The mucosal barrier at implant abutments of different materials

Authors’ affiliation:
Maria Welander, Ingemar Abrahamsson, Tord Berglundh, Department of Periodontology, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden

Correspondence to:
Maria Welander, Department of Periodontology The Sahlgrenska Academy at Göteborg University Box 410 405 30 Göteborg Sweden Tel.: +4631 786 3780 Fax: +4631 786 3791 e-mail: maria.welander@odontologi.gu.se

Key words: biomaterials, dental implants, gold alloy, histology, peri-implant mucosa, soft tissue, titanium, zirconia

Abstract
Objective: The aim of the present study was to analyze the soft tissue barrier formed to implant abutments made of different materials.

Material and methods: Six Labrador dogs, about 1 year old, were used. All mandibular premolars and the first, second and third maxillary premolars were extracted. Three months later four implants (OsseoSpeed™, 4.5 × 9 mm, Astra Tech Dental, Mölndal, Sweden) were placed in the edentulous premolar region on one side of the mandible and healing abutments were connected. One month later, the healing abutments were disconnected and four new abutments were placed in a randomized order. Two of the abutments were made of titanium (Ti), while the remaining abutments were made of ZrO2 or AuPt-alloy. A 5-months plaque control program was initiated. Three months after implant surgery, the implant installation procedure and the subsequent abutment shift were repeated in the contra-lateral mandibular region. Two months later, the dogs were euthanized and biopsies containing the implant and the surrounding soft and hard peri-implant tissues were collected and prepared for histological analysis.

Results: It was demonstrated that the soft tissue dimensions at Ti- and ZrO2 abutments remained stable between 2 and 5 months of healing. At Au/Pt-alloy abutment sites, however, an apical shift of the barrier epithelium and the marginal bone occurred between 2 and 5 months of healing. In addition, the 80-μm-wide connective tissue zone lateral to the Au/Pt-alloy abutments contained lower amounts of collagen and fibroblasts and larger fractions of leukocytes than the corresponding connective tissue zone of abutments made of Ti and ZrO2.

Conclusion: It is suggested that the soft tissue healing to abutments made of titanium and ZrO2 is different to that at abutments made of AuPt-alloy.
based on its ability to promote integration to the connective tissue of the peri-implant mucosa during healing.

The healing around implants made of gold-alloys or ceramic materials were analyzed in experimental studies in animals. The majority of studies described integration between bone and the biomaterial and it was reported that osseointegration to titanium was superior to that at gold-alloys [Albrektsson et al. 1982; Thomsen et al. 1997, Abrahamsson & Cardaropoli 2007]. Healing around ceramic implants made of zirconium, on the other hand, resulted in bone formation in contact with the biomaterial, which was similar to that at implants made of titanium [Albrektsson et al. 1985; Thomsen et al. 1997; Sennerby et al. 2005].

The integration of oral mucosa to implant components of different materials was examined in few studies. Abrahamsson et al. [1998], in an experimental study in dogs, reported that the implant material was of decisive importance for the quality of the attachment that formed between the mucosa and the implant abutment. While abutments made of aluminum-based ceramic provided conditions for a mucosal attachment that was similar to that of titanium, gold-alloy abutments did not promote healing that resulted in integration of connective tissue to the implant component and, in such sites, the mucosal attachment was established apical to the abutment/fixture junction. Conflicting data were reported in an experimental study on dogs by Abrahamsson & Cardaropoli [2007]. In this experiment, the soft tissue dimensions were similar at implants made of gold-alloy and titanium. Furthermore, Vigolo et al. [2006] in a study on 20 subjects reported that no clinical differences in soft tissue conditions were detected between implants with either titanium or gold-alloy abutments.

There is limited information on soft tissue integration to implants made of zirconium. Results presented in clinical reports indicated that favorable soft tissue results were obtained around abutments made of zirconium [Brodbbeck 2003; Kohal & Klaus 2004; Tan & Dunne 2004]. Glauser et al. [2004] in a 4-year follow-up on 18 subjects reported that healthy mucosal conditions and stable marginal bone levels were observed at implants with zirconium abutments.

