Histologic Assessment of Alveolar Bone Remodeling Around Immediate Implants Placed in Single and Multiple Contiguous Extraction Sites

The aim of this study was to histologically assess alveolar bone remodeling around immediate implants placed in single and multiple contiguous extraction sites. Ten dogs were divided into three groups (with six sites per group) on the basis of premolar teeth extraction: group 1 = one tooth extracted, group 2 = two contiguous teeth extracted, and group 3 = more than two contiguous teeth extracted. Immediate implants were placed in each group. Histologic assessment of arches showed no evidence of vertical bone loss (VBL) around implants in group 1 compared with groups 2 \((P < .001)\) and 3 \((P < .001)\). Buccal and lingual bone thicknesses and bone-to-implant contact (BIC) were higher around implants in group 1 compared with groups 2 \((P < .001)\) and 3 \((P < .05)\). Buccal and lingual bone thicknesses, BIC, and VBL are compromised around implants in multiple contiguous extraction sites compared with those in a single extraction site. (Int J Periodontics Restorative Dent 2014;34:413–421. doi: 10.11607/prd.1809)

Tooth extraction is a traumatic procedure that jeopardizes the surrounding alveolar bone. Several studies\(^1\)\(^-\)\(^4\) have shown that tooth extraction is directly associated with buccal bone remodeling. It has been reported that a significant amount of alveolar bone undergoes resorption within the first 3 months of tooth extraction.\(^5\)\(^,\)\(^6\) Likewise, Nevins et al.\(^7\) reported a loss of more than 20% of the buccal plate within the first 12 weeks of tooth extraction. This is due to the fact that the buccal process of alveolar bone is largely composed of bundle bone (that is supplied solely by supraperiosteal vessels and periodontal ligament) that makes it more susceptible to resorption following tooth extraction as compared to the lingual and/or palatal process of alveolar bone.\(^2\)\(^,\)\(^3\)\(^,\)\(^8\)\(^,\)\(^9\)

Recent studies\(^2\)\(^-\)\(^3\)\(^,\)\(^8\)\(^,\)\(^9\) based on micro-computed tomography (micro-CT) technology have shown that extraction of multiple contiguous teeth is associated with more intense buccal bone remodeling compared with single tooth extraction. An explanation in this regard may be derived from a histologic
study on baboons, in which the authors highlighted the histologic finding that the buccal bone also receives a blood supply from the adjacent interdental bone and not merely from the socket side of the alveolus. Results by Al-Askar et al showed that extraction of multiple contiguous teeth is associated with more intense buccal bone remodeling than when fewer teeth are extracted.

Various treatment regimens, including immediate placement of dental implants in fresh extraction sockets, have been proposed in an attempt to minimize alveolar bone resorption that occurs following tooth extraction; however, controversy persists in this regard. Results by Covani et al demonstrated that immediate placement of dental implants in fresh extraction sockets minimizes the rate of alveolar buccal bone resorption. On the contrary, in a recent micro-CT experiment, Al-Shabeeb et al demonstrated that the buccal bone thickness, alveolar bone height, and bone-to-implant contact (BIC) were significantly more compromised around immediate implants placed in multiple contiguous extraction sites compared with those placed in single extraction sites. To the authors’ knowledge, a histologic assessment of alveolar bone remodeling around immediate implants placed in single vs contiguous extraction sites has not yet been performed.

The aim of this histologic experiment was to assess the alveolar bone remodeling around immediate implants placed in single and multiple contiguous extraction sites.

Method and materials

Ethical guidelines

The study protocol was approved by the research ethics review board and the animal experimentation ethics committee of the Engineer Abdullah Bugshan Research Chair for Growth Factors and Bone Regeneration, 3D Imaging and Biomechanical Laboratory, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia.

Study animals and randomization protocol

Ten adult female beagle dogs, with a mean age and weight of 24 ± 0.83 months and 13.8 ± 0.49 kg, respectively, were used. The animals were vaccinated against rabies and infectious hepatitis and kept in individual cages throughout the study period. The subjects were randomly divided into three groups: group 1 = a single tooth extracted, group 2 = two contiguous teeth extracted, and group 3 = more than two contiguous teeth extracted sites, with 6 sites per group.

Nonsurgical protocol

All animals received supragingival scaling twice a week for 2 weeks, using an ultrasonic scaler (NSK). Antibiotics (ampicillin 25 mg/kg of body weight) were administered a day before and at the time of surgery. Antibiotics continued for 5 days after surgery (25 to 50 mg/kg of body weight, every 8 hours). Periapical radiographs of the extraction sites were taken. The animals were draped and the surgical site was swabbed with povidone iodine (Betadin Solution, Purdue Frederick).

Surgical protocol

Full-thickness buccal flaps were raised and extended the mucogingival junction using a sulcular incision with a no. 15 blade. The teeth were atraumatically extracted using piezosurgery (Mectron). All extracted teeth were devoid of dehiscence defects.

