The Effect of Different Dental Implant Surface Characteristics on Bone Immunologic Biomarkers and Microbiologic Parameters: A Randomized Clinical Study

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This study assessed the levels of tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2), receptor activator of nuclear factor kappa B (RANK), RANK ligand (RANKL), osteoprotegerin (OPG), and levels of Fusobacterium nucleatum, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia, and Streptococcus oralis, and levels in the presence of strong acids. The roughness degree is identical to the implants. With the application of acidity, 2- to 4-μm microholes can be formed on their surface. The roughness degree is identical along the implant surface, but it is not used as a crown surface. Modification of dental implants with fluorine is a chemical method in which fluorine, a basic element in bone, is added on the surface to increase osteogenesis. When titanium is modified with fluorine, it gains both surface roughness and the osseointegration-accelerating characteristic of fluorine. Implants with an anodized surface form micro- or nanoporous surfaces as a result of high-intensity (200 A/m²) or high-potential (100 V) potentiostatic or galvanostatic anodization of titanium in the presence of strong acids.
such as \( \text{H}_2\text{SO}_4 \), \( \text{H}_3\text{PO}_4 \), \( \text{HNO}_3 \), and HF.4

Microbial dental plaque accumulation around the implant is the most important cause of implant loss.5 Peri-implant mucositis is a reversible inflammatory reaction in the soft tissue surrounding the functioning implant. Peri-implantitis is an inflammatory reaction characterized by the destruction of the supporting bone around the functioning implant.6 Around the implant areas affected by the disease, gram-negative anaerobic bacterial types are dominant, and they have a microbiologic character similar to that of chronic periodontal infections.7 These bacteria defined by Socransky et al have red complex types (Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia) and orange complex types (Fusobacterium nucleatum and Prevotella intermedia).8

Prostaglandins, especially prostaglandin E2 (PGE2), is accepted as a potent mediator of alveolar bone destruction in periodontitis.9 Many studies have reported that PGE2 levels increase with a dynamic change from health to periodontitis.10 Tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) is a proinflammatory cytokine regulating the gram-negative bacterial response. TNF-\( \alpha \) concentration is an indicator of the bacterial load and the degree of inflammation.11 In areas where peri-implantitis is active, osteoclast presence and activity are needed for bone destruction to occur.12 The formation and activation of osteoclasts is regulated by the interaction of three members of the TNF family: receptor activator of nuclear factor kappa B (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG).13 With the attachment of RANKL on the surface of RANK-presenting osteoclasts and precursors, osteoclast differentiation and activation occur. OPG, which is a soluble protein of the TNF receptors, antagonizes the RANK-RANKL interaction and increases bone formation by inhibiting osteoclastogenesis.14

The objective of this study was to comparatively assess the levels of TNF-\( \alpha \), PGE2, RANKL, RANK, and OPG, as well as \( \text{P} \) gingivalis, \( T \) denticola, \( T \) forsythia, \( F \) nucleatum, \( P \) intermedia, and \( S \) oralis in areas where SLA, fluorde-modified, and anodized implant surfaces are used.

Materials and Methods

Study Population

This study was conducted by calling back patients whose partial missing teeth were treated with implant-supported fixed restorations by same surgeon (E.O.) between 2014 and 2015 at the Department of Periodontology, Faculty of Dentistry, Necmettin Erbakan University, and whose implants had been functioning for at least 1 year. Ethical board approval was obtained from the Clinical Research Ethical Board of the Faculty of Dentistry, Necmettin Erbakan University, under protocol no. 2016/009. All patients were informed about the study, and they were provided informed consent forms; only volunteering participants were included in the study. The study was conducted according to the principles of the Declaration of Helsinki. This study is in compliance with the CONSORT Statement. The study protocol was registered with clinicaltrials.gov (registration no. NCT03693196) prior to its commencement.