The aim of the present study was to further analyze the soft tissue barrier formed to implant abutments made of different materials.

Material and methods

Six Labrador dogs, about 1 year old, were used. The regional Ethics Committee for Animal research, Göteborg, Sweden, approved the study protocol. All surgical procedures were performed using general anesthesia induced with propofol [10 mg/ml, 0.6 ml/kg] intravenously and sustained with N2O:O2 [1:1, 5–2] and isoflurane employing endotracheal intubation.

At the start of the experiment, all mandibular premolars and the first, second and third maxillary premolars were extracted. Three months later, buccal and lingual mucoperiostal flaps were elevated and four implants [OsseoSpeed™, 4.5 ST × 9 mm, Astra Tech Dental, Mölndal, Sweden] were placed in the edentulous premolar region in one side of the mandible. Healing abutments [Zebra™ 6 mm, Astra Tech Dental, Mölndal, Sweden] were connected to the implants and the flaps were adjusted and sutured.

One month after implant placement, the healing abutments were disconnected and four new abutments were placed in a randomized order [Fig. 1]. Two of the abutments were made of titanium (Ti), while the remaining abutments were made of ZrO2 (ceramic) or AuPt-alloy [cast-to]. All abutments had similar dimensions and geometry. A 5-month plaque control program was initiated. The program included cleaning of implants and teeth with a toothbrush and dentifrice once a day, 5 days a week.

Three months after implant surgery, the implant installation procedure and the subsequent abutment shift were repeated in the contra-lateral mandibular region.

Two months later, i.e. 5 months after the first abutment connection procedure, the dogs were euthanized by an overdose of Sodium-Pentothal™ (Abbott Scandinavia AB, Solna, Sweden) and perfused through the carotid arteries by a fixative [Karnovsky 1965]. The mandibles were removed and placed in the fixative. The implant sites were dissected using a diamond saw [Exakt Apparatebau, Norderstedt, Germany]. The tissue blocks that included the implant and the surrounding soft and hard peri-implant tissues, were processed using a modification of the fracture technique [Thomsen & Ericson 1985] as described by Berglundh et al. [1991, 1994]. The tissue samples were placed in ethylenediaminetetraacetic acid (EDTA). Before the hard tissue was fully decalcified incisions, parallel with the long axis of the implant, were made through the peri-implant tissues. Four different units, mesio-buccal, disto-buccal, mesio-lingual and disto-lingual were hereby obtained and carefully separated from the implants. Decalcification was completed in EDTA and dehydration was performed in serial steps of ethanol concentrations. A secondary fixation in OsO4 was performed and the specimens were embedded in epoxy resin [EPON® Fluka Chemie GmbH, Buchs, Switzerland] [Schroeder 1969]. Sections were produced with the microtome set at 3 μm and stained in PAS and toluidine blue (Schroeder 1969).

The histological examination was performed in a Leica DM-RBE® microscope (Leica, Heidelberg, Germany) equipped with an image system Q-500 MC™ (Leica).

Fig. 1. The four abutments [from left: Ti, ZrO2, Ti, Au/Pt-alloy] in place 1 month after implant placement.
Histological measurements

In each section, the following landmarks were identified and used for the linear measurements (Fig. 2): PM, the margin of the peri-implant mucosa; aJE, the apical termination of the barrier epithelium; B, the marginal level of bone to implant contact; A/F, the abutment/fixture borderline. The vertical distances between the landmarks were determined in a direction that was parallel to the long axis of the implant.

