Peri-Implant Defect Augmentation With Autogenous Bone: A Study in Beagle Dogs

Asala F. Al-Sulaimani, BDS, MSc 1*
Sameer A. Mokeem, BDS, MSc, PhD2
Sukumaran Anil, BDS, MDS, PhD2

This study evaluates the success of immediate endosseous implants placed along with autogenous bone graft to fill the peri-implant gap. Thirty-two implants were inserted in 8 beagle dogs. The right and left lateral incisors in the maxilla and the mandible of all animals were extracted, and immediate postextraction implants were placed. In the control sites, no bone grafts or barrier membranes were used. In the contralateral experimental site, autogenous bone graft was used. The implants were retrieved with the jawbone for histomorphometric studies. The histomorphometric measurements were carried out using a computerized image analysis system. All implants were covered by compact, mature bone under examination in light microscopy. A high bone-implant contact percentage and bone density was observed at both grafted and nongrafted implant sites. The sites filled with autogenous bone graft showed a significantly higher crestal bone level and bone density compared to the nonfilled sites. The observations of the study emphasize that the filling of the peri-implant bone defects with autogenous bone grafts showed a better outcome compared to unfilled defects.

Key Words: bone defect, dental implant, histometry, osseointegration, immediate dental implants, autogenous bone

INTRODUCTION

The replacement of lost natural teeth by osseointegrated implants represents one of the most significant advances in dentistry. Early implantation is preferred by many surgeons to achieve long-term stability and a good esthetic result.1 Placement of titanium endosseous implants in fresh extraction sites can result in osseointegration as determined by histologic evidence of the bone closely adapted to the implant surface and lack of clinical mobility.2 Several studies have evaluated and confirmed the successful practice of placing dental implants into fresh extraction sites.3,4 Immediate placement of implants into fresh extraction sockets has the principal advantage of decreasing the overall period required for healing.5 In addition, immediate implant placement may reduce the resorption of the alveolar bone in the extraction area.6

However, when an implant is placed immediately into an extraction socket, it may not completely engage the walls of the socket near the crest of the alveolar ridge.7 The presence of a bone defect around an implant could promote in-growth of soft tissue and compromise osseointegration in the crestal bone area.8 When using immediate postextraction implants, it is almost always necessary to resort to osteopromoting techniques.9,10 Graft materials and barrier membranes have been used extensively when placing immediate implants.11,12 They are often used to prevent soft tissue in-growth and to allow bone-forming cells to exclusively populate osseous defects around the implants.13–16 However, complications such as exposure of the membranes are often reported.17,18 The placement of implants into fresh extraction sites with autogenous bone chips without the use of barrier membranes has been advocated by some
investigators,\textsuperscript{19,20} whereas other researchers found that the combination of barrier membranes with bone substitutes increased the mineralized bone-to-implant contact percentage.\textsuperscript{21} Becker et al\textsuperscript{22} reported 100% survival using autogenous bone grafting without membranes.

Experimental studies have confirmed that a high percentage of bone-implant contact can be achieved in animals when placing implants immediately into extraction defects.\textsuperscript{23–25} Implants placed into fresh extraction sockets have a high survival rate, between 93.9% and 100%.\textsuperscript{26} A variety of materials are used to fill this space between the occlusal part of the implant and the surrounding socket walls, including demineralized freeze-dried bone, autogenous bone, and hydroxyapatite.\textsuperscript{27} However, the placement of the grafting materials in extraction sockets may interfere with the normal healing sequence.\textsuperscript{28}

Autogenous bone is preferred for its osteogenic as well as osteoinductive potential.\textsuperscript{29} Autogenous bone may also have the potential to retain vital cells, which are replaced by the host.\textsuperscript{30} Several studies have shown that the autogenous bone grafts are extremely effective.\textsuperscript{28} The bone may be harvested from surgical bur debris, the lateral bone margin of the implant site, maxillary tuberosity, retromolar area, or the symphysis. The aim of this study is to evaluate the bone-to-implant contact of immediate implants placed with and without autogenous bone in beagle dogs.

