement of the wettability of a surface, expressed by the contact angle, might be a predictive index of cytocompatibility.[1,21] Cell adhesion to and spreading on a biomaterial are dependent, among other factors, on the surface wettability of the biomaterial; therefore, the surface roughness affects the wettability.[22–24] The reason is believed to be that microvilli and filopodia, which work advantageously during the early stage of cell attachment, are needed for the cells to pass through the energy barrier between the material and the cells themselves[25]; therefore, cell attachment in its early stage is affected by physical and chemical properties, including wettability.[26,27]

Oshida and associates[28,29] investigated the effect of surface texture on wettability, evaluated by measuring the surface contact angles. Pre-oxidized biomaterials (in air oxidation at 300°C for 30 minutes), including cpTi, titanium-aluminum-vanadium alloy (Ti-6Al-4V), titanium-nickel alloy (TiNi), pure Ni, 316L stainless steel, and cobalt-chromium alloy (Co-Cr) were subjected to surface contact angle measurements. It was found that: *(1)* all titanium materials were basically covered with mainly tetragonal-structure rutile oxide, which appeared to be responsible for the relatively low initial contact angle and high rate of change in contact angles; and *(2)* other materials were covered with cubic-structure spinel oxides, which were shown to be responsible for the high contact angle and low rate of change. Hence, it was suggested that wettability might be related to the crystalline structure of the oxide films formed on these biomaterials.

Distilled (or deionized) water is normally employed for contact angle measurements,[1,12,21,24,30,31] but glycerol[1,21] and 1.0% sodium chloride (NaCl) solution[28,29] have also been used. The present study included neutrophils as a medium for contact angle measurements, because they are central to early acute and chronic inflammation phase defense and may be critical to implant acceptance in a host. This study also used osteoblast-like cells as a medium, because they are strongly related to osseointegration in implant healing phases.

OXIDE FORMATION

The biocompatibility of titanium is largely related to the characteristics of its oxide layer, as studies have suggested.[28,29] Strong adherence to the substrate, density, and a quick self-healing capacity of the oxide film, which is called "a passive film," give titanium its excellent corrosion resistance. There are 7 possible types of oxide formed on titanium materials. They include: *(1)* amorphous oxide, *(2)* cubic titanium oxide (TiO), *(3)* hexagonal titanium sesquioxide (Ti_2O_3), *(4)* tetragonal titanium dioxide (TiO_2) (anatase), *(5)* orthorhombic TiO_2 (brookite), *(6)* tetragonal TiO_2 (rutile), and *(7)* nonstoichiometric oxides (Ti_xO_y). Normally, anodic oxidation with chromic acid forms amorphous titanium oxide.[32,33] Much work has been done to identify the crystallography of titanium oxides formed with various acids. A mixture of anatase and rutile oxides was identified under wet oxidation using boiling 0.1 weight % H_2SO_4 for 24 hours,[34] while a mixture of anatase and brookite was obtained in boiling 0.2 weight % HCl oxidation for 24 hours.[34] Only anatase-phase oxide was identified under anodization using 0.1 mmol/L H_2SO_4 at 30°C at 12.5 mA/cm^2,[35] or 0.1 mmol/L H_2SO_4 at 5 V.[36] On the other hand, solely rutile-structure oxide was obtained by wet oxidation using boiling 10 weight % HCl,[37] boiling 10 weight % H_2SO_4,[38] or anodization using 0.5 mmol/L (1N) H_2SO_4 at 5 to 10 V.[39]

MATERIALS AND METHODS

Titanium Materials

In this study, 3 commonly used dental implant materials were used: *(1)* cpTi (ASTM Grade 2), *(2)* Ti-6Al-4V, and *(3)* TiNi alloy. Plates (10×30×2 mm) were prepared for each material. Specimens were polished using #800-grit silicon carbide (SiC) metallographic papers, washed in distilled water, cleaned in an ultrasonic bath, and dried at room temperature; these served as control samples. Prior to various treatments, all samples were treated with the same procedures done for the control group. For each surface modification (see below) of the 3 titanium materials, 10 sample plates were prepared; 8 of these (2 for each of 4 media) were used for contact angle measurements. The first plate (to which 3 droplets were applied) was used for the first observer and the second plate (again with 3 droplets) for the second observer; accordingly, the sample size for contact angle measurements for each of the 4 media was n = 6. The remaining 2 treated sample plates were used for measurements of surface roughness. Five readings for each plate were collected, giving a sample size of n = 10. For the electron diffraction method, 1 of the roughness-tested sample plates was used for chemical isolation of surface oxide from the substrates.