The composition of the connective tissue compartment of the peri-implant mucosa that was in contact with the different abutments and interposed between aJE and B was assessed using a stereological technique as described previously (Schroeder & Münzel-Pedrazzoli 1973; Berglundh et al. 1991; Abrahamsson et al. 1999). The analysis was confined to an 80-μm-wide tissue zone lateral to the abutment interface. A lattice comprising 100 light points was superimposed over the epithelium at a magnification of ×1000 and the relative proportions of the connective tissue occupied by collagen (Co), fibroblasts (Fi), vascular structures (V), leukocytes (Leu) and residual tissue (R) (e.g., nerves, matrix components and unidentified structures) were determined. The relative volume of infiltrating leukocytes within the barrier epithelium was assessed according to methods described by Schroeder [1973] and Berglundh et al. [1992a, 1992b]. A lattice comprising 400 points was superimposed over the epithelium at a magnification of ×1000 and the percentage of infiltrating leukocytes in relation to the volume of the barrier epithelium was determined.

Data analysis

Mean values from the different assessments were calculated for each abutment type and animal (n = 6). Differences were analyzed using the two-way analysis of variance (ANOVA) and the Student–Newman–Keuls test. The null hypothesis was rejected at P < 0.05.

Results

One implant in one dog was lost during the healing after implant surgery. Healing at the 47 remaining implant sites was uneventful.

Histological observations

The soft tissue interface to the different implant abutments comprised an epithelial and connective tissue portion that followed the geometry of the devices made of titanium (Ti), ZrO2 (ceramic) and AuPt-alloy (cast-to). In sections from Ti- and ceramic-abutment sites, the dimensions of epithelial and connective tissue components remained stable between 2 and 5 months of healing. At cast-to sites, however, an apical shift of the barrier epithelium and the level of marginal bone occurred in most sites between 2 and 5 months of healing. The results from the linear measurements are presented in Table 1. Thus, the barrier epithelium at Ti- and ceramic abutments at 2 months of healing extended to a distance apical of PM that was 1.80 and 1.60 mm, while at 5 months the corresponding dimensions were 1.83 and 1.75 mm, respectively. In sections representing cast-to abutments, however, the mean PM to aJE distance increased from 1.56 to 2.07 mm between 2 and 5 months of healing. Similar observations were made regarding the position of marginal bone level at the different abutments. Thus, at Ti- and ceramic-abutment sites the distance between A/F and B was 1.17 and 1.07 mm at 2 months, and 1.02 and 0.95 mm at 5 months, respectively. The corresponding distances at cast-to abutment sites representing 2 and 5 months of healing were 1.05 and 1.71 mm, respectively.

The results from the assessments of the composition of the connective tissue compartment in contact with the different abutments are presented in Tables 2 and 3. The barrier epithelium extended to a position that was apical of the A/F borderline in four out of six cast-to abutment sites at 5 months of healing and, hence, impeded connective tissue analysis. In sites representing 2 months of healing, the volume fractions occupied by collagen and fibroblasts were significantly smaller at cast-to sites than at Ti and ceramic sites. Conversely, the proportions of leukocytes and residual tissue were significantly larger

Table 2. The composition of the connective tissue zone between aJE and A/F at 2 months of healing

<table>
<thead>
<tr>
<th>%</th>
<th>Ti</th>
<th>Ceramic</th>
<th>Cast-to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>41.4 (10.7)</td>
<td>43.5 (7.7)</td>
<td>18.6 (6.9)*</td>
</tr>
<tr>
<td>V</td>
<td>7.3 (5.1)</td>
<td>6.6 (3.1)</td>
<td>3.9 (3.6)</td>
</tr>
<tr>
<td>Fi</td>
<td>29.2 (9.1)</td>
<td>35.0 (9.0)</td>
<td>15.9 (6.9)*</td>
</tr>
<tr>
<td>Leu</td>
<td>7.5 (4.0)</td>
<td>6.5 (3.9)</td>
<td>25.3 (6.4)*</td>
</tr>
<tr>
<td>R</td>
<td>14.6 (9.3)</td>
<td>8.5 (7.2)</td>
<td>36.5 (17.0)*</td>
</tr>
</tbody>
</table>

Mean values and standard deviations. *P < 0.05 between cast-to and Ti and between cast-to and ceramic.

