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RESEARCH AND EDUCATION

Changes in the esthetic, physical, and biological properties of a titanium alloy abutment treated by anodic oxidation

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ABSTRACT

Statement of problem. The grayish appearance of titanium abutments adversely affects peri-implant esthetics in patients with thin mucosa, impacting patient satisfaction with implant-supported restorations in esthetic regions.

Purpose. The purpose of this in vitro study was to change the color of titanium alloys with anodic oxidation and to evaluate alterations in the esthetic, physical, and biological properties of the anodized titanium alloys.

Material and methods. Pink and yellow titanium alloys produced by anodization were the experimental groups, and the untreated titanium alloy and zirconia were used as the control groups. Pig gingiva was placed on the tested specimens to evaluate the esthetic effect by recording the color change in the gingiva. Physical properties including morphology, chemical composition, roughness, and contact angle were evaluated by scanning electron microscopy, atomic force microscopy, and a contact angle analysis system. Biological properties were evaluated by observing the cell behaviors of human gingival fibroblasts, using scanning electron microscopy, fluorescence microscopy, a live/dead viability assay, and a cell counting assay.

Results. A variety of colors can be produced on the surfaces of titanium alloys by anodization at different voltages. Titanium alloys anodized at 60 and 65 V exhibited yellow and pink appearances, respectively. Color differences of gingiva caused by anodized titanium alloys were lower than those of the untreated titanium alloy, but they were higher than those of zirconia. Compared with the untreated titanium alloy, the anodized titanium alloys exhibited grain formation, a lower contact angle, and higher roughness. Cell morphology, proliferation, and viability on surfaces of anodized titanium alloys were similar to those of the untreated titanium alloy but lower than those of zirconia.

Conclusions. Anodization could change the color of titanium alloys to pink or yellow at different voltages. Grain formation, roughness, and hydrophilicity were increased after treatment. The esthetics and biocompatibility of anodized titanium alloys were not as good as that of zirconia, but the pink and yellow titanium alloys treated by anodization achieved better gingival esthetics than the untreated titanium alloy. (J Prosthet Dent 2019;121:156-65)

Replacing missing teeth with dental implant restorations is considered an effective treatment.[1] The abutment, which connects the dental implant to the superstructure, forms the transmucosal profile of a crown or fixed prosthesis. The long-term clinical survival rates and good biocompatibility of titanium have made this material the gold standard for dental implant abutments.[2] From an esthetic viewpoint, a shortcoming of titanium is its grayish appearance through thin mucosa, which can reduce patient satisfaction with implant-supported restorations in the esthetic regions.[3-5]

To overcome this drawback, various abutment materials, such as gold, nitride-treated titanium (TiN), alumina, and zirconia, have been introduced to reduce discoloration of peri-implant soft tissue.[6-10] However, the abutment may cause

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Clinical Implication

Titanium abutments colored by anodic oxidation may be an effective method to achieve excellent esthetics in esthetic regions when missing teeth were replaced with dental implant restorations.

discoloration of peri-implant soft tissue regardless of the chosen type of materials. Ishikawa-Nagai et al[11] reported that gingival esthetics could be improved by coloring the implant neck, most effectively with light pink. Happe et al[12] suggested that zirconia abutments veneered with fluorescent ceramic could decrease the color difference between peri-implant soft tissue and natural gingiva. Thoma et al[13] concluded that veneering zirconia abutments with pink ceramic positively influenced the color of peri-implant mucosa.

In addition to the esthetics of peri-implant soft tissue, a strong biological seal around the implant is important for ensuring the long-term survival of the dental implant. A tight biological seal can form an effective barrier that protects the implant from invasion by pathogenic bacteria, which is regarded as a crucial trigger for peri-implantitis.[14,15] The surface characteristics of the abutment have an influence on the adhesion and proliferation of human gingival fibroblasts (HGFs), which are important in forming peri-implant soft tissue seals.[16] Fibroblasts are the preferred cells to form a collagen-rich connective tissue to repopulate the wound after dental implants are installed.[17] Meanwhile, surface characteristics of the abutment affect the amount, type, conformation, orientation, and binding strength of bound proteins secreted by HGFs.[18-20]

Anodic oxidation is a surface modification technique for titanium. An oxide film of variable thickness can form on the titanium surface at different voltages, and the films display different colors due to light interference in the titanium dioxide layer.[21-25] Additionally, the titanium surface demonstrates various surface morphologies due to different voltages.[26] These characteristics may influence the biological seal formed by HGFs.

The purpose of this in vitro study was to explore the impact of colored titanium alloys treated by anodic oxidation on gingival esthetics and to investigate the alteration of the alloys' physical and biological properties after anodic oxidation. The null hypothesis was that the esthetics and biocompatibility of anodized titanium alloys would be similar to those of the control groups.

MATERIAL AND METHODS

A total of 153 titanium alloy disks (Ti6Al4V; Baoji Haibao Special Metal Materials, Co, Ltd), 10 mm in width and 2 mm in thickness, were ground with SiC paper in successive grades from 400 to 2000 grit. The disks were degreased with an acetone solution, chemically washed in a solution of nitric acid and hydrofluoric acid for 20 seconds and rinsed in deionized water for 10 minutes.

A total of 54 Ti6Al4V disks were used to screen for appropriate anodization conditions for subsequent tests. They were divided evenly into 18 groups, with 3 disks in each group. The Ti6Al4V disks were electrochemically colored in a temperature-controlled container with 1 M phosphoric acid as the electrolyte.[27,28] The Ti6Al4V disks were fixed on the anode, and the cathode was made from a stainless steel plate. The feed voltages supplied by a regulated power supply (FD-A; Fudi High Technology Co, Ltd) were arranged from 5 to 90 V, with an incremental voltage of 5 V at each step. The anodizing time was 60 seconds. Specimens were subsequently cleaned in deionized water for 10 minutes, sealed in boiled deionized water (100°C) for 30 minutes, dried under a stream of cool air, and stored in a desiccator until required for further analysis.[29] Specimens anodized at 60 and 65 V were chosen for study in the subsequent experiments as their colors matched tooth and gingiva colors.