Surface Modifications

All 3 titanium materials were treated by 13 different methods, as described below.

Mechanical Treatment Group. The specimens in this group were subjected to 1 of 2 treatments:

1. Sandblasting on one side of the plate with 50-μm alumina particles at 20 psi for 1 minute with a fixed distance (1 cm) between the sample of the surface and blasting tip
2. Shot-peening on one side of the plate with glass beads (an average shot-peening dimension of 50 μm or less)

Chemical Treatment Group. Sample plates were chemically treated by one of the following immersion methods:

3. Boiling (10 weight %) HCl for 6 hours
4. $HF/HNO_3/H_2O$ (1/1/2) for 10 seconds
5. Boiling 3% hydrogen peroxide (H_2O_2) for 6 hours
6. Boiling 5% H_2SO_4 for 15 hours
7. 70°C 5 mmol/L sodium hydroxide (NaOH) for 24 hours

Mechanochemical Treatment Group. Only one treatment protocol was used in this group.

8. Sandblasting followed by chemical treatment in boiling $HCl/H_2SO_4/H_2O$ (20 mL/20 mL/260 mL) for 3 hours

Oxidation Treatment Group. Four different methods were used for these samples.

9. Treatment with NaOH (ie, surface preparation #7), followed by in-air oxidation at 600°C for 1 hour
10. In-air oxidation at 600°C for 1 hour
11. In-air oxidation at 165°C for 1.5 hours
12. Treatment by $HF/HNO_3/H_2O$ (ie, surface preparation #4), followed by treatment #9

Control Group (Treatment #13). The control group was untreated except for mechanical polishing with #800-grit SiC metallographic paper, as described previously.

Media and Preparation for Surface Contact Angle Measurements

Four different media were chosen for measuring the surface contact angles: *(1)* distilled water, *(2)* 1% NaCl aqueous solution, *(3)* neutrophil suspension, and *(4)* osteoblast-like cell suspension.

For preparation of the neutrophil suspension, routine procedures were followed.[40,41] The cells were suspended to the final concentration of 1.0×106 cells/mL. The MG-63 osteoblast-like cell suspension was prepared according to the process described in the literature.[42] Confluent MG-63 cells were trypsinized, pelleted, and resuspended in minimum essential medium (MEM) without FBS at a density of 1.65×106 cells/mL. As a control, the MEM without cells was used.

Contact Angle Measurement

One drop (1 μL) of medium was deposited on the surface of the treated sample and photographed

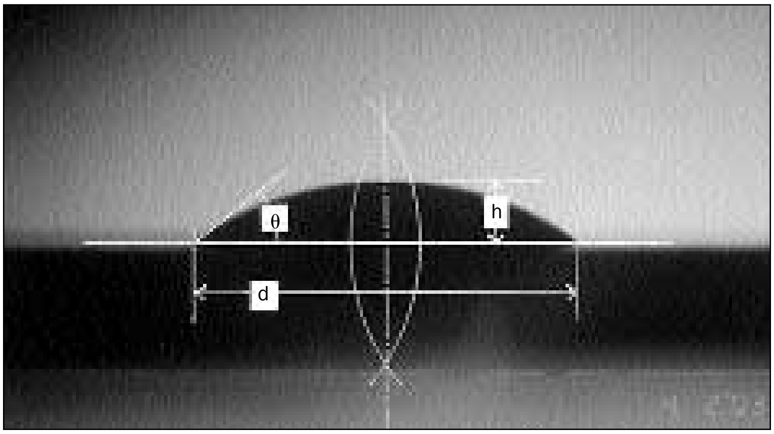

Fig 1 On a photograph, the height (h) and diameter (d) of each drop were measured. The surface contact angle (θ) was calculated by the equation $\theta = \tan^{-1}(2h/d)$.

immediately by an optical microscope coupled with a conventional camera. Two observers measured and calculated the contact angles of 3 drops for each media. The contact angles (θ) were obtained by the following equation (Fig 1): $\theta = \tan^{-1}(2h/d)$.