Table 1. Dimensions of the peri-implant mucosa at 2 and 5 months of healing

<table>
<thead>
<tr>
<th>Landmarks</th>
<th>Ti 2 m</th>
<th>Ti 5 m</th>
<th>Ceramic 2 m</th>
<th>Ceramic 5 m</th>
<th>Cast-to 2 m</th>
<th>Cast-to 5 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM–B</td>
<td>3.13 (0.33)</td>
<td>2.85 (0.37)</td>
<td>3.08 (0.39)</td>
<td>2.82 (0.38)</td>
<td>3.04 (0.34)</td>
<td>3.54 (1.05)</td>
</tr>
<tr>
<td>PM–aJE</td>
<td>1.80 (0.29)</td>
<td>1.83 (0.22)</td>
<td>1.60 (0.31)</td>
<td>1.75 (0.27)</td>
<td>1.56 (0.40)</td>
<td>2.07 (0.51)</td>
</tr>
<tr>
<td>A/F–B</td>
<td>1.17 (0.26)</td>
<td>1.02 (0.34)</td>
<td>1.07 (0.27)</td>
<td>0.95 (0.27)</td>
<td>1.05 (0.41)</td>
<td>1.71 (1.30)</td>
</tr>
</tbody>
</table>

Mean values and standard deviations.
at cast-to abutment sites than at tissue units obtained from Ti- and ceramic-abutment sites. The tissue composition in sections prepared from the 5-month specimens from cast-to abutment sites was similar to that assessed in the 2-month samples. At Ti- and ceramic-abutment sites, however, the densities of collagen increased and leukocytes decreased between 2 and 5 months of healing [Figs 3a–c and 4a–c]. The large differences in tissue composition at 2 months between sites representing cast-to abutments on the one hand and Ti- and ceramic abutments on the other, persisted at 5 months of healing. Thus, in cast-to sites available for connective tissue analysis the densities of collagen and fibroblasts remained smaller, while the proportions of leukocytes and residual tissue were found to be larger than in Ti- and ceramic-abutment sites (Fig. 5a–c).

The results from the assessments of leukocytes residing in the barrier epithelium of the peri-implant mucosa are reported in Table 4. At 2 months, the relative volume of infiltrating leukocytes within the epithelium was 6.7–6.8% at Ti and cast-to abutments and 5.2% at ceramic-abutment sites. This difference was statistically significant. At 5 months of healing, the densities of leukocytes had decreased and varied between 3.5% and 4.5%.

### Discussion

In the present study, soft tissue healing to implant abutments made of different materials was examined previously. Thus, Abrahamsson et al. [1998] placed six Brånemark implants in the mandibular premolar regions of five dogs. Abutments made of c.p. titanium, ceramic (highly sintered Al₂O₃), gold-alloy and dental porcelain were connected in a second stage surgery 3 months later. Biopsies were obtained after 6 months of plaque control. It was reported that healing at ceramic and titanium abutments allowed the formation of a mucosal attachment that included an epithelial and a connective tissue portion that were about 2 mm and 1–1.5 mm high, respectively. Abrahamsson et al. (1998) also reported that no proper mucosal attachment was formed to

<table>
<thead>
<tr>
<th>%</th>
<th>Ti</th>
<th>Ceramic</th>
<th>Cast-to**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>50.1 (5.3)</td>
<td>48.5 (6.1)</td>
<td>18.1 (25.6)</td>
</tr>
<tr>
<td>V</td>
<td>6.5 (1.2)</td>
<td>8.0 (7.6)</td>
<td>4.9 (6.9)</td>
</tr>
<tr>
<td>Fi</td>
<td>36.8 (6.9)</td>
<td>34.2 (9.5)</td>
<td>16.8 (22.3)</td>
</tr>
<tr>
<td>Leu</td>
<td>1.6 (2.0)</td>
<td>2.6 (2.0)</td>
<td>29.8 (23.5)</td>
</tr>
<tr>
<td>R</td>
<td>5.1 (1.8)</td>
<td>6.8 (7.4)</td>
<td>31.4 (31.3)</td>
</tr>
</tbody>
</table>

Mean values and standard deviations.