The inclusion criteria for the study were as follows: not having any systemic disorders that could affect bone metabolism and wound healing; being older than 18 years; having prostheses in the posterior area; having received a cement-retained implant prosthesis in which a standard abutment was used; having an implant prosthesis that had been functioning for at least 1 year; not having received advanced implant surgery or a bone augmentation procedure during implant surgery; not having received periodontal treatment during the previous year; controlled oral hygiene (overall plaque score < 20%); and having received one SLA, fluorde-modified, or anodized implant. The exclusion criteria were as follows: uncontrolled diabetes mellitus and other uncontrolled diseases; pregnancy; lactation; aggressive periodontitis; overdenture; poor oral hygiene (overall plaque score ≥ 20%); and parafunctional habits such as bruxism. In the study, 71 implants from 37 patients (24 women, 13 men) were assessed. The patients were divided into three groups according to the surface characteristics of the implants:

- Group 1: Titanium implants whose surfaces were roughened with SLA (sandblasted and acid-etched titanium surface; Straumann)
• Group 2: Implants whose surfaces were roughened by modifying with fluorine (OsseoSpeed, Astra Tech)
• Group 3: Implants whose surfaces were roughened by anodization (NobelReplace Conical Connection, TiUnite, Nobel Biocare)

The implants included were categorized into three groups; healthy, peri-implant mucositis, and peri-implantitis.\textsuperscript{15}

**Clinical Periodontal Measurements**

The indices and measurements used in the present study were measured in a specific order and recorded on data-recording forms prepared according to such order. All measurements were made using a Williams-type periodontal plastic probe (PCPNU-15, Hu-Friedy) with a diameter of 0.5 mm and a calibration of 1 mm. Gingival Index (GI),\textsuperscript{16} pocket depth (PD), bleeding on probing (BOP),\textsuperscript{17} clinical attachment level (CAL; distance from the junction implant/crown to the most apically probable portion, measured in millimeters),\textsuperscript{18} and keratinized tissue width around the implant (KTW) were recorded. The panoramic radiograph of the implant-abutment joint acquired during the prosthetic loading process and radiographs acquired at least 1 year later were accepted as fixed reference points. Bone loss was calculated in millimeters by measuring the distance between the implant-abutment joint and the crest using a panoramic radiography program (Veraviewepocs 3D F40, Morita).

**Collecting the PICF and Subgingival Plaque Samples**

The plaques and soft additions around the implants were removed; thereafter, the area was isolated using cotton rolls, and the teeth were dried with air. Peri-implant crevicular fluid (PICF) was collected from the mesiobuccal area of the implant using paper tapes (PerioPaper, Oraflow; Fig 1). Paper tapes were placed 1 to 2 mm inside the peri-implant sulcus using a dental tweezer. After they were kept for 30 seconds, the paper tapes were placed in sterile microcentrifuge tubes containing 200 µL phosphate-buffered saline (PBS). The tubes were kept at –80°C until the day of analysis. PICF analysis

Commercial enzyme-linked immunosorbent assay kits (ELISA Kit, Elabscience Biotechnology) were purchased for measuring TNF-α, PGE2, RANKL, RANK, and OPG, and assays were carried out according to
the manufacturers’ recommendations. The detection limits for the assays were as follows: TNF-α, 7.81 to 500 pg/mL; PGE2, 31.25 to 2,000 pg/mL; RANKL, 0.16 to 10 pg/mL; RANK, 0.16 to 10 pg/mL; and OPG, 0.16 to 10 pg/mL.

Preparation and Assessment of Genomic DNA

For DNA extraction, the collected subgingival plaque samples were processed using a commercially available kit (GF-1 Bacterial DNA Extraction Kit, Vivantis Technologies) according to the manufacturer’s instructions (Fig 3). Total DNA from the target bacterial species was used as standards. The concentrations of standard bacterial DNA were adjusted to 100 µg/mL in distilled water and prepared as four additional serial dilutions (1:10). Genomic DNA was stored at 4°C until use.

Real-Time Quantitative PCR

Selected putative periodontal pathogens (P. intermedia, T. forsythia, T. denticola, F. nucleatum, P. gingivalis, and S. oralis) and total bacterial load in the subgingival biofilms were detected as previously described.19 In order to identify each bacterium and to observe the proliferation curves using real-time polymerase chain reaction (PCR), primer probes were identified, and primer probe sequences were used (Table 1). DNA amplification detection was performed using a real-time PCR system (LightCycler 480 Instrument II, Roche Life Science) by using a master mix (SYBR Green Master Mix, Roche Life Science) for the amplification reaction. The PCR cycling parameters used were 10 minutes at 95°C, 40 cycles of 30 seconds at 95°C, and 1 minute at 60°C. DNA content was calculated from the above-standard curves.
Statistical Analysis

Before the study, power analysis was conducted, and the reliability of significance values was calculated. Power value was found as 80% of the comparison of three groups.