The other 99 disks were used to evaluate their esthetic effect, surface characteristics, and biocompatibility. They were divided into 3 groups (n=33): anodized at 60 V, anodized at 65 V, and unaltered control group. In addition, 33 zirconia disks (Doceram; Doceram Medical Ceramics GmbH), of the same dimensions, manufactured using computer-aided design and computer-assisted manufacturing (CAD-CAM), were also used as a control group.

Three pig maxillae were used to evaluate the esthetic effect of tested specimens.[30,31] The maxillae were stored in a humidistat to preserve freshness. The titanium or zirconia specimens were placed under a 1-mm thickness of pig gingiva harvested from the buccal gingiva. Change of gingiva color was measured by using a spectrophotometer (Crystaleye CE100-DC/EU; Olympus Campus) in a black cassette.[32] Each specimen was measured 3 times, and Commission Internationale de l'Eclairage (CIE) L* (luminosity), a* (green-red), and b* (blue-yellow) values were recorded. Color differences (ΔE) between the tested specimens and natural gingiva were calculated according to the formula: $\Delta E=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$.

Surface morphology and chemical composition were evaluated using scanning electron microscopy (SEM) (S-4800; Hitachi). Roughness was analyzed by atomic force microscopy (NanoScope MultiMode IIIa; Veeco). Water contact angles were measured using the sessile drop method, using a contact angle analysis system (SL2008; Solon Tech).[33]

HGFs were cultured in high-glucose Dulbecco modified Eagle medium (HyClone), 10% fetal bovine serum (Gibco), and 1% penicillin/streptomycin (HyClone). When cells grew from the tissue and reached 80% to 90% confluence,

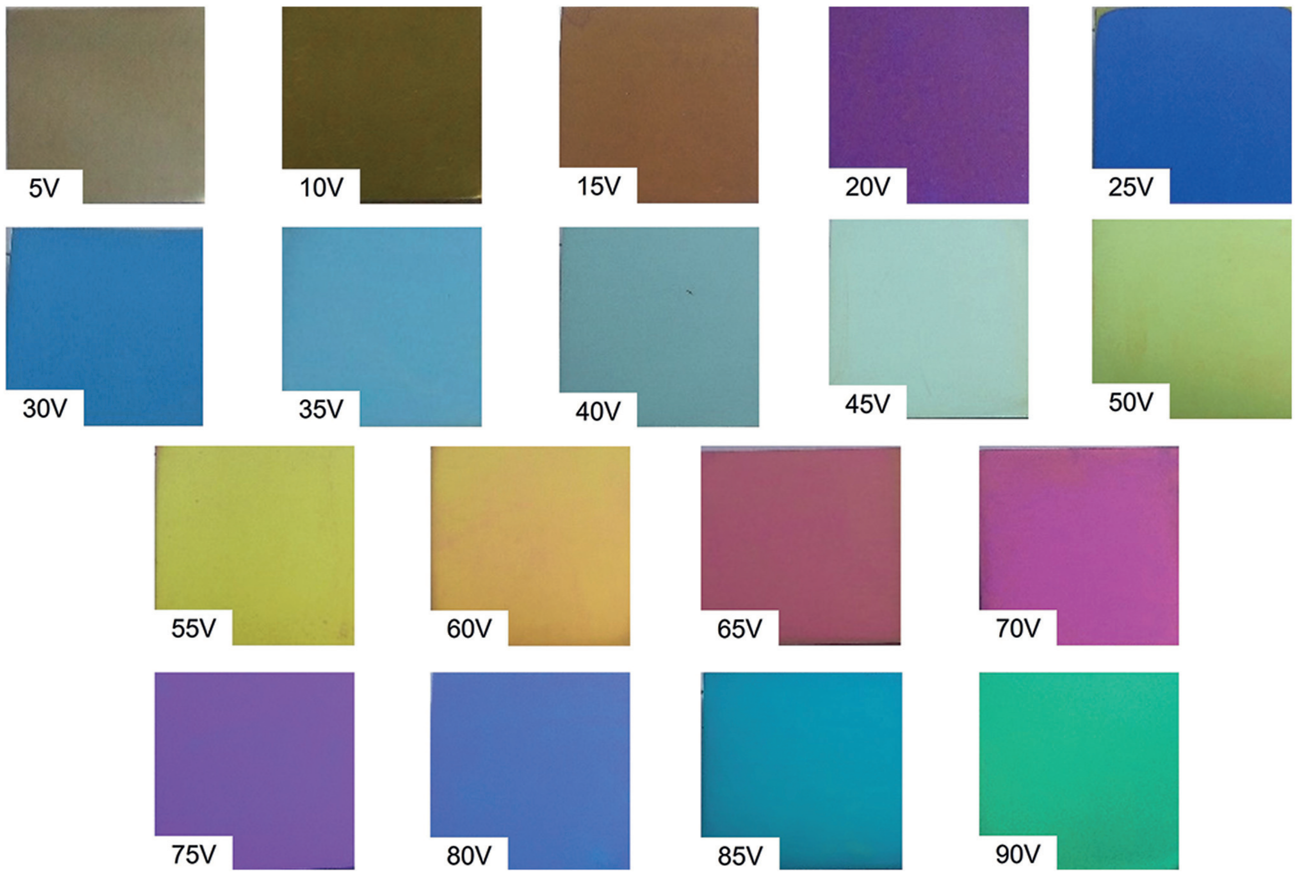

Figure 1. Colors of Ti6Al4V specimens treated by anodic oxidation with different voltages (0-90 V; 1 mmol/L H_3PO_4; 25°C-27°C; time=60 seconds).

they were passaged and cultured.[34] Cells at passages 5 to 10 were used in the subsequent experiments.