**Data obtained from two animals.

Fig. 3. (a) Buccal-lingual section of the peri-implant tissues at a titanium abutment at 5 months of healing. Original magnification × 25. (b) Detail given in (a) showing the barrier epithelium with a few infiltrating leukocytes and the underlying connective tissue. Original magnification × 400. (c) Detail given in (a) showing the collagen rich connective tissue with fibroblasts and few vessels in the abutment/connective tissue interface area. Original magnification × 400.

Fig. 4. (a) Buccal-lingual section of the peri-implant tissues at a ceramic abutment at 5 months of healing. Original magnification × 25. (b) Detail given in (a) showing the barrier epithelium with a few infiltrating leukocytes and the underlying connective tissue. Original magnification × 400. (c) Detail given in (a) showing the collagen rich connective tissue with fibroblasts and few vessels in the abutment/connective tissue interface area. Original magnification × 400.
abutments made of gold-alloy and dental porcelain and at such sites, recession of the mucosal margin and bone resorption occurred. The finding in the study by Abrahamsson et al. (1998) that healing to abutments made of gold-alloy was different than that at ceramic and titanium abutments is supported by observations made in the current experiment. Thus, the barrier epithelium and the marginal bone level were found to extend to a more apical position at cast-to [Au/Pt-alloy] than at Ti and ceramic abutments at 5 months of healing. This finding indicates that healing at 2 months at cast-to abutments was incomplete and that the apical shift of epithelium and marginal bone that was observed at 5 months in the present study represents a stage of tissue reaction to establish a mucosal seal that extended apical to the A/F border, i.e., in contact with the titanium surface of the implant. In the present study, it was also demonstrated that the connective tissue portion of the mucosa that was in contact with cast-to abutments contained smaller amounts of collagen and fibroblasts and larger proportions of inflammatory cells and residual tissue than the corresponding tissue unit at ceramic and Ti abutments. The connective tissue composition at the cast-to abutments revealed in the current study may thus resemble an insufficient mucosal adaptation resulting in epithelial proliferation and bone loss.

The finding presented in the present experiment and in the study by Abrahamsson et al. (1998) that soft tissue healing to implant components made of gold-alloy was insufficient, is not in agreement with data reported in a recent study by Abrahamsson & Cardaropoli (2007). They evaluated soft and hard tissue healing to implants made of titanium or gold-alloy. Implants were placed in the mandibular premolar regions of four dogs and biopsies were collected after 6 months. The histological analysis of the ground sections revealed that the dimension of the barrier epithelium and the position of the marginal bone were similar at sites representing titanium and gold-alloy implants. The absence of differences in soft tissue dimensions between the two implant types in the study by Abrahamsson & Cardaropoli (2007) is partly consistent with observations made in sites from 2 months of healing in the present study. Thus, the values describing the apical extension of the barrier epithelium and the position of the marginal bone at cast-to [Au/Pt] abutments were comparable to those at Ti and ceramic sites. The evaluation of the composition of the connective tissue portion in contact with the abutment material, however, revealed large differences between cast-to sites on the one hand and Ti and ceramic sites on the other. No qualitative assessments of the soft tissue portion in contact with the implants were made in the study by Abrahamsson & Cardaropoli (2007).

