Tissue Reactions to Abutment Shift: An Experimental Study in Dogs

Ingemar Abrahamsson, DDS, OD;* Tord Berglundh, DDS, OD;+
Satoshi Sekino, DDS;+ Jan Lindhe, DDS, OD*

ABSTRACT

Background: Standard protocols for the clinical use of dental implants often include the placement of healing abutments prior to standard or custom-made abutments. The tissue response to a single shift from a healing abutment to a permanent abutment has not been studied.

Purpose: The aim of the present experiment was to study tissue reactions that may occur following the removal of a healing abutment and the placement of a permanent abutment.

Materials and Methods: In six beagle dogs, all mandibular premolars were extracted. Three months later three fixtures of the Astra Tech Implants® Dental System (Astra Tech AB, Mölndal, Sweden) were installed in each edentulous premolar region. An additional 3 months later, the first abutment connection was performed. In two sites on each side of the mandible, healing abutments were placed; in the remaining site, a Uni-abutment® (Astra Tech AB) was used. The two healing abutments were removed 2 weeks later, and one Uni-abutment and one prepable abutment were placed. A plaque-control period was initiated, and 6 months later block biopsies were obtained. The biopsies were prepared for histometric and morphometric examination. Radiographs were obtained at fixture placement, 2 weeks after the first abutment connection, and 6 months later.

Results: The length of the barrier epithelium, the height of the connective tissue attachment, and the level of the marginal bone did not differ between the three abutment groups. The major part of the radiographic bone loss during the experiment took place prior to or immediately after abutment connection; only small bone level alterations occurred during the subsequent 6-month period.

Conclusions: The shift from a healing abutment to a permanent abutment resulted in the establishment of a transmucosal attachment, the dimension and quality of which did not differ from those of the mucosal barrier formed to a permanent abutment placed during a second-stage surgery.

KEY WORDS: abutment-ICT, animal experiment, dental implant, healing abutment, histometry, morphometry, periimplant mucosa, radiographs

Findings from studies in humans and from animal experiments have revealed that the mucosal interface to dental implants made of commercially pure (c.p.) titanium is composed of two units, one epithelial and one connective tissue. The barrier epithelium at implants and the junctional epithelium at teeth have many features in common, and hemidesmosomes are suggested to form the attachment between epithelial cells and titanium. Berglundh and colleagues and Moon and colleagues reported that, in experiments in dogs, the connective tissue portion located between the barrier epithelium and the marginal bone was poor in vascular structures but rich in collagen fibers. This connective tissue immediately lateral to the titanium surface was, however, rich in fibroblasts that appeared to be in close contact with the abutment. Abrahamsson and colleagues, in a study in beagle dogs, demonstrated that repeated dis- and reconnection of implant abutments resulted in an apical shift of the marginal bone level and in soft tissue recession. It was suggested that the repeated injury to the mucosal barrier was associated with an apical proliferation of the epithe-
lium, and that bone resorption occurred to reestablish a connective tissue attachment of a sufficient dimension.

Standard protocols for the clinical use of dental implants often include the placement of healing abutments 1 to 3 weeks prior to the insertion of standard or custom-made abutments. The tissue response to such a single shift from a healing abutment to a permanent abutment has not been studied.

The aim of the present experiment was, therefore, to study tissue reactions that may occur in the transmucosal attachment following the change of a healing abutment to a permanent abutment.

MATERIALS AND METHODS

In the present experiment,* six beagle dogs, about 1 year old, were used. All surgical procedures were performed under general anesthesia induced with propofol (10 mg/mL, 0.6 mL/kg) intravenously and maintained with N₂O:O₂ (1:1.5–2) and isoflurane employing endotracheal intubation.

All mandibular premolars (4P₄, 3P₃, 2P₂, 1P₁) were extracted. After 3 months of socket healing, three fixtures of the Astra Tech Implants® Dental System (TiOblast™, Astra Tech AB, Mölndal, Sweden; 8 × 3.5 mm) were installed in each edentulous premolar region. A crestal incision was made, and buccal and lingual full-thickness flaps were elevated. The implants were placed in such a way that the fixture margin coincided with the bone crest. Radiographs were obtained immediately after fixture installation using a custom-made film-holder device® connected to the posterior implant. Cover screws were placed, and the flaps were sutured to cover the fixtures. The sutures were removed after 2 weeks.