**Materials and Methods**

**Animals**

Eight adult dogs aged 16 to 18 months were used. The dogs weighed 10 to 15 kg. The design of the study was approved by the Ethics Committee on Animal Research, College of Dentistry Research Center and King Saud University.

**Extraction procedure**

Teeth were extracted under general anesthesia under sterile conditions in an operating room. An intramuscular injection of ketamine hydrochloride (5 mg/kg) and diazepam (1 mg/kg) was used to sedate the animals prior to the procedure. The oral cavities of the animals were rinsed with a 1:1 mixture of povidone iodine 10% and chlorhexidine solution. The area around the lower premolars was locally anesthetized with an injection of lidocaine 2% with 1:100 000 epinephrine. Following complete anesthesia of tissues, the maxillary and mandibular lateral incisors were extracted. Thin elevators were used to luxate the teeth and then extracted using forceps with rotary movements to avoid trauma of the alveolar ridges.

**Autogenous bone harvest**

Autogenous graft was procured from the ramus of the mandible. A horizontal incision extending from the first to the last molar was placed 2 mm below the cementoenamel junction. The mucoperiosteal flap was reflected. A $2 \times 3$ mm block of bone was harvested by drilling holes with a small round bur and connected by a fissure bur (Figure 1). The harvested autogenous bone block was kept in saline mixed with blood. At the time of implant insertion, a bone mill was used to grind the autogenous bone into small granules. The surgical site was closed with continuous matrix sutures.

**Implant placement and defect creation**

The implant sites were prepared according to the manufacturer’s instructions. After the final drill, the implant sites were widened with a round bur to a depth of 5 mm from the crest of the preparation to create the peri-implant defect (Figure 2). Threaded titanium implants of 3.3 mm in diameter and 9 mm in length (Allfit System, Dr Ihde Dental, GmbH, Gommiswald, Switzerland) were placed into both the test and control sites. The resulting gap (coronally) between the bone and the implant was filled with autogenous bone (harvested bone and bone collected from drilling burs) on one side of the maxillary and the mandibular incisor area. In the contralateral side (maxillary and mandibular lateral incisor) no graft material was placed, and it served as the control for comparison (Figure 3). The dogs were numbered from 1 to 8, and the odd numbers received graft in the right side and the even numbers on the left side.

After the placement of implants, the flaps were closed with interrupted sutures using 4-0 braided synthetic absorbable Vicryl, and primary soft tissue closure was achieved without any additional procedure. A broad-spectrum antibiotic (gentamycin 4 mg/kg body weight) was administered intramuscularly for 7 days. The dogs were kept on
a soft diet for 2 weeks after the surgical procedure. None of the implants in this study were loaded.

Periodic clinical examinations were carried out at 2, 4, 6, and 8 months. Eight months after insertion of the implants, the dogs were sacrificed to harvest the jawbones with the implants. After premedication with a combination of haloperidol and fentanyl, the dogs were anesthetized using 30 mg/kg thiopental after which 0.5 mL/kg Thromboliquine was injected intravenously, followed by a lethal dose of thiopental. The vascular system was perfused with physiologic saline, followed by 4% neutral formaldehyde as a fixative. After perfusion, the mandibles were dissected out and immersed in 4% neutral formaldehyde. The implants and adjacent tissues were removed en bloc for histomorphometric analysis.

**Preparation of the specimens for histomorphometric analysis**

The specimens were stored in 10% buffered formalin and processed to obtain thin ground sections. Each block was sectioned mesiodistally through the center of the implant. The sections were fixed in 10% neutral buffered formalin for an additional 24 hours; this was followed by dehydration in a graded series of alcohols for 9 days. Following dehydration, the specimens were infiltrated with a light-curing embedding resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany) using a precision parallel control oscillating specimen mounting system. Three sections were made of each implant. Each section was polished to a thickness of 40 μm using a series of polishing discs followed by a final polish with 0.3 μm alumina. During all procedures, care was taken to preserve the original implant-tissue interface. Finally, all of the sections were stained with 1% toluidine blue solution and examined by light microscopy.