In the study by Abrahamsson et al. (1998) referred to above, it was demonstrated that soft tissue healing to a ceramic abutment made of Al2O3 was similar to that of c.p. titanium. This finding is interesting in relation to observations made in the present experiment. It should be pointed out, however, that the ceramic abutments used by Abrahamsson et al. (1998) were aluminum based, while the ceramic abutments in the current study were made of ZrO2. Although the ceramic material was different in the two studies, soft tissue healing in terms of tissue dimensions and composition in both studies was similar to that at abutments made of titanium. Corroborating data were presented in an experimental study by Kohal et al. (2004b). They analyzed soft and hard tissues around implants made of either titanium or zirconium in six monkeys. Biopsies were obtained 9 months after implant placement. It was reported that soft tissue dimensions were similar at titanium and zirconium implants.

In the present study, the relative volume of infiltrating leukocytes in the barrier epithelium was analyzed. The presence of migrating polymorphonuclear leukocytes and other inflammatory cells in the junctional/barrier epithelium in the mucosa surrounding implants or teeth is a result from a microbial challenge in adjacent sulcus areas. The assessment of leukocytes in the barrier epithelium was applied in previous experiments to evaluate differences in epithelial attachment to implant abutments or tooth enamel (Berglundh et al. 1989, 1992a, 1992b). Recently, Welander et al. (2007) applied this technique to compare epithelial attachment to titanium implants with or without an ultrathin collagen coating. It was reported

<table>
<thead>
<tr>
<th>Table 4. Percentage of leukocytes residing in the barrier epithelium at 2 and 5 months of healing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Ti</td>
</tr>
<tr>
<td>Ceramic</td>
</tr>
<tr>
<td>Cast-to</td>
</tr>
</tbody>
</table>

Mean values and standard deviations. *P < 0.05 between ceramic and Ti and between ceramic and cast-to at 2 months of healing.

![Fig. 5.](https://example.com/fig5.png) [a] Buccal-lingual section of the peri-implant tissues at a cast-to abutment at 5 months of healing. Original magnification × 25. [b] Detail given in [a] showing the barrier epithelium with a few infiltrating leukocytes and the underlying connective tissue. Original magnification × 400. [c] Detail given in [a] showing the connective tissue with large numbers of fibroblasts and leukocytes and few collagen fibers in the abutment/connective tissue interface area. Original magnification × 400.
that the density of leukocytes in the barrier epithelium varied between 5% and 7% at coated and uncoated implants after 4 and 8 weeks of healing. This result is consistent with data presented in the present study. Thus, at 2 months [8 weeks] of healing the volume fraction occupied by leukocytes in the barrier epithelium varied between 5.2% and 6.8%. In the specimens representing 5 months of healing, however, the density of leukocytes in the epithelium had decreased at all abutment types. The proportion of leukocytes in the barrier epithelium at ceramic [ZrO₂] abutments was smaller than that at Ti and cast-to-abutments. This observation indicates that the ZrO₂ material provided appropriate conditions for epithelial attachment in the establishment of a proper mucosal seal. Another explanation may be related to differences in bacterial colonization on the abutment surfaces. Such a hypothesis was proposed by Rimondini et al. [2002] and Scarano et al. [2004]. Rimondini et al. [2002] evaluated microbial colonization on titanium and zirconium discs in vitro and in vivo. While only small differences were detected in bacterial adherence between the two surfaces in the in vitro test, the results from the in vivo model revealed that significantly larger amounts of bacteria were found on titanium than on zirconium discs. Similar findings were reported by Scarano et al. [2004]. They analyzed the percentage surface covered by bacteria on titanium and zirconium discs. The discs were mounted to removable acrylic devices that were adapted to the premolar–molar regions of 10 subjects. Scanning electron microscopy analysis of the discs that was performed after 24 h revealed that the surface area covered by plaque was significantly smaller at zirconium than at titanium discs.

In summary, the present study demonstrated that abutments made of titanium and ZrO₂ promoted proper conditions for soft tissue healing, whereas abutments made of Au/Pt-alloy failed to establish appropriate soft tissue integration.

Acknowledgements: The present study was supported by grants from Astra Tech AB, Möln达尔, Sweden.

References