Surface Roughness Measurement

With a profilometer (Surtronic 3P, Taylor-Hobson, Leicester, England), the maximum roughness (R_{max}) and the average roughness (R_a) were measured and recorded at a traverse speed of 0.5 mm/second with a diamond-tipped stylus running parallel to the long axis of the specimen. The transverse length was 4.5 mm, the cutoff length was 0.8 mm, and the stylus radius was 5 μm (which conforms to the requirements of ISO Standard R468).

Transmission Electron Diffraction on Surface Oxide Film

All treated samples were immersed in $HF/HNO_3/H_2O$ (1/1/1) to isolate the surface oxide film from the substrate.[28,29,43] The peeled-off thin oxide film was then subjected to transmission electron diffraction (TED) tests under an accelerated voltage of 100 kV. For the electron diffraction method, the Bragg's diffraction formula can be reduced to the following equation[28,29,43]: $2r^{Au}d^{Au}$ = constant = $2r^{sample}d^{sample}$. Accordingly, if a standard sample was used (in this study, gold foil with known d-spacings), d-spacings for test samples could be calculated, from which the crystal structure can be identified.

Statistical Analysis

The agreement between surface contact angle measurements for the 2 observers was measured using intraclass correlation coefficients, and the measurements from the 2 observers were averaged for the group comparisons. A 1-way analysis of variance (ANOVA) of the surface roughness and surface contact angle was performed. Individual comparisons among group mean values were accompanied through the application of Tukey's HSD (honestly significant difference). This test shows whether the differences in results from the ANOVA were significant, as well as which groups were significantly different. To statistically determine whether a correlation existed between the surface roughness and surface contact angle in the study, the Spearman rank correlation test was performed for each of the 4 media (distilled water, 1.0% NaCl solution, a suspension of human neutrophils, and a suspension of MG-63 osteoblast-like cells).

RESULTS

Surface Roughness Measurements and Contact Angles

The relationship between measured contact angles for the 4 different media and average surface roughness for cpTi is presented in Fig 2. The same relationship for Ti-6Al-4V is shown in Fig 3. Figure 4 shows the relationship between contact angles and surface roughness for TiNi. In all 3 figures, 4 different marks are indicated for the 4 different media used for contact angle measurements.

Despite the 4 different media, there was little difference in contact angle measurement values. The differences resulting from these processes were not significant according to 1-way ANOVA. This result suggests that, in the case of initial contact angle measurements, any of the materials used could be selected as a contact angle medium, and the same trend could then be observed in the experimental system.

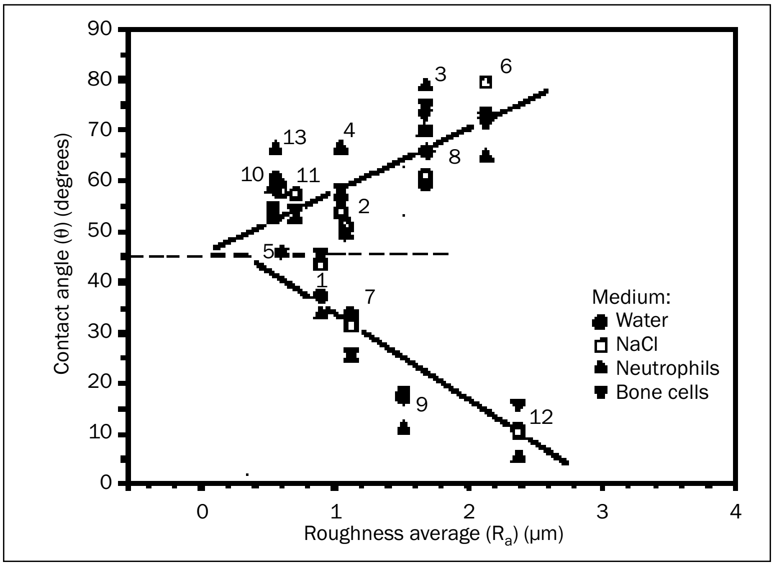

Fig 2 Relationship between contact angle and surface average roughness of variously treated cpTi (ASTM grade 2) with 4 different media, showing a positive relation and hydrophobic behavior when θ is greater than 45 degrees and a negative relationship and hydrophilic behavior when θ is less than 45 degrees.