**Histomorphometric analysis**

Photomicrographs were taken using a Wild M400 Photomakroskop (Wild Heerbrugg, Gais, Switzerland). The pictures were digitalized and loaded in the software for analysis. The analysis of the image was done using Scion Image analysis system (Scion Corporation, Frederick, MD).

**Bone-to-implant contact**

Crestal bone levels at the mesial and distal sites of each image were assessed visually with the linear measurement tool function of the National Institutes of Health image software to measure the vertical distance (in millimeters) from the top margin of the cover screw of the implant to the most apical initial point of contact observed between the implant and bone. Measurements were done at the mesial and distal site of each image. The measurements were repeated twice and the data tabulated and compared.

**Bone density**

The bone density was determined as the percentage of bone found within a rectangular grid measuring 9600 mm² (16 × 6 cm) each, superim-
posed over the image in a uniform manner (Figure 4). The images were loaded into imaging software (Jasc Paint Shop Pro, Corel Inc, Mountain View, Calif). A standard grid was superimposed over the image using the built-in facility of the software, and the percentage of bone coverage was calculated and tabulated for statistical analysis. Standardization of the image was done with the known measurements taken from the implant size.

Reproducibility of measurements

Histomorphometric measurements were repeated after 2 weeks on 10 randomly selected samples. All measurements of bone-to-implant contact, both in the coronal and apical 4 mm were within 5% of initial measurements, with the exception of 1 measurement, which differed by 6%. All measurements of the highest bone-to-implant contact were within 0.2 mm of the initial measurements. Eight of 10 measurements were exactly the same. A false numbering system was used for the slides and only the study supervisor (S.A.) was aware of the exact specimen and details.

Statistical analysis

The data were tabulated in a spreadsheet and the mean values were calculated for the control and experimental site (maxilla and mandible) for each dog. The statistical analysis was performed using InStat GraphPad software (InStat, GraphPad Software, Inc, San Diego, Calif). Paired t test was used to evaluate the differences in the bone-to-implant contact and percentage bone fill between the study groups. A P value less than .05 was considered statistically significant.

RESULTS

Clinical observations

Thirty-two implants were placed in 8 beagle dogs. After an 8-month healing period following implant placement, the healing progressed uneventfully. All implants, control and experimental, were clinically immobile at the time of sacrifice, and there were no signs of significant inflammation or other signs of pathology. The implants were stable throughout the study and demonstrated clinical osseointegration. Radiographically, all implants were surrounded by normal-appearing bone, and they showed no signs of the preexisting radiolucency lesions on the experimental sites.

Histomorphometric observations

Bone-to-Implant Contact

The mean bone to first implant contact (BIC) is shown in Table 1. The mean BIC for the control site
was 4.62 ± 0.59, and for the experimental site it was 4.15 ± 0.60 (Table 1). The BIC was significantly higher in implant sites filled with autogenous graft compared to the sites left unfilled ($P < .05$) (Figures 5 and 6).

**Bone Fill**

The percentage of bone fill analyzed with the grid showed 82.29% at the control site (SD = 5.36) compared to 86.11% (SD = 3.57) at the site filled with autogenous graft (Table 2). The bone volume was found to be statistically significantly higher in the graft site compared to the sites left unfilled ($P < .001$).

**DISCUSSION**

Immediate implants may provide advantages such as preventing bone resorption, maintaining width and height of alveolar crest, reducing surgical procedures and treatment time, and moreover providing good esthetic results, as the implant can be seated according to the natural tooth angulation and aligned with the adjacent teeth.\textsuperscript{14,32} Though the implants placed immediately after tooth extraction offer several advantages, the problems in filling the residual gap between the implants and the socket walls remains a major concern to immediate implant placement.\textsuperscript{33} The use of grafting material and barrier membranes to fill the peri-implant space has been studied extensively with varying results.\textsuperscript{34} The present study was undertaken to analyze and compare bone formation around immediate implants placed with and without any grafting material in beagle dogs. The histomorphometric analysis of the bone adjacent to implants with and without autogenous graft showed considerable gain in bone healing around the implants. The results of the study are in agreement with Schwartz-Arad and Chaushu\textsuperscript{18} who showed that immediate implants installed with autogenous bone to fill the peri-implant defect can have a high survival rate without using barrier membranes. In the present study, autogenous bone graft material was used to fill the peri-implant space. The advantages of using autogenous graft material has gained much attention and is considered to be superior to many other commercially available materials in terms of osseointegration.\textsuperscript{6,35,36}