The cell morphology of HGFs was assessed with SEM. Before the test, the specimens were sterilized in an autoclave at 121°C for 30 minutes, followed by ultraviolet irradiation for 2 hours. HGF suspensions (3×10^4 cells/mL) were cultured on the specimens at 37°C with 5% CO_2 in air for 24 hours and then rinsed 3 times with phosphate-buffered saline (PBS). HGFs were fixed with 4% glutaraldehyde for 60 minutes at room temperature, dehydrated in a series of ethanol concentration gradients (50%, 70%, 80%, 90%, 95%, and 100%), freeze dried, coated with a 15- to 20-nm layer of gold, and examined using SEM.

The growth pattern of HGFs was observed under fluorescence microscopy (Nikon ECLIPSE80i; Nikon Corp). HGF suspensions (3×10^4 cells/mL) were cultured on the specimens for 72 hours. Specimens were rinsed 3 times with PBS, fixed with 4% glutaraldehyde for 60 minutes, permeabilized with 0.1% Triton-X (Sangon; Biotech) for 5 minutes, and blocked with 1% bovine serum albumin (Sigma) for 30 minutes. The cells were then incubated with vimentin antibody (ab195877; Abcam) at a dilution of 1:500 for 30 minutes. Nuclear DNA was labeled with 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (Abcam).

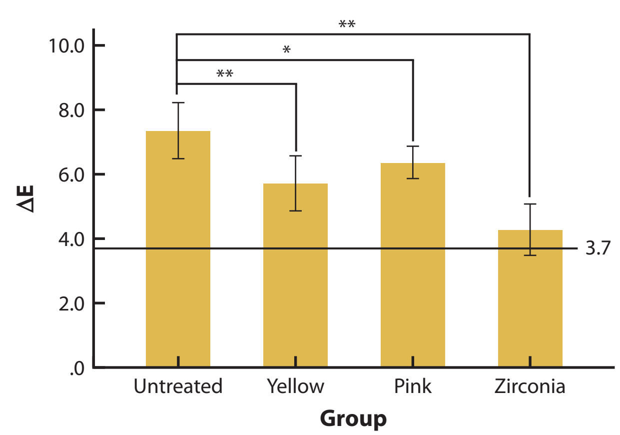

Figure 2. ΔE values for different materials evaluated under pig gingiva. Line at ΔE=3.7 is a reference representing a critical ΔE threshold for intraoral color distinction as perceived by naked eye.[30] Data are means; bars indicate SD. *$P<.05$; **$P<.01$ indicated statistically significant differences between groups. ΔE, color differences; SD, standard deviation.

Ability of cells to proliferate on the specimens was evaluated by using a cell counting assay kit (CCK-8; Dojindo Laboratories Inc). HGF suspensions (3×10^4 cells/mL) were cultured on the specimens for 1, 3, 5, and 7 days. At each time point, the specimens were moved to new 24-well plates, and 10% CCK-8 was added for

