Keywords: dental implant, laser ablation, inductively coupled plasma, titanium alloy

Introduction

The use of dental implants in the treatment of edentulism to recover aesthetic and functional losses is a frequently used treatment modality. The features considered as required for dental implant materials are: resistance to corrosion, non-allergenicity, sterility, harmony with the surrounding tissues, and resistance to mechanical loads (5). In the past, dental implants made of metal and metal alloys, polymer-based materials, ceramics, glass and carbon materials were used. Today, titanium (Ti) and its alloys are generally used in the production of dental implants and prosthetic upper structure fragments. Ti is generally considered to be an inert metal due to its titanium oxide (TiO₂) layer (3, 21, 29); but, like other metals that are in contact with biological systems, Ti may corrode and release ion and other particles (8, 33). Such particles may be accumulated on the adjacent tissues (17, 24, 25) or may be carried to distant organs with the blood (15, 26, 27). The main parts of the dental implants, which are embedded in the jaw bone, release ions directly to the body. Gingiva formers or implant covers are in contact especially with the soft tissue. Therefore, they may cause local or systemic side effects (23). Released ions may activate the immune system, thus causing hypersensitivity reactions, tumors and other pathologies (1, 2, 18, 30, 36, 37). There are limited data available about the local and systemic effects of dental implant materials made of Ti and its alloys. Friskén et al. (13) evaluated the titanium release into regional lymph nodes, lungs, spleens and livers following the surgery of a single dental implant into the mandibles of sheep. The animals with successful implants did not show any statistically significant different levels of titanium in any organ compared to controls. On the other hand, two animals with failed implants were shown to have higher titanium levels in the lungs (2.2–3.8 times the mean of the controls) and regional lymph nodes (7–9.4 times the levels in controls). Du Preez et al. (9) reported a case who lost all 6 dental implants due to titanium hypersensitivity. After histological evaluation, chronic inflammatory response with concomitant fibrosis was found around all implants and foreign body giant cell reaction was found around two implants. Egusa et al. (11) reported facial eczema in a patient after surgery of two implants on the mandible to support the overdenture prosthesis. Sicilia et al. (28) encountered titanium allergy in 9 out of 1500 patients who were treated with a titanium dental implant. Recently, Flatebo et al. (12) evaluated the titanium particles in peri-implant oral mucosa. They revealed that 6 months after implant surgery there were more Ti particles at the implant sites than at control sites according to a probability test in the deeper part, rather than in the epithelial part.

In the light of the above data, this study aimed to determine the level of titanium element in soft tissues that are in contact with the dental implant cover screw and to make a comparison with a control group.

Materials and Methods

Patient selection

Thirty generally healthy, non-smoking patients who were treated in Istanbul University, Faculty of Dentistry, Department of Oral Implantology, between May 2010 and January 2011 were included in the study. The study protocol was approved by the Ethical Committee of Istanbul University in accordance with the Helsinki Declaration of 1975, as revised in 2000. Written consents were obtained from all participants.
Twenty patients with single tooth edentulism, treated with dental implants, were included in the study as a test group. The control group comprised of 10 volunteers who needed gingivoplasty and had not received any treatment with titanium or titanium alloys previously.

**Dental implant surgery**

All dental implant surgery procedures were carried out under infiltrative local anesthesia (Ultracain DS Fort, Sanofi Avantis, Istanbul, Turkey). Full thickness flap was elevated in all surgeries. Osteotomy and implant installation were performed according to the manufacturer’s surgical protocol (Straumann Dental Implant Systems, Basel, Switzerland). Cover screws (code number: 048.371V4, Straumann Dental Implant Systems, Basel, Switzerland) were used during the healing phase. A two-stage surgical protocol was chosen in all cases. Primary wound closure was completed using silk interrupted sutures (Dogsan Medical Supplies Industry, Trabzon, Turkey).

**Second-stage surgery and sampling for the test group**

Second-stage surgery was performed under infiltrative local anesthesia (Ultracain DS Fort, Sanofi Avantis, Istanbul, Turkey) three months after the dental implant surgery. Implants were exposed with the aid of a no. 15 lancet (Braun, Aesculap AG, Tuttingen, Germany). Gingiva samples from the test group were placed in Eppendorf tubes and stored at -18 °C. Cover screws were removed and gingiva formers were placed.

**Sampling for the control group**

Gum samples (volume of 4 mm×4 mm) were taken from edentulous areas in patients who needed gingivoplasty. All procedures were done under infiltrative local anesthesia (Ultracain DS Fort, Sanofi Avantis, Istanbul, Turkey) and biopsies were stored like the gum samples in the test group.