Three months later the first abutment connection was performed. Crestal incision and flap elevation were performed. In two of the three implant sites in each mandibular quadrant, healing abutments (3.0 mm; Astra Tech AB) were placed, whereas in the remaining site a permanent Uni-abutment® (height: 1.5 mm, angle: 20°; Astra Tech AB) was used (control). Two weeks later the two healing abutments were removed and one pristine Uni-abutment ("shift") and one preparable abutment (Astra Tech AB; "shift/prepare") were placed.

Prior to insertion, the preparable abutment was cut by a dental technician, subsequently cleaned in an ultrasonic bath, and finally autoclaved. Radiographs from all implant sites were obtained. A plaque-control program was initiated. This included daily cleaning of all teeth and exposed implant surfaces using a toothbrush and dentifrice.

After another 3 months, clinical examinations including assessments of plaque and soft tissue condition were performed, and radiographs were obtained from all implant sites. The clinical and radiographic examinations were repeated 3 months later, that is, after 6 months of plaque control.

The animals were sacrificed with an overdose of thiopental sodium (Pentothal) and perfused with a fixative through the carotid arteries. The fixative consisted of a mixture of 5% glutaraldehyde and 4% formaldehyde buffered to pH 7.2.¹⁰ The mandibles were removed and placed in the fixative. Each implant region was dissected using a diamond saw (Exakt®, Kulzer & Co. GmbH, Wehrheim, Germany), placed in ethylenediaminetetraacetic acid (EDTA), and further processed according to a modification of the "fracture technique" described by Berglundh and colleagues.¹¹ Before the hard tissue was fully decalcified, incisions were placed at the mesial and distal aspects of the implants. The cuts penetrated the entire periimplant tissue and were made parallel with the long axis of the implant. Each specimen was divided into one buccal and one lingual unit. These units were further separated into one mesiobuccal, one distobuccal, one mesiolingual, and one distolingual portion. Decalcification was completed in EDTA, and dehydration was performed in serial steps of ethanol concentrations. Secondary fixation in OsO₄ was carried out, and the units were finally embedded in EPON® (Fluka Production GmbH, Buchs, Switzerland).¹² Sections were produced from each tissue unit with the microtome set at 3 µm. The sections were stained in periodic acid–Schiff and toluidine blue.¹² Five sections, selected to represent each of the four units (ie, all 20 sections from each implant site) were used for the histologic examination. Hence, all aspects (mesial, distal, buccal, and lingual) of the periimplant tissues were included in the analysis. All histometric and morphometric measurements were performed with a Leica DM-RBE® microscope (Leica, Wetzlar, Germany) equipped with an image system Q-500 MC® (Leica).

Radiographic Measurements

In the radiographs the distance between the most coronal part of the fixture (ie, the abutment–fixture junction) and the most coronal bone judged to be in contact with the implant surface was determined at the mesial and distal aspects of each implant. The measurements were determined using a Leica DM-RBE microscope equipped with an image system (Q-500 MC).

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¹The protocol of the present study was approved by the regional Ethics Committee for Animal Research, Gothenburg, Sweden.
The radiographic measurements were analyzed and presented as bone level alterations during three different phases: phase I, the time from implant placement to abutment connection; phase II, the time from abutment connection to 3 months of plaque control; and phase III, the time from 3 months of plaque control to biopsy (at 6 mo).

**Histometric Measurements**

The histometric analysis included assessments of some vertical dimensions of the soft and mineralized peri-implant tissues. The following landmarks were used for the linear measurements (Figure 1): the marginal position of the peri-implant mucosa (PM), the apical termination of the barrier (junctional) epithelium (aJE), the marginal level of bone-to-implant contact (B), and the level of the abutment-fixture border (A/F). The distances between the various landmarks were determined. In addition, the height and size of an inflammatory lesion present at the level of A/F (ie, abutment [ie, abutment-infiltrated connective tissue] ICT) were determined.

**Morphometric Measurements**

The morphometric measurements were performed within a 200 µm wide compartment of the “zone of connective tissue integration,” located between the aJE and the B. The measurements were performed in two different zones, namely zone A, the “inner” 40 µm wide zone close to the titanium surface, and zone B, the “outer” 160 µm wide zone that was continuous with zone A. The composition of the connective tissue was analyzed with respect to the content of collagen, vessels, fibroblasts, and residual tissue (the remaining tissue constituents such as leukocytes, nerves, and matrix components lumped together). A point-counting procedure was performed using a lattice comprising 100 light points superimposed over the connective tissue area at a magnification of × 1,000.