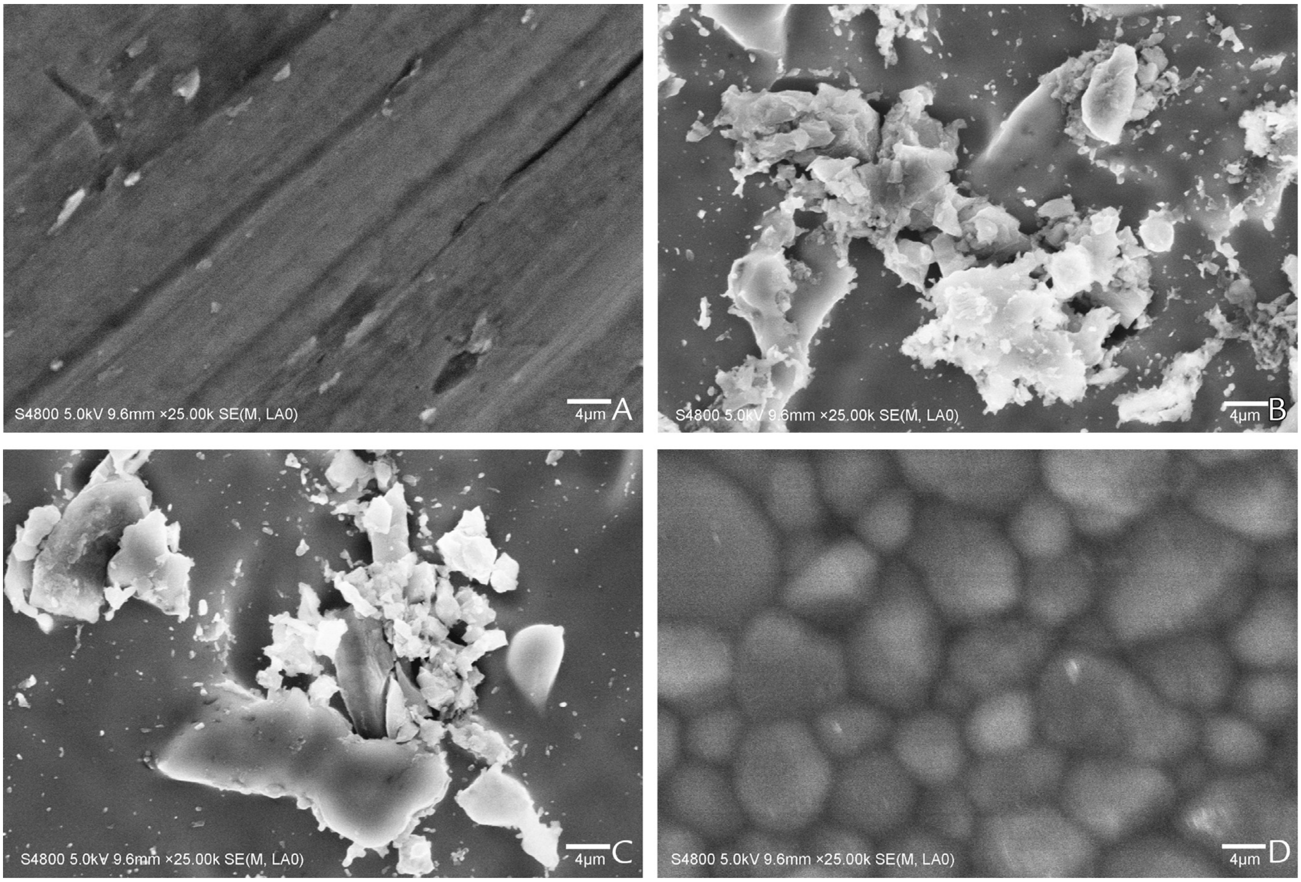

Figure 3. Scanning electron micrographs of specimens. A, Untreated Ti6Al4V. B, Yellow Ti6Al4V. C, Pink Ti6Al4V. D, Zirconia. (Original magnification ×25 000).

incubation for 2 hours. The optical density of the resulting solution was measured by using a microtiter plate reader (TECAN Infinite 200 PRO; Tecan Group Ltd.) at wavelength of 450 nm.

The viability of HGFs was evaluated using a live/dead viability assay (live/dead Double Staining Kit; KeyGEN BioTECH Inc) according to the manufacturer's protocol. HGF suspensions (3×10^4 cells/mL) were cultured on the specimens for 24 hours, and specimens were rinsed twice with PBS and incubated in the live/dead solution (1 mM Live-Dye and 1 mM propidium iodide) for 30 minutes. Then cells were examined with fluorescence microscopy (Nikon ECLIPSE80i; Nikon Corp).

Data means ±SD. All statistical analysis was conducted using statistical software (IBM SPSS Statistics, v20.0; IBM Corp). Color differences, roughness, contact angles, and cell viability were analyzed by 1-way ANOVA, followed by the Tukey honestly significant difference (HSD) test; cell proliferation data were compared by 2-way ANOVA (specimens versus time) and by the Tukey HSD test[35] (α=.05).

RESULTS

The colors on the Ti6Al4V surfaces changed synchronously with the alteration of anodization voltages (Fig. 1).

Table 1. Chemical composition (wt%) of untreated and anodized Ti6Al4V (pink)

Ti6Al4V	Ti	O	Al	V	Si	P	C
Untreated	85.18	0	6.20	3.35	0.93	0	4.34
Anodized (pink)	65.73	21.12	4.43	5.15	0.14	0.51	2.92

The Ti6Al4V surface exhibited a yellow appearance at 60 V, and the surface changed to pink at 65 V. Color differences of all specimens in each group were above the critical ΔE threshold of 3.7 for intraoral color distinction by the naked eye (Fig. 2). Color differences with the anodized specimens (including yellow and pink) were lower than on the untreated specimens, but they were higher than with zirconia. Zirconia showed the lowest ΔE value (4.28), whereas the untreated specimen exhibited the highest ΔE value (7.35).

Uniform scratches in the same direction were observed on the untreated specimen, whereas many grains formed on the anodized specimens. Cracks were found between grains at high magnification. Spherical particles were observed on the zirconia surface (Fig. 3). Grains on the anodized specimens contained oxygen and phosphorus. All Ti6Al4V specimens contained small amounts of aluminum, vanadium, carbon, and silicon

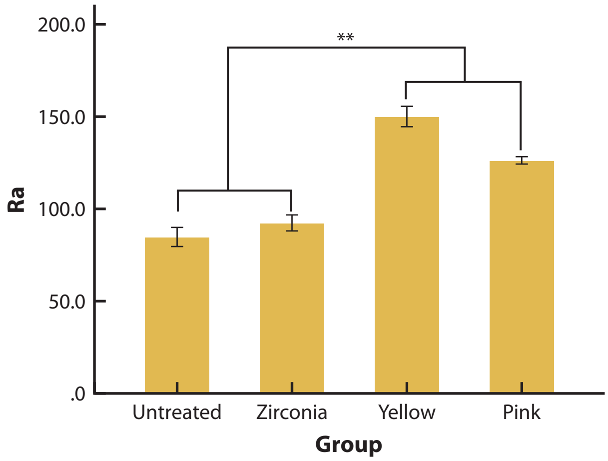

Figure 4. Surface roughness of untreated, yellow, and pink Ti6Al4V and zirconia surfaces. Data are means; bars indicate standard deviation. **P<.01 indicated statistically significant differences between experimental and control groups.

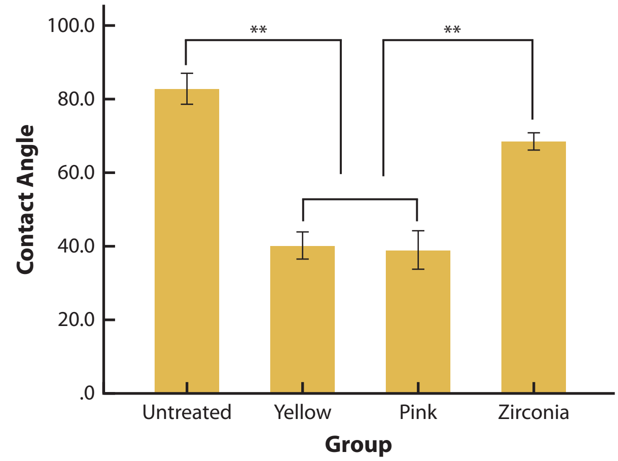

Figure 5. Contact angles of untreated, yellow, and pink Ti6Al4V and zirconia surfaces. Data are means; bars indicate standard deviation. **P<.01 indicated statistically significant differences between experimental and control groups.