Overall, 48 immediate implants (10.5 mm long and 3 mm in diameter) (BioHorizons Laser-Lok) were placed in the maxillae and
mandibles (16 implants per group) in accordance with the extraction socket classification\(^2\) and at bone level placement (Fig 1). Briefly, in group 1, bilateral first and third premolars were extracted and replaced with immediate implants. In group 2, two adjacent premolars were extracted with one immediate implant placement in the mesial socket in the maxilla and in the distal socket in the mandible. This was done to avoid contacting the root of the distally curved canine root in the mandible using four dogs. In group 3, three teeth were extracted and immediate implants were placed in the central socket using four dogs. All immediate implants were placed in a submerged position inclined toward the lingual/palatal side, and healing screws were used. Immediate implants lacking primary stability at the time of placement were excluded from the study. In each group, a minimum distance of 1.5 mm was maintained between the implant and the adjacent tooth, and the mean gap between the implant and extraction socket was 0.75 mm. Suturing was performed using resorbable sutures (Ethicon).

Postoperative management

All animals received injections of amoxicillin (5 mg/kg of body weight once a day for 3 days) (Betamox LA, Norbrook Laboratory). Plaque control procedures were executed twice weekly by topical application of a 0.2% chlorhexidine digluconate solution (GUM, Sunstar Americas). Periapical radiographs were taken immediately after surgery to assess the relationship of the implants with the adjacent vital structures. Antibiotics were continued for 3 days after surgery (25 to 50 mg/kg of body weight, every 8 hours).

Euthanasia and jaw sectioning

After 16 weeks, all subjects were sacrificed with an overdose of 3% sodium pentobarbitol. The jaw segments containing the dental implants and associated mesial and distal tooth structures were removed en bloc using an electric saw (Leica SP 1600) and fixed in 10% neutral formalin solution.

Light microscopy

Jaw segments were decalcified in a solution containing equal parts 50% formic acid and 20% sodium citrate for 10 weeks. The decalcified specimens were washed in running water, dehydrated in an ascending ethanol series, and embedded in paraffin. Polymerized blocks were primarily ground to bring the tissue components closer to the cutting surface. A section of 100-\(\mu\)m thickness attached to the second slide was cut with a diamond blade saw under a pressure of 50 to 100 g. An ultimate thickness of 40 \(\mu\)m was attained by grinding and polishing with 1200-, 2400-, and 4000-grit sandpaper. From each block, sections were stained with toluidine blue/pyronin G as described elsewhere.\(^{11}\)

Histomorphometry

Measurements were performed with a light microscope linked to a video camera and computer using image analysis software (Omnimet 9.5). A new periodontal probe (Hu-Friedy) was placed over a histologic section to serve as a scale prior to image acquisition. A 1-mm portion of the periodontal probe was calibrated into pixels. Buccal bone thickness (BBT), lingual bone thickness (LBT), and BIC were determined using previously established criteria.\(^{12–14}\) Vertical bone loss (VBL) was measured by drawing a line from the cementoenamel junction of teeth adjacent to the implant to the crest of the alveolar bone.\(^2,15\)

Statistical analysis

The data were statistically analyzed using a software program (SPSS version 18, IBM). One-way analysis of variance and Bonferroni post hoc multiple comparison tests were used to determine if differences existed in the BBT, LBT, VBL, and BIC among the three experimental groups. Statistical significance was predetermined at level of \(\alpha = .05\).
Results

Histologic

Immediate implants placed in group 1 demonstrated healthy peri-implant soft tissues (see Fig 1). Figure 2a demonstrates BIC coronal to the interface gap and extending to the Laser-Lok abutment (LLA). There was no evidence of inflammatory cell infiltrate or absence of crestal bone resorption. Higher-power magnification shows very effective BIC with no gap and evidence of native and new bone (Figs 2b and 2c). The junctional epithelium extended only to the most coronal part of the laser-microetched grooved surface area (Fig 2d). Subsequently, the healthy connective tissue fibers have close proximity to the LLA surface, running in a perpendicular pattern. Evidence of bone regeneration can be seen on the LLA surface and the elimination of the interface gap through the osseointegration of the LLA with bone in the absence of any junctional epithelium on both buccal and lingual sides, respectively (Figs 2e and 2f).

Immediate implants placed in group 2 showed healthy peri-implant soft tissue with no inflammatory cell infiltrate (Fig 3a). Crestal bone loss with a mean exposure of two threads can be seen (see Fig 3a). The junctional epithelium ended up at the coronal part of the grooved area of the LLA and with healthy connective tissue fibers below, running perpendicular to the LLA surface (Fig 2b). The histologic results showed healthy connective tissue to be in direct contact with the laser-microetched implant abutment microchannels with fibers running in a perpendicular pattern with absence of long junctional epithelium (LJE) (Fig 3d). At $\times 100$ magnification, high and excellent percentages of BIC were noted. Native and regenerated bone (Figs 3c and 3e) were also noted.