SPSS for Windows version 19.0 (IBM) was used for the statistical analyses. The normality test for continuous numerical variables was conducted using Kolmogorov-Smirnov analysis method. Because all of the variables were not normally distributed, nonparametric methods were preferred in the analyses. Mann-Whitney U test was preferred for two independent groups, while Kruskal-Wallis test was used for multiple groups. Monte Carlo–corrected chi-square analysis was used to identify the association between categorical variables. \( P < .05 \) was considered statistically significant.

Results

Demographic and Clinical Findings

Seventy-one dental implants from 37 individuals were assessed. Demographic characteristics were compared according to the implant groups (Table 2). Groups were found to be similar in terms of age, sex, and smoking status.

Tables 3 and 4 show the clinical characteristics of the implant groups. The groups showed no statistically significant differences in GI, PD, BOP, CAL, KTW, and peri-implant status \( (P > .05) \).

Immunologic Findings

The total amounts of PGE2, TNF-\( \alpha \), RANKL, RANK, and OPG and the RANKL/OPG ratio were not significantly different between groups \( (P > .05) \). The PGE2, TNF-\( \alpha \), and RANKL measurements had higher average values in Group 2. OPG was higher in Group 1, while RANK was higher in Group 3 (Table 5).

Microbiologic Findings

The DNA concentrations of \( F \) nucleatum, \( T \) forsythia, \( P \) intermedia, \( P \) gingivalis, \( S \) oralis, and \( T \) denticola
### Table 2  Patient Demographics of Each Implant Group

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4 (33.3)</td>
<td>4 (28.6)</td>
<td>5 (45.5)</td>
<td>.560</td>
</tr>
<tr>
<td>Female</td>
<td>8 (66.7)</td>
<td>10 (71.4)</td>
<td>6 (54.5)</td>
<td></td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td>就要</td>
<td>要</td>
<td>要</td>
<td>9</td>
</tr>
<tr>
<td>Smoker</td>
<td>4 (33.3)</td>
<td>3 (21.4)</td>
<td>3 (27.3)</td>
<td>.735</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>8 (66.7)</td>
<td>11 (78.6)</td>
<td>8 (72.7)</td>
<td></td>
</tr>
<tr>
<td>Age (y), mean ± SD</td>
<td>52.50 ± 3.46</td>
<td>47.79 ± 2.41</td>
<td>45.36 ± 3.73</td>
<td>.332</td>
</tr>
</tbody>
</table>

Group 1 = titanium implants whose surfaces were roughened with SLA; Group 2 = implants whose surfaces were roughened by modifying with fluorine; Group 3 = implants whose surfaces were roughened by anodization.

### Table 3  Clinical Characteristics at the Implant Sites

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 24)</th>
<th>Group 2 (n = 24)</th>
<th>Group 3 (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival Index</td>
<td>1.37 ± 0.06</td>
<td>1.42 ± 0.08</td>
<td>1.60 ± 0.07</td>
<td>.076</td>
</tr>
<tr>
<td>Probing depth, mm</td>
<td>3.40 ± 0.15</td>
<td>3.43 ± 0.19</td>
<td>3.95 ± 0.22</td>
<td>.104</td>
</tr>
<tr>
<td>Clinical attachment level, mm</td>
<td>3.40 ± 0.15</td>
<td>3.60 ± 0.21</td>
<td>3.95 ± 0.22</td>
<td>.194</td>
</tr>
<tr>
<td>Keratinized tissue width, mm</td>
<td>3.04 ± 0.36</td>
<td>2.50 ± 0.43</td>
<td>3.65 ± 0.45</td>
<td>.179</td>
</tr>
</tbody>
</table>

Group 1 = titanium implants whose surfaces were roughened with SLA; Group 2 = implants whose surfaces were roughened by modifying with fluorine; Group 3 = implants whose surfaces were roughened by anodization.

Values are shown as mean ± SD.

### Table 4  Additional Clinical Characteristics of the Implants

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 24)</th>
<th>Group 2 (n = 24)</th>
<th>Group 3 (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding on probing, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (30.6)</td>
<td>8 (34.7)</td>
<td>8 (34.7)</td>
<td>.150</td>
</tr>
<tr>
<td>No</td>
<td>17 (35.4)</td>
<td>16 (33.3)</td>
<td>15 (31.3)</td>
<td></td>
</tr>
<tr>
<td>Peri implant status, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>18 (37.6)</td>
<td>15 (31.2)</td>
<td>15 (31.2)</td>
<td></td>
</tr>
<tr>
<td>Peri-implant mucositis</td>
<td>3 (25.0)</td>
<td>5 (41.7)</td>
<td>4 (33.3)</td>
<td>.550</td>
</tr>
<tr>
<td>Peri-implantitis</td>
<td>3 (27.4)</td>
<td>4 (36.3)</td>
<td>4 (36.3)</td>
<td></td>
</tr>
</tbody>
</table>