(Table 1). Degrees of surface roughness (Ras), from low to high, were: untreated specimen, zirconia, pink specimens, and yellow specimens. The differences among Ras values of the anodized specimens and untreated specimen were statistically significant (P<.001) (Fig. 4). The contact angles of the anodized specimens were lower than those of the untreated specimen (P<.001) (Fig. 5).

The morphologies of the HGFs are shown in Figure 6. At low magnification, the HGFs were spindle-shaped and exhibited a flat shape with elongated lamellipodia connecting adjacent cells. At high magnification, HGFs demonstrated a veil-like appearance while in tight contact with underlying specimens. The HGFs on the zirconia surface were spread more evenly than on the other specimens.

The growth patterns of HGFs are shown in Figure 7. On the surfaces of the anodized specimens and zirconia, the filopodia of the HGFs were in contact with each other and formed a multidirectional network. On the untreated specimen, HGFs were observed to align parallel to each other. The orderly parallel pattern of HGFs was guided by the uniform scratches on the untreated specimen surface.

No significant differences between cell proliferation in the untreated and yellow specimens (P=.761) or between the untreated and pink specimens (P=.881) (Fig. 8) were found. However, cells seeded on the zirconia achieved a higher cell proliferation than on the other specimens (P<.001).

Cell viability of HGFs, shown as number of live-to-total cell ratio, was assessed to evaluate the biocompatibility of tested specimens. The percentages of dead cells remained low in both the experimental and control groups (Fig. 9). No significant difference was observed between the yellow and untreated specimens (P=.370) or between the untreated and pink specimens (P=.998). However, the percentage of dead cells on the zirconia surface was lower than on the surfaces of the other specimens (P<.001) (Fig. 10).

DISCUSSION

The dark color of the conventional titanium dental implant abutment can shine through the mucosa in patients with high smile lines or with a thin gingiva biotype and adversely affect esthetics. The problem can be addressed by changing the color of the abutment with thermal oxidation, chemical oxidation, TiN coating, or anodic oxidation. Thermal oxidation can form various colors on the titanium surface, but the color uniformity and reproducibility are poor.[36] The oxide film has poor durability and poor corrosion resistance to chemical oxidation.[37] A TiN coating can improve the esthetics by coloring the titanium surface gold, but this coating might induce an allergic reaction in certain patients.[10] Anodic oxidation not only forms various colors on the titanium surfaces but also improves its corrosion resistance because of the increased thickness of the surface oxide layer.[23] Therefore, compared with other techniques, anodic oxidation is a more suitable titanium surface coloring technique, which is why this technique was chosen for this study.

The colored appearance is mainly due to light interference in the transitional oxide layer of titanium. Thus, colors formed on the titanium surfaces after anodization are known as interference colors.[24] Our results showed that various colors could form by anodic oxidation. Additionally, anodized specimens (yellow and pink) showed smaller color differences under the gingiva than those of untreated