The abutment-ICT was analyzed with respect to its content and relative distribution of collagen, vessels, fibroblasts, macrophages, lymphocytes, plasma cells, polymorphonuclear cells, and residual tissue.

**Statistical Analysis**

Mean values for the different variables were calculated for each implant and animal. Differences between the three implant units were analyzed using analysis of variance and the t-test for paired observations. The null hypothesis was rejected at p < .05.

**RESULTS**

**Clinical Observations**

Healing following implant installation and subsequent abutment connection was uneventful. The examinations performed 3 and 6 months after abutment connection disclosed that the abutment surfaces were virtually free from plaque and that the mucosas at all implant sites were clinically healthy (Figure 2).

**Radiographic Measurements**

The results from the radiographic measurements are reported in Table 1. The marginal bone level following fixture installation was similar in all three groups. During phase I (implant installation–abutment connection), bone loss of about 1.1 mm was recorded at the control sites, whereas at the shift and shift/preparable sites, the corresponding bone loss measured about 0.7 mm. During the following 6 months of plaque control, the control sites gained 0.2 mm of marginal bone; during the corresponding period, no change in the bone level was observed in the two other groups. The differences between the three groups regarding bone level alteration during phases I to III were not statistically significant.

**Histologic and Histometric Observations**

The histometric measurements are reported in Table 2. The peri-implant mucosa (Figure 3) was on average between 3.11 and 3.25 mm high, and the height of the barrier epithelium (PM to aJE) varied between 1.72 and 1.80 mm. The connective tissue portion of the peri-implant mucosa that was located between aJE and B (“zone of connective tissue integration”) was 1.3 to 1.5 mm high.
at control and shift sites and contained large amounts of collagen and few vascular structures and cells. Although the corresponding connective tissue compartment at shift/preparable sites had a similar dimension to the control and shift sites, this connective tissue portion consistently contained inflammatory cells.

The distance between A/F and B was 1.00 mm (control), 0.72 mm (shift), and 0.87 mm (shift/preparable). A small and well-defined inflammatory cell infiltrate (abutment-ICT; Figure 4B) was frequently identified in the connective tissue at the level of A/F at the control and shift sites. This abutment-ICT was about 0.54 to 0.55 mm high and occupied a surface area that varied between 0.07 and 0.15 mm² at the control and shift sites, respectively. In 8 of 12 shift/preparable sites, however, the abutment-ICT was continuous with an inflammatory lesion located in the connective tissue lateral to the barrier epithelium. The mean height of the abutment-ICT at shift/preparable sites was 1.89 mm, and the area occupied by this ICT was 0.64 mm². The difference of the height and the area of the abutment-ICT at shift/preparable sites compared with control and shift sites was statistically significant.

Morphometric Observations

The results from the morphometric measurements performed in the zone of connective tissue integration in the control and shift sites are presented in Table 3.

In zone A (the inner 40 μm) the collagen density was 61.6% at control and 60.4% at shift sites, whereas the corresponding figures in zone B (the outer 160 μm) were 78.3% and 73.1%. Zone A was almost devoid of vascular units, that is, 0.4% and 0.5% for control and shift sites, respectively, whereas the tissue of zone B at both sites included about 4.2% vessels. The proportion of the tissue that was occupied by fibroblasts amounted to 30.6% at the control sites and 31.5% at the shift sites of zone A (Figure 4A and C). In zone B the corresponding fibroblast densities were 11.1% and 13.5%.

The results from the assessments of the abutment-ICT are reported in Table 4. The relative proportions of collagen, fibroblasts, and vascular units were 55.3%, 17.6%, and 18.3%. Among the inflammatory cells, polymorphonuclear leukocytes dominated, making up about 12.4% of the ICT volume, whereas macrophages, lymphocytes, and plasma cells occupied 3.2%, 0.5%, and 0.6%, respectively.

DISCUSSION

In the present experiment it was demonstrated that the change of a healing abutment to a permanent abutment resulted in the reestablishment of a transmucosal attachment with a dimension and quality similar to those characterizing the mucosal barrier at an abutment placed during the second-stage surgery. It was further demonstrated that the largest radiographic bone level alteration occurred during the first 3 months of healing following installation, that is, before the implants were exposed to the oral environment.