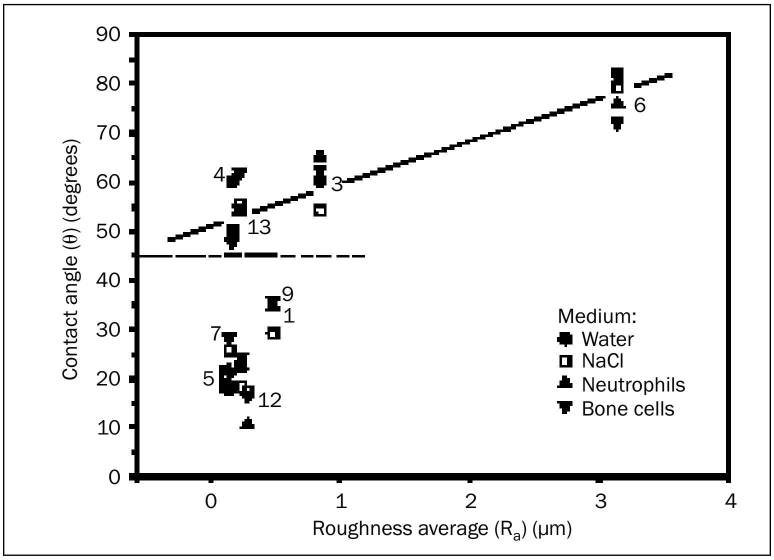

Fig 3 Relationship between contact angle and surface average roughness of variously treated Ti-6Al-4V alloy with 4 different media, showing a positive relationship when θ is greater than 45 degrees and grouped data points when θ is less than 45 degrees.

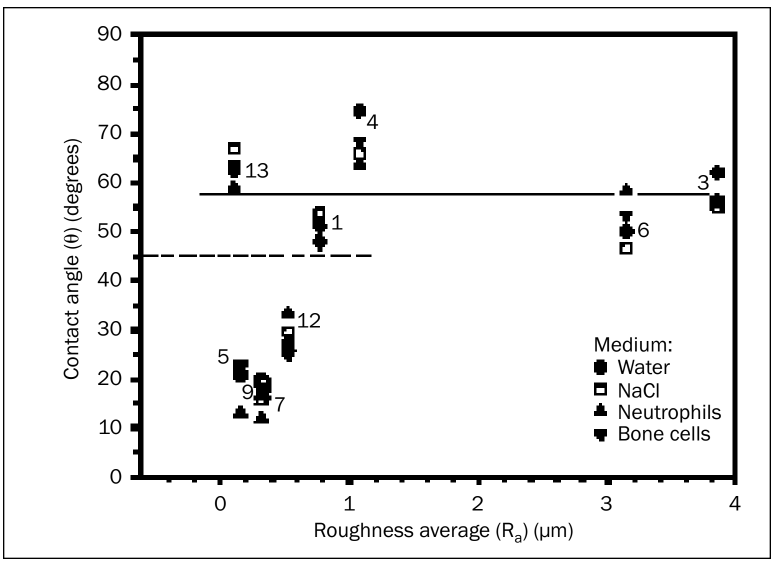

Fig 4 Relationship between contact angle and surface average roughness of variously treated TiNi alloy with 4 different media, showing contact angles to be independent of roughness when θ is greater than 45 degrees and grouped data points when θ is less than 45 degrees.