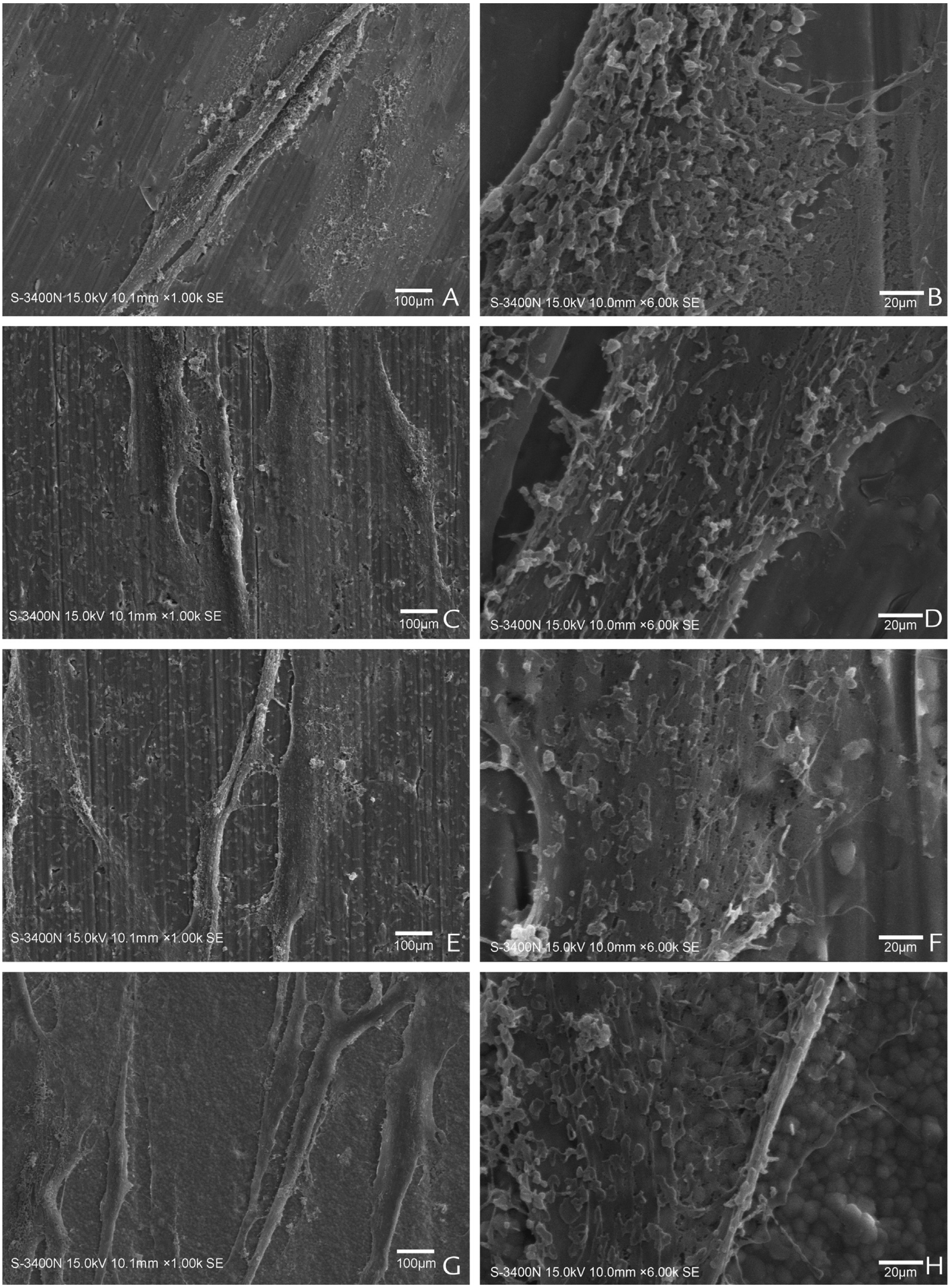

Figure 6. Low- and high-magnification scanning electron micrographs of human gingival fibroblast grown for 24 hours on surfaces. A, B, Untreated Ti6Al4V. C, D, Yellow Ti6Al4V. E, F, Pink Ti6Al4V. G, H, Zirconia. A, C, E, G, Original magnification ×1000; B, D, F, H, ×6000.

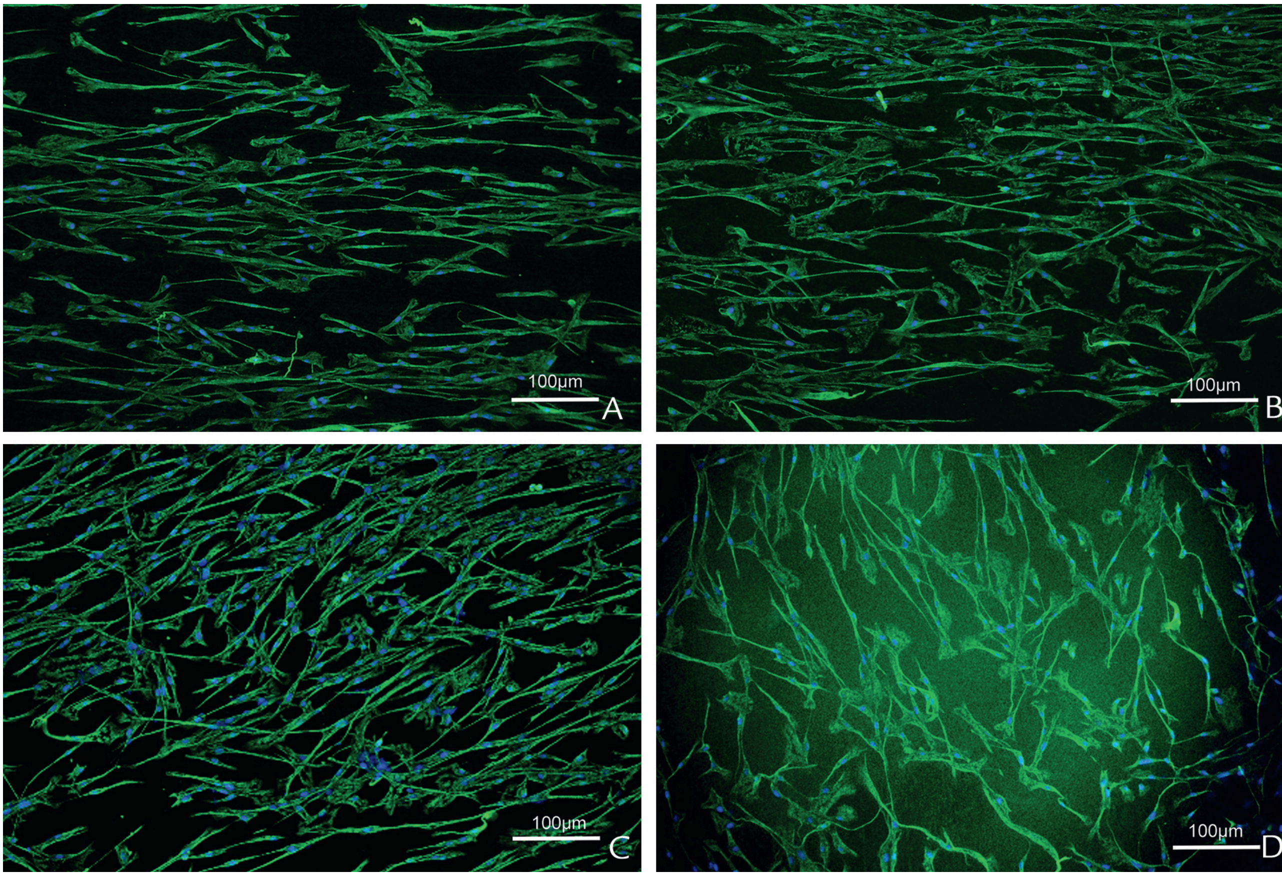

Figure 7. Fluorescence microscopy showing human gingival fibroblasts growing on surfaces. A, Untreated Ti6Al4V. B, Yellow Ti6Al4V. C, Pink Ti6Al4V. D, Zirconia. Antivimentin staining (original magnification ×100).