The observation that the mechanical trauma produced to the perimplant mucosa during the removal of a healing abutment and the placement of the Uni-abutment did not result in detectable bone loss is partly in agreement with findings reported in clinical studies.16,17

Engquist and colleagues16 performed a retrospective analysis of 82 Bränemark® implants (Nobel Biocare AB, Gothenburg, Sweden) used for single-tooth replacement.

| TABLE 1 Radiographic Bone Level Alterations during Phases I to III* |
|------------------------|------------------------|------------------------|
|                        | Mean (SD) in Control (mm) | Mean (SD) in Shift (mm) | Mean (SD) in Shift/Preparable (mm) |
| Phase I                | −1.10 (0.25)            | −0.69 (0.33)            | −0.67 (0.56)            |
| Phase II               | +0.11 (0.44)            | +0.09 (0.18)            | −0.07 (0.37)            |
| Phase III              | +0.10 (0.23)            | −0.06 (0.31)            | +0.03 (0.13)            |
| Phases I–III           | −0.89 (0.45)            | −0.66 (0.48)            | −0.71 (0.39)            |
| Phases II–III          | +0.21 (0.48)            | +0.03 (0.39)            | −0.04 (0.46)            |

+ = gain in bone; − = bone loss.

*Phase I = implant installation–abutment shift; phase II = abutment shift–3 mo plaque control; phase III = 3 mo plaque control–sacrifice.
Initially placed healing abutments were replaced by permanent abutments after 4 to 6 weeks. Radiographic examinations were performed at crown delivery and at 1 year of follow-up. The investigators concluded that the bone levels recorded at crown delivery (1.6 mm apical to A/F) and at the 1-year follow-up (2.2 mm apical to A/F) at implants where a healing abutment had been replaced by a permanent abutment were similar to bone levels found with "conventional" two-stage surgery.

Jemt studied bone level alterations at single-tooth implants in 14 subjects. The implants were provided with temporary abutments that were replaced by crown restorations fitted to the fixtures. The author stated that the bone levels assessed in radiographs at 1 month and 1 year after crown placement (1.3 mm and 1.7 mm apical to A/F, respectively) "compare favorably with other single-implant techniques."

There are, however, obvious differences related to the design of the present animal experiment and to the radiographic examinations performed in the clinical studies to which we refer. Although baseline measurements of bone levels at the implants were obtained at second-stage surgery (ie, the first abutment connection) in the current animal experiment, the starting point for the bone level assessment in the clinical studies was related to the placement of the definitive crown that was carried out about 2 to 3 months following the second-stage surgery.

In a previous study in beagle dogs, it was observed that repeated abutment removal and replacement severed the mucosal attachment and resulted in marginal bone loss. Thus, at sites where abutments were dis- and reconnected five times during a 6-month period, the bone level was found to be located 1.5 mm apical of the abutment-fixture junction. The corresponding distance (A/F to B) at control sites at which the abutments were left unchanged was 0.8 mm. In this context it should be observed that the A/F to B distance in the three implant groups analyzed in the current experiment varied between 0.7 and 1.0 mm, variations similar to the bone level data reported for control sites in the previous study. Furthermore, dimensions describing the length of the barrier epithelium (PM to aJE) and the height of the connective tissue attachment (aJE to B) did not differ between the three abutment groups of the present experiment. It may, therefore, be suggested that the

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**Table 2: Histometric Measurements**

<table>
<thead>
<tr>
<th>Distance Measured</th>
<th>Mean (SD) in Control (mm)</th>
<th>Mean (SD) in Shift (mm)</th>
<th>Mean (SD) in Shift/Prepable (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM-B</td>
<td>3.25 (0.31)</td>
<td>3.20 (0.34)</td>
<td>3.11 (0.28)</td>
</tr>
<tr>
<td>PM-aJE</td>
<td>1.80 (0.17)</td>
<td>1.72 (0.15)</td>
<td>1.76 (0.34)</td>
</tr>
<tr>
<td>aJE-B</td>
<td>1.45 (0.16)</td>
<td>1.48 (0.31)</td>
<td>1.35 (0.33)</td>
</tr>
<tr>
<td>A/F-B</td>
<td>1.00 (0.43)</td>
<td>0.72 (0.27)</td>
<td>0.87 (0.33)</td>
</tr>
<tr>
<td>Area of abutment-ICT</td>
<td>0.07 (0.05)</td>
<td>0.15* (0.13)*</td>
<td>0.64 (0.38)</td>
</tr>
<tr>
<td>Height of abutment-ICT</td>
<td>0.55 (0.32)</td>
<td>0.54* (0.19)</td>
<td>1.89 (0.38)</td>
</tr>
</tbody>
</table>