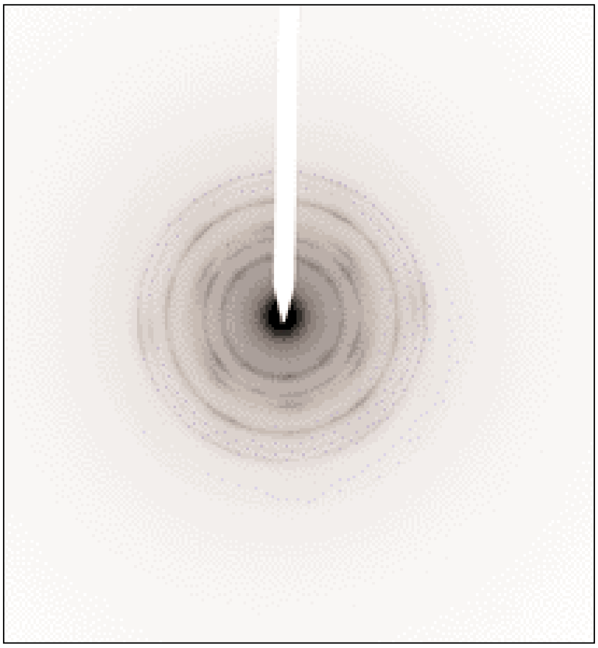

Fig 5 Electron diffraction pattern of oxide film formed on cpTi treated with sulfuric acid (5% boiling H_2SO_4 for 15 hours), showing that the oxide was identified as rutile-type TiO_2 only.

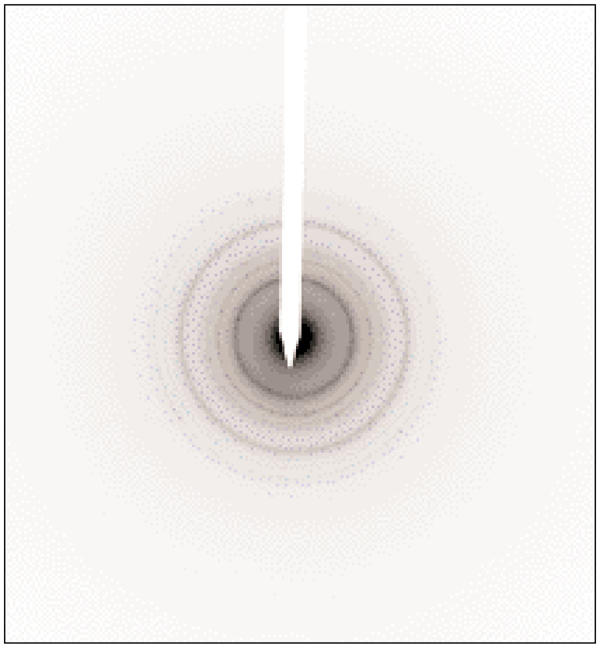

Fig 6 Electron diffraction pattern of oxide film formed on sulfuric acid-treated (5% boiling H_2SO_4 for 15 hours) Ti-6Al-4V alloy, showing that the oxide was identified as rutile-type TiO_2 only.

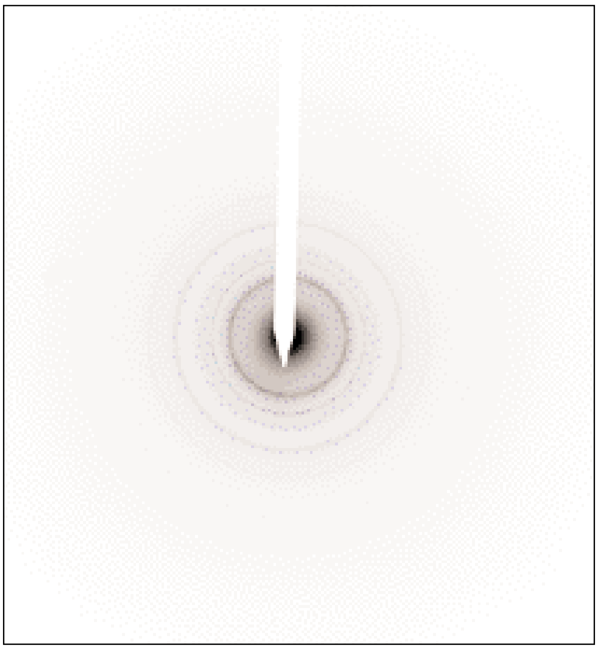

Fig 7 Electron diffraction pattern of oxide film formed on TiNi alloy treated with sulfuric acid (5% boiling H_2SO_4 for 15 hours), showing that the oxide was identified as rutile-type TiO_2 only.