specimens, which demonstrated that the anodization of titanium alloys may improve the peri-implant esthetics of titanium abutments. Zirconia showed the lowest discoloration among the tested specimens because of its good light transmittance, but zirconia abutments are brittle and cannot be extensively milled, making them contraindicated in a narrow space.[38]

The abrasive resistance, corrosion resistance, and color stability of anodized titanium oxide films are important to the long-term maintenance of color in the oral environment. Li et al[39] reported that anodized titanium dentures in the oral environment for half a year retained good color stability. In addition, the color of titanium after prolonged exposure to ethanol was stable, demonstrating that the passivation oxide film was resistant to degradation.[25] Additionally, anodic oxidation improved the corrosion resistance of the titanium surface due to the increased thickness of the oxide layer on the surface and also increased the durability of the abutment. Little research has been carried out on the influence of abrasive wear on the color stability of anodized titanium surfaces. However, the wear resistance of the material may have had much to do with its hardness, especially surface hardness.[40] Moreover, incorporating the oxide

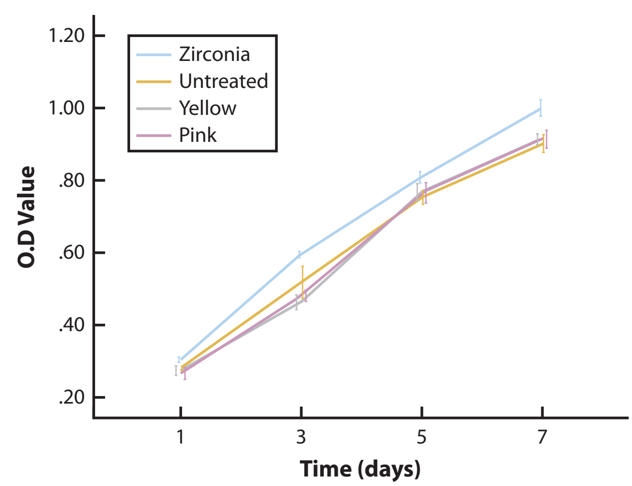

Figure 8. Human gingival fibroblast proliferation activity on different surfaces detected by cell counting kit assay after 1, 3, 5, and 7 days. Data are means, bars indicate standard deviation.

into the titanium alloy after anodic oxidation improved the surface hardness adjacent to the oxide layer.

The elemental analysis of the titanium alloy indicated that the electrolyte solution was incorporated into the film formation. Kern et al[27] noted that anodization

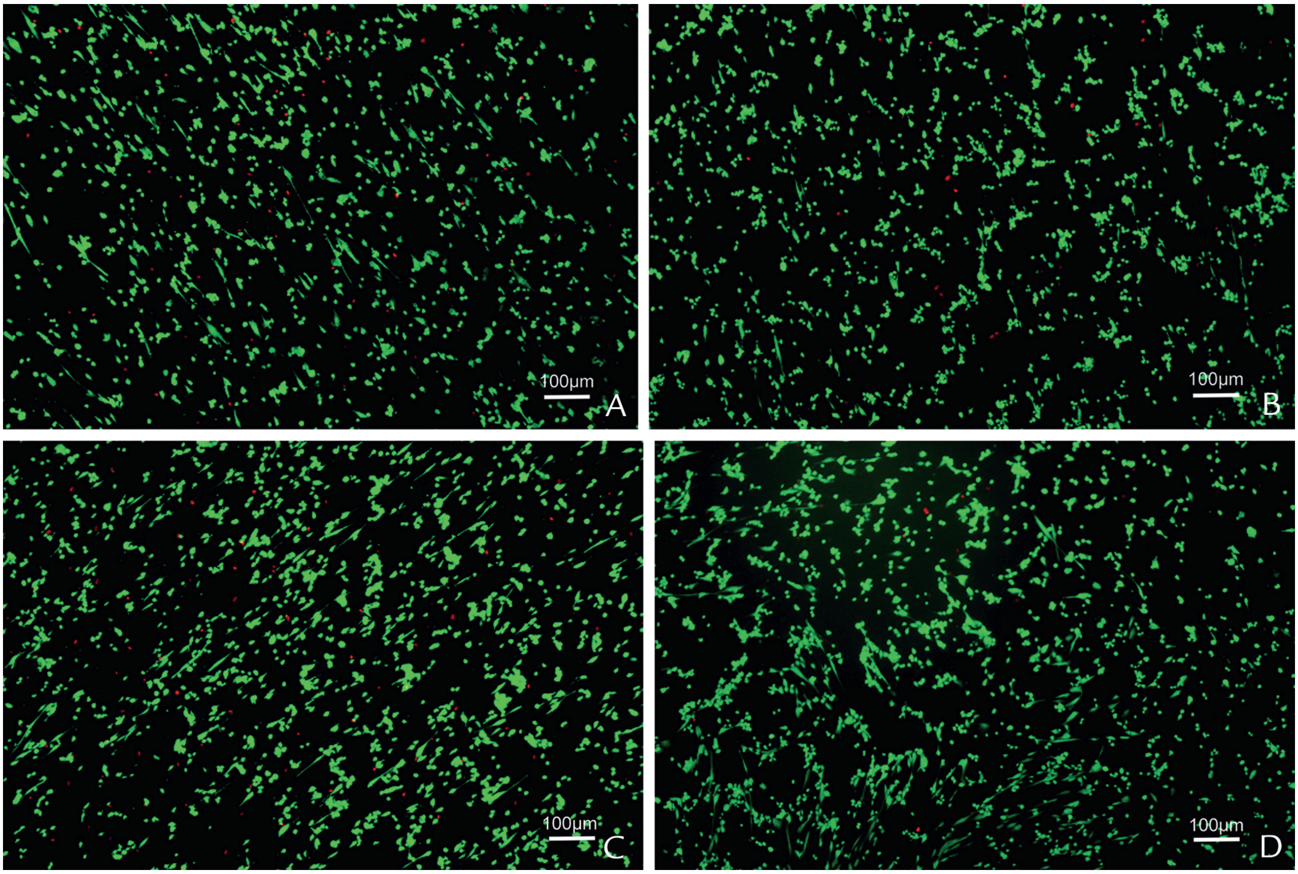

Figure 9. Cell viability of human gingival fibroblasts grown on surfaces. A, Untreated Ti6Al4V. B, Yellow Ti6Al4V. C, Pink Ti6Al4V. D, Zirconia. Live/dead viability assay staining (original magnification ×40).