A/F = abutment-fixture border; aJE = apical termination of the junctional epithelium; B = marginal bone-to-implant contact; ICT = infiltrated connective tissue; PM = marginal position of the perimplant mucosa; histologic landmarks are illustrated in Figure 1.

* *p < .05.

**Figure 3** Buccolingual cross-section of the marginal perimplant tissues of one control site. The areas outlined by the rectangles are shown in Figures 4A–C. A/F = abutment-fixture border; aJE = apical termination of the junctional epithelium; B = marginal bone-to-implant contact; PM = marginal position of the perimplant mucosa (×25 original magnification; stained with periodic acid–Schiff and toluidine blue).
change from a healing abutment to a permanent abutment did not compromise the mucosal attachment and did not result in additional bone loss and that the connective tissue attachment to the titanium abutment was reestablished following a single abutment shift.

In the present experiment, it was demonstrated that most of the marginal bone loss detected occurred before the implants were exposed to the oral environment. This finding is in agreement with observations previously reported from animal experiments and clinical studies. Abrahamsen and colleagues, in a study of beagle dogs, assessed bone level alterations in radiographs before and after abutment connection at Astra Tech implants. It was suggested that the major part of the marginal bone loss that occurred took place before or immediately after abutment connection and that only small bone level alterations could be detected during a subsequent 6-month period of monitoring. Similar observations were also made in another animal experiment in which implants of the 3i type were included. Thus, about 1 mm of bone loss was recorded during the "submerged" phase (3 mo), whereas during the following 6 months after abutment connection, the additional amount of bone loss was only 0.25 mm.

In a study that included 62 partially edentulous patients restored with Steri-Oss (Nobel Biocare, Yorba Linda, CA, USA) and 3i implants, Jung and colleagues analyzed radiographic bone level alterations during a 1-year interval following abutment connection. The authors concluded that more than 50% of the total amount of bone loss was recorded during the first 3 months. It was suggested that the damage caused during the initial surgical procedure and the trauma caused by the abutment connection procedure were factors of importance in the process of initial bone loss.

In the present study, only minute changes of the marginal bone level were observed during the 3 months that followed the initial abutment connection (phase II; see Table 1) and during the final 3 months of monitoring (phase III; see Table 1). In other words, during this particular 6-month interval, the remodeling of the bone around the implants occurred with an unchanged

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean (SD) in Zone A (% volume)</th>
<th>Mean (SD) in Zone B (% volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Shift</td>
</tr>
<tr>
<td>Collagen</td>
<td>61.6 (1.9)</td>
<td>60.4 (4.2)</td>
</tr>
<tr>
<td>Vascular structures</td>
<td>0.4 (0.7)</td>
<td>0.5 (0.8)</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>30.6 (2.9)</td>
<td>31.5 (3.8)</td>
</tr>
<tr>
<td>Residual tissue</td>
<td>7.4 (1.1)</td>
<td>7.6 (2.0)</td>
</tr>
</tbody>
</table>

*The percent volume of the connective tissue occupied by collagen, vascular structures, fibroblasts, and residual tissue.
TABLE 4 Morphometric Measurements of the Abutment-ICT*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean (SD) in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>35.3 (6.0)</td>
</tr>
<tr>
<td>Vascular structures</td>
<td>18.3 (4.5)</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>17.6 (6.6)</td>
</tr>
<tr>
<td>Macrophages</td>
<td>3.2 (1.5)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.5 (0.3)</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0.6 (0.5)</td>
</tr>
<tr>
<td>Polymorphonuclear leukocytes</td>
<td>12.4 (3.4)</td>
</tr>
<tr>
<td>Residual tissue</td>
<td>12.1 (3.1)</td>
</tr>
</tbody>
</table>

*The percent volume of the abutment-ICT occupied by collagen, vascular structures, fibroblasts, macrophages, lymphocytes, plasma cells, polymorphonuclear leukocytes, and residual tissue.