ANOVA and Tukey multiple-range tests were used to identify significant differences in contact angle measurements for each treated sample of Ti and its alloys. There were significant differences according to 1-way ANOVA, and the Tukey multiple-range test determined these differences at the level of $P < .05$.

To determine whether a statistical correlation existed between the surface roughness and contact angle, the Spearman rank correlation test was performed for each of the 4 media. For cpTi, when θ was lower or higher than 45 degrees, the 2 values had a significant relationship (Fig 2). When θ was greater than 45 degrees with Ti-6Al-4V, the 2 values (θ and R_a) also had a significant relationship (Fig 3). On the other hand, when θ was smaller than 45 degrees with Ti-6Al-4V, the 2 values did not correlate (Spearman rank correlation test) (Fig 3). For the TiNi material, when θ was greater than 45 degrees, the contact angle appeared to be independent of surface roughness, and when it was smaller than 45 degrees, a trend similar to that seen for Ti-6Al-4V was observed (Fig 4).

Crystal Structure Identification of Surface Oxides

Figures 5 to 7 are electron diffraction patterns of oxides formed on sulfuric acid–treated surfaces (ie, treatment no. 6) of cpTi, Ti-6Al-4V, and TiNi,

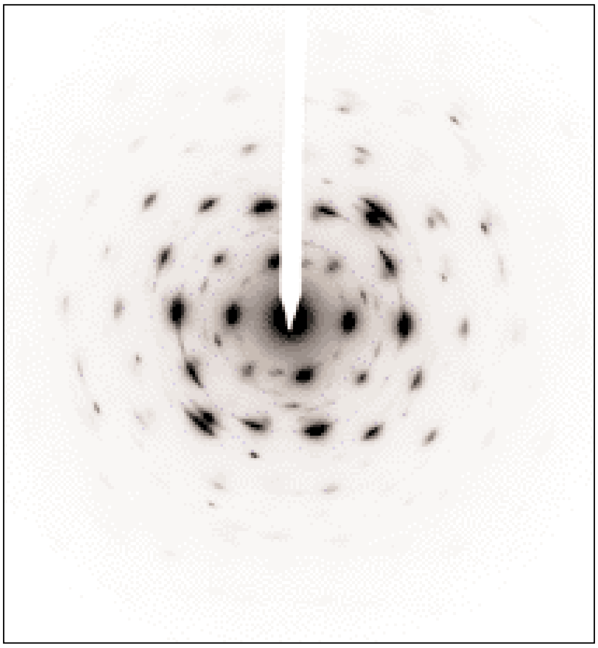

Fig 8 Electron diffraction pattern of oxide film formed on treated cpTi (in $HF/HNO_3/H_2O$ for 10 seconds → 70°C 5 mmol/L NaOH for 24 hours → in-air oxidation at 600°C for 1 hour), showing that the formed oxide was identified as predominantly rutile-type TiO_2 mixed with anatase-type TiO_2.

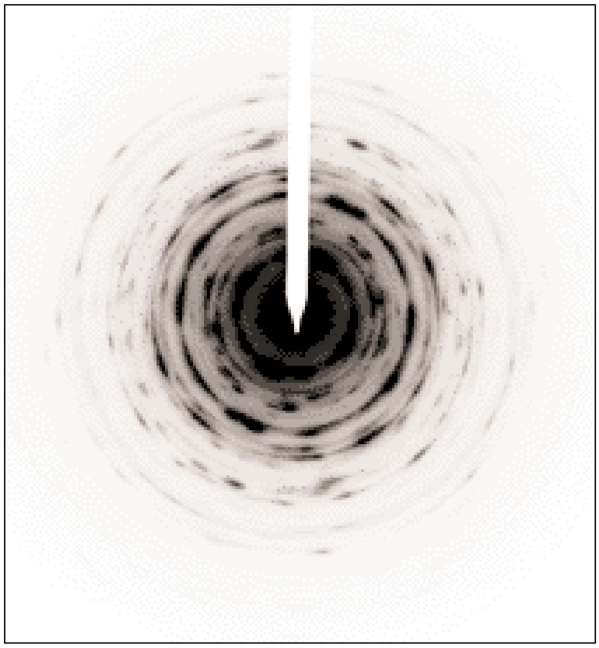

Fig 9 Electron diffraction pattern of oxide film formed on treated Ti-6Al-4V alloy (in $HF/HNO_3/H_2O$ for 10 seconds →70°C 5 mmol/L NaOH for 4 hours → in-air oxidation at 600°C for 1 hour), showing that the formed oxide was identified as predominantly rutile-type TiO_2 mixed with anatase-type TiO_2.

respectively. All these oxides were identified as only rutile-type (tetragonal) TiO_2. It was also found that oxides formed on treated surfaces (treatments no. 2, 3, 4, 8, 10, and 13) were identified as only rutile-type TiO_2.