experiments with electrolyte solutions containing only phosphoric acid showed the reproducible presence of P in the corresponding oxides. Phosphate ions incorporated from the electrolyte solution were present in the outer 62% of the total film thickness.[28] Phosphoric acid was selected as the electrolyte solution in this experiment due to its good biocompatibility as one of the basic components of organisms.[41] The TiO_2 film formed at the alloy-film interface and the oxygen produced at the oxide-electrolyte interface were the result of migration of ions at the alloy-film-electrolyte interfaces during anodization.[42,43] The grain structures observed in the SEM images were formed by oxygen ions, titanium ions, and phosphate ions in the electrolyte solution. Cracks were observed between the grains at high magnification because of oxygen bubbles formed by the oxygen evolution reaction during anodization.

The topography of the abutment surface influenced the adhesion and proliferation of HGFs. Regularly roughened surfaces with specific dimensions can selectively manipulate the activity of fibroblasts and alter their proliferation pattern by means of contact guidance. HGFs showed better adhesion strength, a more mature morphology, and greater proliferation and differentiation on rough titanium surfaces than on smooth surfaces.[18] However, others have reported contradictory results.[44,45] The optimal surface roughness for fibroblast growth is still a matter of debate, although much

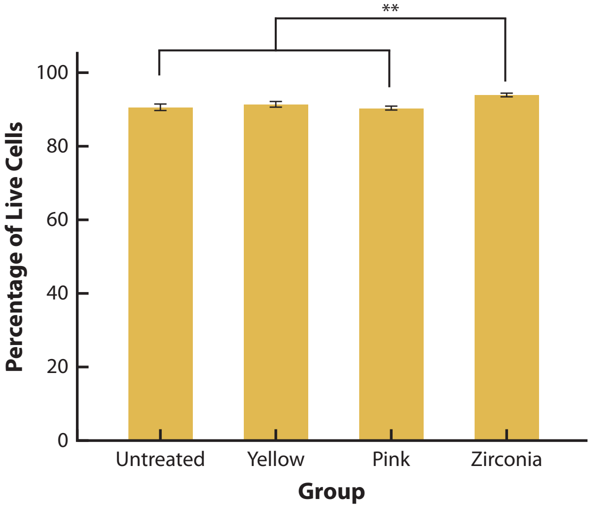

Figure 10. Live cell percentages of untreated Ti6Al4V, anodized Ti6Al4V, and zirconia. Data are means; bars indicate SD. *P<.05 indicated statistically significant differences among tested specimens. SD, standard deviation.

information is available. The surface roughness of the anodized specimen was higher than that of the untreated specimen, but the proliferation of HGFs in these 2 groups was not significantly different. This result may be due to chemical composition, surface morphology, or surface wettability. By creating similar surface topographies on the zirconia and titanium, Nothdurft et al[45] found that fibroblasts exhibited significantly higher proliferation rates on zirconia than on titanium alloy, in agreement with the current findings. Animal and clinical studies have indicated that zirconia was better than titanium in terms of heathy soft tissue, osseointegration, and bacterial adhesion.[2,46]

The initial attachment of fibroblasts could be greatly affected by alterations in surface wettability. The more hydrophilic the surface, the more fibroblasts adhered in the initial stage.[19] The contact angles of the anodized specimens were smaller than those of the untreated specimen, which is consistent with the results of previous research.[35,47] As initially hypothesized, the surface wettability represented by the contact angle improved considerably after anodization. Cell proliferation on the anodized specimens was similar to that on the untreated specimen over 24 hours, which may indicate that Ti6Al4V retains good biocompatibility after anodization.

Pig gingiva was chosen to detect the discoloration of gingiva caused by different specimens because it is similar in color and texture to human keratinized gingiva.[30] The mucosa thickness also plays a vital role in peri-implant esthetics. With a gingiva thickness of 1 mm, the abutments made from zirconia, titanium, gold, or other materials demonstrated color differences above the critical threshold of Δ3.7.[30,31,48] Therefore, a gingival thickness of 1 mm was chosen in this study. An in vitro study revealed that the color differences observed with the pink specimen were lower than for the yellow specimen, which contradicted this result.[49] Although the pig gingiva was harvested and the experiment was conducted shortly after the pig was sacrificed, the pig gingiva was whiter than human gingiva because of lack of blood flow.

Data were analyzed using CIE parameters L*, a*, and b*.[49] ΔE were determined by using the mean values of L*, a*, and b* in the equation: $E=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$. The pink specimen demonstrated higher values of Δa* under the whiter pig gingiva than under human gingiva. This result might explain why the color differences caused by pink specimen were greater than those of the yellow specimen in this study.

CONCLUSIONS

Based on the findings of this in vitro study, the following conclusions were drawn:

1. Anodization changed the color of titanium alloys to pink or yellow at different voltages. Grain formation, roughness, and hydrophilicity increased after treatment.
2. The esthetics and biocompatibility of anodized titanium alloys were not as good as those with zirconia, but the pink and yellow titanium alloys treated by anodization achieved better esthetics for gingiva than untreated titanium alloy.

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