Botticelli et al,\textsuperscript{37} studied the peri-implant bone changes following immediate implant placement, and they found that a marginal gap that occurs between the implant and the bone may predictably heal with new bone formation and defect resolution. Peri-implant defects (1–2.5 mm) were created in a dog model and implants were installed. The defects were closed with a resorbable membrane (Bio-Gides, Geistlich AG, Wolhusen, Switzerland) placed over the defect and surrounding 3–4 mm of bone tissue. No bone grafts were used in the study. They documented that wide and deep marginal gaps in buccal and palatal/lingual locations could be resolved through new bone formation from the inside of the defects and bone resorption from the outside of the ridge. Clinical and animal studies showed that the defect that occurs between the implants and bone interface might heal without the use of space-maintaining barrier membranes or filler material.\textsuperscript{38–40} In a clinical study, Wilson et al\textsuperscript{41} demonstrated that peri-implant gaps less than 4 mm around implants with a sand-blasted large-grit acid-etch surface could heal.

### Table 1

<table>
<thead>
<tr>
<th>Bone-to-implant contact measured from the top of the implant coverscrew to the initial bone-implant contact</th>
<th>Mean ± SD</th>
<th>Sample size, n</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (defect untreated)</td>
<td>4.62 ± 0.59</td>
<td>16</td>
<td>.0473</td>
</tr>
<tr>
<td>Experimental group (defect treated with autogenous bone graft)</td>
<td>4.15 ± 0.60</td>
<td>16</td>
<td></td>
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</table>

### Table 2

<table>
<thead>
<tr>
<th>Area of bone fill</th>
<th>Mean ± SD</th>
<th>Sample Size, n</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (defect untreated)</td>
<td>82.29 ± 5.36</td>
<td>16</td>
<td>.0080</td>
</tr>
<tr>
<td>Experimental group (defect treated with autogenous bone graft)</td>
<td>86.11 ± 3.57</td>
<td>16</td>
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These observations are in agreement with the present study, which exhibited bone healing and formation at the control site where the peri-implant defect was left unfilled.

The observation in the present study revealed that bone healing and adaptation of the implant is possible with or without the use of any graft material. However, a better bone level appears to have been achieved when the implant is placed along with autogenous bone grafts. The differences in BIC between experimental and control site were statistically significant. On histomorphometric examination, it was observed that in both the control and graft sites the implants had consolidated bone and soft tissue after a healing period of 8 months. Earlier studies have shown that autogenous bone is one of the most suitable materials for filling the defects around the implants.\(^{18}\) Haas et al\(^{42}\) compared homogenous and heterogeneous bone graft material for their osteogenic potential to improve bone to implant healing. From histologic and histomorphometric observation, they concluded that demineralized freeze-dried bone homograft and heterograft with cancellous bone from the iliac graft showed better bone-to-implant contact than homogenous or heterogeneous bone graft alone.

Some studies claim that a peri-implant gap of \(\leq 2\) mm can be left untreated during immediate implant placement.\(^{33,43–45}\) However, these reports were based on reentry surgeries done in humans without histologic evaluation. Covani et al\(^{46}\) studied the buccolingual changes after immediate and delayed implant placement. From their observations, they concluded that the peri-implant defects healed without membranes or a graft. Their study, however, only focused on the remodeling of buccolingual bone width and the vertical bone changes around the top of the implant.

Most of the available literature supports the observations of the present study, which suggest improved healing outcomes following grafting of the peri-implant defects.\(^{37,47,48}\) The consensus statement and recommendations of the third ITI symposium advocated the use of graft and/or membrane-supporting materials at peri-implant defects more than 2 mm.\(^{47}\) Further studies, however, are required to establish the advantages of autogenous bone grafts along with immediate implants to obtain improved osseointegration.

Abbreviation

**BIC:** bone-to-implant contact

References

18. Schwartz-Adar D, Chaushu G. Placement of implants into