Figures 8 to 10 show electron diffractographs of oxide films formed on mixed-acid–treated surfaces (no. 12) of cpTi, Ti-6Al-4V, and TiNi, respectively. These oxides consisted mainly of a rutile-type with an anatase-type (tetragonal) TiO_2. Oxides formed on NaOH-treated (ie, treatment nos. 7 and 9) cpTi, Ti-6Al-4V, and TiNi were also identified as a mixture of predominantly rutile-type and anatase-type TiO_2.

DISCUSSION

In summarizing the findings obtained from the relationships between contact angle and surface roughness for all tested samples (Figs 3 to 5), several points can be identified. It was found that cpTi samples given treatments 7, 9, or 12 exhibited an exactly opposite trend from the other treatments (eg, 3, 4, 6, 8); namely, an increase in surface roughness resulted in a decrease in contact angle. Although the same treatments performed on Ti-6Al-4V and TiNi did not show a similar relationship between surface

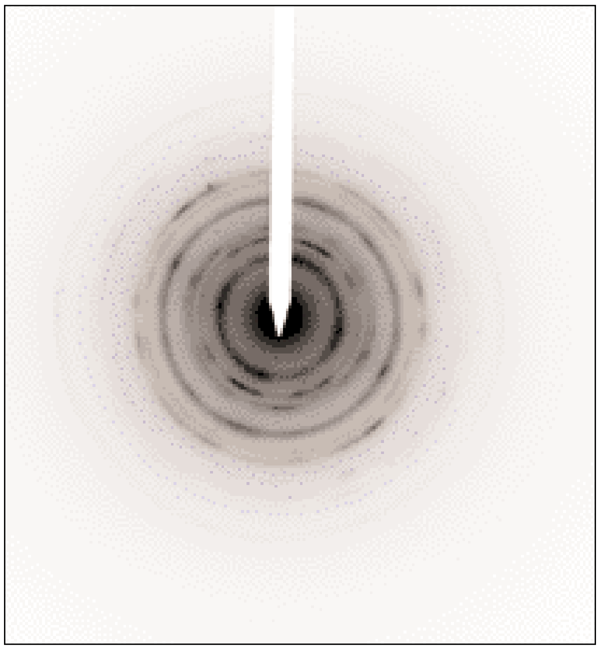

Fig 10 Electron diffraction pattern of oxide film formed on treated TiNi alloy (in $HF/HNO_3/H_2O$ for 10 seconds → 70°C 5 mmol/L NaOH for 24 hours → in-air oxidation at 600°C for 1 hour), showing that the formed oxide was identified as predominantly rutile-type TiO_2 mixed with anatase-type TiO_2.

roughness and contact angle, all treated samples of these alloys showed a lower contact angle. Because titanium alloys (Ti-6Al-4V and TiNi) had different chemical reactions to acid or alkaline and differing resistance (ie, different surface hardness) to sandblasting and shot-peening than did cpTi, treatments no. 5, 7, 9, and 12 did not make a larger deviation in terms of surface roughness, as seen in Figs 4 and 5. When the contact angle was greater than 45 degrees, the relationship between θ and R_a in TiNi was different from that seen for cpTi and Ti-6Al-4V. Although there was no significant difference ($P < .05$) among TiNi samples given treatments 1, 3, 4, 6, or 13, it appeared that the contact angle was independent of surface roughness. In the present study, alkaline treatment with NaOH (7 and 9) produced lower contact angles. According to current practices in implant dentistry, acid treatment of implant surfaces is one of the common modifications used in the commercial market. There is no implant system available in which samples are treated by alkaline. Further study will be required.