Group 1 = titanium implants whose surfaces were roughened with SLA; Group 2 = implants whose surfaces were roughened by modifying with fluorine; Group 3 = implants whose surfaces were roughened by anodization.
were compared among the groups, and the results showed significant differences (Table 6). F nucleatum, T forsythia, P intermedia, P gingivalis, and T denticola were significantly higher in Group 3 implants (P < .05). F nucleatum, T forsythia, and T denticola DNA concentrations were not significantly different between Groups 1 and 2. P intermedia and P gingivalis DNA concentrations showed significant differences, with the value in Group 2 significantly higher than Group 1. S oralis DNA concentrations were higher in Group 2 (P < .05).

**Table 5 Biochemical Data Obtained from the PICF Samples**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 24)</th>
<th>Group 2 (n = 24)</th>
<th>Group 3 (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE2 (pg/30 sn)</td>
<td>25.92 ± 1.22</td>
<td>27.75 ± 1.28</td>
<td>26.47 ± 1.28</td>
<td>.534</td>
</tr>
<tr>
<td>TNF-α (pg/30 sn)</td>
<td>34.69 ± 0.44</td>
<td>36.13 ± 0.92</td>
<td>35.55 ± 0.51</td>
<td>.297</td>
</tr>
<tr>
<td>OPG (pg/30 sn)</td>
<td>1.62 ± 0.029</td>
<td>1.60 ± 0.005</td>
<td>1.59 ± 0.005</td>
<td>.676</td>
</tr>
<tr>
<td>RANK (pg/30 sn)</td>
<td>0.67 ± 0.036</td>
<td>0.75 ± 0.058</td>
<td>0.93 ± 0.091</td>
<td>.178</td>
</tr>
<tr>
<td>RANKL (pg/30 sn)</td>
<td>0.80 ± 0.007</td>
<td>0.81 ± 0.005</td>
<td>0.80 ± 0.006</td>
<td>.576</td>
</tr>
<tr>
<td>RANKL/OPG</td>
<td>0.41 ± 0.015</td>
<td>0.46 ± 0.038</td>
<td>0.58 ± 0.056</td>
<td>.198</td>
</tr>
</tbody>
</table>

Group 1 = titanium implants whose surfaces were roughened with SLA; Group 2 = implants whose surfaces were roughened by modifying with fluorine; Group 3 = implants whose surfaces were roughened by anodization.

Values are shown as mean ± SD.

**Table 6 Microbial Profile of Subgingival Plaque Biofilm**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 24)</th>
<th>Group 2 (n = 24)</th>
<th>Group 3 (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P intermedia</td>
<td>5.6^{104} ± 2.6^{104} (6^{101})_{a,b}</td>
<td>7.5^{104} ± 2.6^{104} (1.065^{103})_{a,c}</td>
<td>1.12^{106} ± 5.7^{105} (5.38^{104})_{a,c}</td>
<td>.002*</td>
</tr>
<tr>
<td>T forsythia</td>
<td>7.6^{104} ± 4.1^{104} (6^{101})_{a}</td>
<td>3.9^{104} ± 3.4^{104} (5.65^{102})_{b}</td>
<td>5.5^{105} ± 3.2^{105} (5.23^{102})_{b}</td>
<td>.029*</td>
</tr>
<tr>
<td>T denticola</td>
<td>2.7^{105} ± 1.4^{105} (5.41^{103})_{a}</td>
<td>3.4^{105} ± 1.4^{104} (1.025^{104})_{b}</td>
<td>1.5^{104} ± 8.8^{105} (4.16^{104})_{b}</td>
<td>.001*</td>
</tr>
<tr>
<td>F nucleatum</td>
<td>4.29^{103} ± 2.4^{104} (7.37^{102})_{a}</td>
<td>5.32^{103} ± 2.3^{104} (1.405^{103})_{b}</td>
<td>4.06^{104} ± 3.09^{105} (6.09^{104})_{a,b}</td>
<td>.001*</td>
</tr>
<tr>
<td>P gingivalis</td>
<td>3.2^{105} ± 2.9^{105} (3)^{b}</td>
<td>9.0^{105} ± 4.4^{105} (3.4^{102})_{a,c}</td>
<td>1.1^{106} ± 1.1^{105} (5.9^{107})_{a,c}</td>
<td>.006*</td>
</tr>
<tr>
<td>S oralis</td>
<td>3.1^{104} ± 1.6^{104} (1.52^{103})_{a}</td>
<td>2.0^{105} ± 8.8^{104} (3.56^{102})_{b}</td>
<td>1.18^{105} ± 4.3^{104} (1.26^{103})_{a,b}</td>
<td>.033*</td>
</tr>
</tbody>
</table>