**Sample preparation and analysis**

Instrumental analysis was carried out in Istanbul University, Institute of Forensic Science, Forensic Toxicology Laboratories, using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS). The same sample preparation processes were conducted for both the test group and the control one. Samples stored at -18 °C until analysis were adhered on laminas with the Cyanoacrylate adhesive. Attention was paid to leave the gingiva surfaces that are in contact with titanium on the upper side. Then fixed samples were dried in an incubator at 90 °C for 2 h. In addition, adhesive was applied on lamina without any tissues and this sample was used as blank lamina. Certified Standard Material (CRM), NIST 612 glass matrix (Gaithersburg, MD) was used for a quality control sample with the 50.1 mg/kg certified Ti level. The relative standard deviation (RSD, %) was calculated from the results of six replicated CRM Glass samples for stability of the method. Values under 15 % RSD were considered acceptable. This study was carried out using Thermofisher Scientific X Series II (Germany) ICP-MS coupled to Nd:YAG laser unit operating 213 nm wavelength (New Wave Research Laser Ablation System, Merchantek, USA). LA-ICP-MS semi-

**Statistical analysis**

Statistical analyses were performed using SPSS for Windows (Version 17.0, SPSS Inc, Chicago, IL, USA). Student t-test was used to compare the two study groups in terms of age, gender and sampling region. Mann–Whitney U-test was used to compare the Ti levels of the two groups. Values were considered significant when the p value was <0.05.

**Results and Discussion**

Our study examined the level of titanium element in 20 gum samples from the test group having contact with the cover screw during 3 months and in 10 gum samples from the control group having no contact with titanium element.

### Table 1

**ICP-MS parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma gas</td>
<td>13.0 L/min</td>
</tr>
<tr>
<td>Auxiliary gas</td>
<td>0.77 L/min</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>0.77 L/min</td>
</tr>
<tr>
<td>Additional gas (He)</td>
<td>214 L/min</td>
</tr>
<tr>
<td>Dwell time</td>
<td>10 ms</td>
</tr>
<tr>
<td>Cones</td>
<td>Ni (Sample and skimmer)</td>
</tr>
</tbody>
</table>

**LA parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>213 nm</td>
</tr>
<tr>
<td>Scan speed</td>
<td>20 µm/sec</td>
</tr>
<tr>
<td>Spot size</td>
<td>20 µm</td>
</tr>
<tr>
<td>Pulse frequency</td>
<td>10 Hz</td>
</tr>
<tr>
<td>Number of pulses/analysis point</td>
<td>20</td>
</tr>
<tr>
<td>Transport gases</td>
<td>Argon–Helium</td>
</tr>
<tr>
<td>Focus</td>
<td>On sample surface (autofocus)</td>
</tr>
</tbody>
</table>

**Table 2**

Information about the patients who participated in the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test group (n = 20)</th>
<th>Control group (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9 (45 %)</td>
<td>4 (40 %)</td>
</tr>
<tr>
<td>Male</td>
<td>11 (55 %)</td>
<td>6 (60 %)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>37.8</td>
<td>41.1</td>
</tr>
<tr>
<td>Sampling Region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>23–52</td>
<td>32–52</td>
</tr>
<tr>
<td>Maxilla</td>
<td>7 (35 %)</td>
<td>4 (40 %)</td>
</tr>
<tr>
<td>Mandible</td>
<td>13 (65 %)</td>
<td>6 (60 %)</td>
</tr>
</tbody>
</table>

Information about the participants in terms of age, gender, and sampling region is shown in Table 2. The differences between the age, gender, and sampling region in the two groups
were not statistically significant \((p = 0.39; p = 0.40; p = 0.08,\) respectively). No titanium element was found in blank laminas. The result of the CRM Glass sample was 5.6 % RSD.

The average values of the semi-quantitative Ti levels were 50.4 µg/g ± 23.5 µg/g in the test group and 37.1 µg/g ± 1.0 µg/g in the control group. This difference was not found to be statistically different \((p = 0.075)\). Fig. 1 shows the result from the analysis of one of the ablated gingival samples in the test group. The ablated region demonstrated Ti response of mass detected in the figure, whereas the gas blank was evaluated as a non-response. Additionally, different titanium levels among patients were obtained from the gum samples in contact with the titanium implant as shown in Fig. 2. In the test group, patients coded from 16 to 20 were separately evaluated because their Ti levels were higher than the average value \((50.4 \mu g/g)\), but no significant difference was found for these five patients in terms of age or gender compared to others \((p = 0.447; p = 0.267,\) respectively).