Because roughness of the surface plays a predominant role in cell adhesion during the implant healing phases, this factor should be considered in the manufacturing of endosseous implants. However, the results of this study led to the hypothesis that surface roughness is not as important as other surface properties in biologic responses. For example, the highest R_a value on cpTi was measured for sample #12, but the contact angle at this rough surface was significantly lower than that obtained by other samples that have lower R_a. In contrast, sample #6 also gave a high value for R_a but exhibited the highest contact angle value. Other surface properties should also be considered important in the biologic response and may be more critical parameters of biocompatibility than surface roughness.

In oxide film identification, the main oxide film formed on cpTi, Ti-6Al-4V, and TiNi was TiO_2. However, TiO_2 has 3 distinct crystal structures: tetragonal anatase, orthorhombic brookite, and tetragonal rutile. As seen in Figs 6 to 10 and Figs 3 to 5, it was found that oxides that formed on the surfaces and behaved in a hydrophobic manner (regardless of type of titanium materials and surface modifications) were identified as only rutile TiO_2. On the other hand, oxides that formed on surfaces and had a hydrophilic character (for examples, samples given treatments 1, 5, 7, 9, or 12) were identified as a mixture of dominant rutile-type and anatase-type oxides. It is known that rutile-type oxide has a tetragonal structure with similar axial lengths (ie, a_o = 4.58 Å, c_o = 2.98 Å), while the same tetragonal anatase type has a large axial ratio (ie, a_o = 3.78 Å, c_o = 9.50 Å).[44] It is not clear that this structural difference between rutile and anatase oxides will contribute to the observed differences between hydrophilic and hydrophobic behaviors in terms of the relationship between surface roughness and contact angle for all tested samples of 3 different titanium materials. Nevertheless, the data suggest that the contact angle may be related to the crystalline structure of the oxide films formed on titanium and its alloys.

SUMMARY

In this study, surfaces of cpTi, Ti-6Al-4V, and TiNi were treated mechanically, chemically, by oxidation, and mechanochemically. The relationship between surface roughness and contact angle measurement was investigated with 4 different media (distilled water, 1.0% NaCl solution, a suspension of human neutrophils, and a suspension of MG-63 osteoblast-like cells). In addition, surface oxides were identified by the transmission electron diffraction (TED) method.

Within the limited scope of the experimental study, the following conclusions were drawn:

1. All tested specimens showed contact angles between 0 and 90 degrees, and the differences among the 4 different media were not significant ($P < .05$; 1-way ANOVA).
2. For cpTi, a combined treatment ($HF/HNO_3/H_2O \rightarrow NaOH \rightarrow$ air-oxidation at 600°C for 1 hour) showed the lowest θ of 10.51 degrees in water, 9.84 degrees in NaCl solution, 4.89 degrees in neutrophils, and 15.61 degrees in osteoblast-like cells; the surface average roughness, R_a, was 2.38 µm. The cpTi surfaces treated with HNO_3 showed the highest contact angle values: 72.99 degrees in water, 78.95 degrees in NaCl solution, 64.33 degrees in neutrophils, and 71.20 degrees in osteoblast-like cells, with an R_a value of 2.15 µm.
3. The alkali-treated samples showed lower contact angle values.
4. An influence of surface roughness parameters on the contact angle was found in this study. For cpTi, the contact angle increased linearly with R_a when θ was greater than 45 degrees, while it decreased linearly with R_a when θ was less than 45 degrees.
5. For Ti-6Al-4V, the same trend was seen at angles greater than 45 degrees. For angles less than 45 degrees, the samples clustered (or grouped) within the lower roughness range.

6. In the case of TiNi, the contact angle decreased linearly with R_a when θ was greater than 45 degrees. For angles less than 45 degrees, the samples grouped within the lower roughness range.
7. Oxides formed on surfaces that behaved in a hydrophobic nature were identified as only rutile-type titanium oxide, while oxides formed on surfaces behaving in a hydrophilic character were identified as a mixture of dominant rutile and anatase oxides.
8. It is suggested that the surface energy represented by the contact angle for titanium and its alloys appears to be related to the crystalline structure of the oxide films formed on the surfaces.

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