Group 1 = titanium implants whose surfaces were roughened with SLA; Group 2 = implants whose surfaces were roughened by modifying with fluorine; Group 3 = implants whose surfaces were roughened by anodization; F nucleatum = Fusobacterium nucleatum; T forsythia = Tannerella forsythia; P intermedia = Prevotella intermedia; P gingivalis = Porphyromonas gingivalis; S oralis = Streptococcus oralis; T denticola = Treponema denticola.

The same superscript letters indicate groups that are different from each other. Values are shown as mean ± SD. Numbers in parentheses represent the difference between the two values above it.

*Statistically significant difference (P < .05).

**Discussion**

The results of this study have shown that changes in PICF levels of PGE2, TNF-α, RANKL, RANK, OPG, and the RANKL/OPG ratio were not statistically significant in any of the groups, while the DNA concentrations of F nucleatum, T forsythia,
P intermedia, P gingivalis, and T denticola were significantly higher in Group 3 implants.

Heitz-Mayfield et al evaluated crestal bone protection 12 weeks after they placed implants in a pig model. The reported extent of bone protection in fluorine-modified and SLA surface implants were higher than that in anodized implants.20 Jimbo and Albrektsson reviewed 71 articles with at least 5 years of follow-up and reported that SLA surface implants had less marginal bone loss than anodized or processed-surface implants.21 When these studies are taken into consideration, the general view is that marginal bone loss in the anodized surface is higher than that in the fluorine-modified and SLA surfaces. Similar to those results, the present authors found that the concentrations of F nucleatum, T denticola, T forsythia, P intermedia, and P gingivalis, which are responsible for bone loss, were significantly higher in anodized surfaces. This finding can help predict future bone loss.

Derks et al examined rates of early- and late-period implant loss according to the brands of implants in a 9-year follow-up study. Using the SLA surface as the reference (odds ratio [OR]: 1.0), early loss rates were found to be 1.9 times higher on the anodized surface and 2.1 times higher on the fluorine-modified surface. In late implant loss, compared with the SLA surface, the implant loss rate was 6.1 times higher for the anodized surface and 5.2 times higher for the fluorine-modified surface.22 In another study by Derks et al on the same patient population, peri-implantitis prevalence at the end of 9 years was examined. When the SLA surface was taken as the reference (OR: 1.0), moderate/advanced peri-implantitis was found to be 3.8 times higher on the anodized surface and 3.6 times higher on the fluorine-modified surface.23 When these rates were taken into consideration, long-term clinical success rates of anodized implants were found to be lower. Similarly, in the present study, F nucleatum, T forsythia, P intermedia, P gingivalis, and T denticola, which cause bone loss, were higher in the anodized-surface group.

Previous studies have shown that the total amount of gingival crevicular fluid is a better indicator than is its concentration. In these studies, the researchers argued that concentration is directly influenced by sample size, and the total amount gives a more objective result.24 In the present study, the total amount of PICF was assessed.

RANK/RANKL/OPG interaction, TNF-α, and PGE2 are complex processes that can affect systemic health, hormonal and metabolic states, drug use, and bone metabolism.25–27 In the present study, the sample size is small. Therefore, the difference between groups in the total amounts of RANKL, RANK, OPG, TNF-α, and PGE2 may not be significant. Studies with a greater sample size can better and more clearly understand the impact of implant surface properties. However, as the bacterial study is performed by PCR, it is not possible to distinguish the number of viable or nonviable bacteria, which is another limitation of this study.

Conclusions

Within the limitations of this study, SLA- and fluorine-modified implant surfaces may be more successful than anodized-surface implants. It remains unclear whether the surface characteristics and physiochemical structure of dental implants affect the microbial content of biofilms around the implants and whether it affects PICF biomarkers. To better understand this effect, large-scale studies with longer follow-up periods are warranted.

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References