![Fig. 1. Titanium response of gingiva surface in contact with the implant cover screw for 3 months.](image)

![Fig. 2. Ti levels in samples from patients in the test group.](image)

In many studies about orthopedics and dermatology; Ti particles have been found in the peri-implant area (18) and in lymph nodes among cases that lack a Ti-based implant (13, 25, 32) and some of these studies reported an increased level of titanium in the serum (16). Meningaud et al. (22) studied the metal release from titanium miniplates in maxillofacial surgery and found no correlation between Ti levels and duration of plating. They suggested that released Ti may precipitate locally as in physiological conditions; Ti ions cannot be chelated for transportation. Different Ti levels were reported according to the sensitivity of the techniques and contamination probability in many studies (13, 20). Platebo et al. (12) demonstrated the presence of Ti particles both in the peri-implant oral mucosa and normal oral mucosa by LA-ICP-MS. The authors compared the quantity, size, element signature and distribution of Ti element and showed the presence of Ti particles both in control and test samples, implying contamination with TiO\(_2\), which is widely used as a whitening agent or as a safe ingredient in cosmetics, pharmaceuticals and food products. For this reason, we determined the particular Ti level in the oral cavity of our control group patients, despite that they did not have a Ti implant.

In our study, different Ti levels were detected in the test group, although all gingival biopsies had been in contact with the implant for 3 months. This may have resulted from different metabolic rates of the individuals, different pH values of their oral cavity depending on nutrition, age, smoking, etc. Ti values may also increase with extending the period of contact with the implant or with increasing the number of implants. Future studies should include more samples and examine the various results depending on the age, gender, number of dental implants and contact period with titanium.

It is very difficult to measure the concentration of ions in the tissues around the implant (19). In the past, ion presence was determined through the acidulation of all of the tissue to be examined. However, it is impossible to obtain detailed results with these techniques (14). LA-ICP-MS is a technique determining the ion release in the solid materials reliably and in a short time (31). LA-ICP-MS also gives the opportunity for multi-element analysis of solid materials and is being used effectively in many fields (4, 10). With LA-ICP-MS, along with food, marine speciation, solids and sediments, archaeological findings, botanical tissues, and environmental materials, it is now possible to directly examine biological samples such as teeth, hair and nails as well. In this study, the success of the LA-ICP-MS technique was examined in practice as well as through the analysis of elements. The parameters used in our study were sufficient to obtain the required data. Therefore, the results of our study could be taken as a reference in future studies. In this technique, during the analysis, no additional chemicals were used and no additional preliminary preparation was done. Gingiva samples were placed in eppendorf tubes and stored at -18 °C; then they were dried in the incubator and analyzed. In this way, the reliability of the process was higher and time was saved. Since gingival biopsy materials are of a very low amount, Laser Ablation may be the best method to determine the inorganic profile by ablating the low amounted tissue surface. Otherwise it would be difficult to provide a sufficient amount of gingival sample for acid digestion for liquid analysis. After the evaluation of blank lamina, it was demonstrated that the adhesive did not contain any titanium contamination and it can be concluded that this may be a suitable sample preparation process for solid samples, as well as biological tissue, to be studied using Laser Ablation.

Although Ti is defined as a biocompatible element, it could directly or indirectly induce necrosis and bone resorption. The study of Mine et al. (23) found that titanium ions may have an adverse effect on the bone remodeling at the interface between the dental implant and the tissue, and that titanium...
ion change the gene expression rates related to the osteoclast concentration. Despite these data, the study of Velasco Ortega et al. (34) showed that a Ti-6Al-4V alloy had no cytotoxic effect on rat and human fibroblasts and had high biocompatibility. Our results are in agreement with those of some studies which show that metal ions are released from the implant materials in the short or long term (6, 7, 35). However, there is no sufficient data about the effect of Ti ions on human health. Also, the toxic effect may increase with the period of exposure to the above-mentioned factors. Dental implants are placed in a patient’s mouth to function for a long time (if possible, for a lifetime). For this reason, Ti toxicity should be well evaluated in various study designs.

Conclusions
This study found that different amounts of titanium may penetrate into the gingiva in as short as three months. LA-ICP-MS is a safe, fast and practical technique to examine the presence of trace element in the soft tissue samples.

Acknowledgements
There was no financial sponsor for this study except the authors. Implants and surgical costs were covered at the patients’ expenses and by the health insurance system. We declare that there are no conflicts of interest.

REFERENCES