Fig 2 Histologic results of immediate laser-ablated implants placed in group 1. (a) Healthy peri-implant soft tissue with no evidence of long junctional epithelium formation, buccal or lingual crestal bone loss, or inflammatory cell infiltrate. Osseointegration of all the implant threads was observed (toluidine blue/pyronin G; original magnification $\times 4$). (b and c) Excellent BIC on the buccal side, which is similar to the BIC on the lingual side. (d) Healthy connective tissue in proximity to the laser-ablated abutment microchannels with connective tissue fibers running in a perpendicular pattern (toluidine blue/pyronin G; original magnification $\times 80$). (e and f) Evidence of buccal and lingual BIC on the laser-ablated abutment surface (toluidine blue/pyronin G; original magnification $\times 60$).
Immediate laser-ablated implants placed in group 3 showed healthy peri-implant soft tissue with no evidence of LJE or inflammatory cell infiltrate (Fig 4a). Loss of vertical bone height was observed on the buccal side with a mean exposure of three threads (arrows) was observed (toluidine blue/pyronin G; original magnification ×4). Absence of LJE and evidence of a direct contact between connective tissue matrix and laser-etched abutment surface with implant threads in a functional pattern (toluidine blue/pyronin G; original magnification ×20). Direct contact between the connective tissue and implant threads preventing the long junctional epithelium to the beginning of BIC with no evidence of soft tissue encapsulation (toluidine blue/pyronin G; original magnification ×40). Finally, a high percentage of BIC and evidence of newly formed bone (dark stain) (toluidine blue/pyronin G; original magnification ×100).
Vertical bone loss

On the mesial aspect, the mean VBL was significantly higher in groups 2 (1.62 ± 0.67 mm) \((P < .001)\) and 3 (2.70 ± 0.83 mm) \((P < .001)\) compared with group 1 (0.37 ± 0.07 mm). On the distal aspect of the implant, the mean VBL was significantly higher in groups 2 (2.07 ± 0.30 mm) and 3 (2.76 ± 0.94 mm) compared with group 1 (0.38 ± 0.12 mm). These results are shown in Table 1.

### Table 1 \(\text{VBL (mean ± SD) around immediate implants placed in single and multiple contiguous teeth extraction sites} \)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mesial VBL (mm)</th>
<th>Distal VBL (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.37 ± 0.07</td>
<td>0.38 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>1.62 ± 0.67 *</td>
<td>2.07 ± 0.30 *</td>
</tr>
<tr>
<td>3</td>
<td>2.70 ± 0.83</td>
<td>2.76 ± 0.94</td>
</tr>
</tbody>
</table>

VBL = vertical bone loss.

\(*P < .01\)

### Table 2 \(\text{Mean ± SD of BBT, LBT, and BIC around immediate implants placed in single and multiple contiguous teeth extraction sites} \)

<table>
<thead>
<tr>
<th>Group</th>
<th>LBT (mm)</th>
<th>BBT (mm)</th>
<th>BIC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.81 ± 0.88§</td>
<td>1.29 ± 0.44*†</td>
<td>56.2#**</td>
</tr>
<tr>
<td>2</td>
<td>1.77 ± 0.34¶</td>
<td>0.64 ± 0.69*¶</td>
<td>41.23#††</td>
</tr>
<tr>
<td>3</td>
<td>1.29 ± 0.98*¶</td>
<td>0.23 ± 0.17†‡</td>
<td>23.84**††</td>
</tr>
</tbody>
</table>

BBT = buccal bone thickness; LBT = lingual bone thickness; BIC = bone-to-implant contact.

\(*)P < .01\); \(†P < .01\); \(‡P < .05\); \(§P < .05\); \(¶P < .01\).

### Fig 5 \(\text{Mean buccal and lingual bone thicknesses around immediate implants placed in single and multiple contiguous teeth extraction sites.} \)

### Fig 6 \(\text{Mean BIC around immediate implants placed in single and multiple contiguous teeth extraction sites.} \)

### BB T, LBT, and BIC

The mean BBT was significantly higher around immediate implants placed in group 1 (1.29 ± 0.44 mm) compared with groups 2 (0.64 ± 0.69 mm) \((P < .01)\) and 3 (0.23 ± 0.17 mm) \((P < .01)\). The mean BBT was significantly higher around immediate implants placed in group 2 (0.64 ± 0.69 mm) compared to those placed in group 3 (0.23 ± 0.17 mm) \((P < .05)\) (Table 2 and Fig 5).

The mean LBT was significantly higher around immediate implants placed in group 1 (2.81 ± 0.88 mm) compared with groups 2 (1.77 ± 0.34 mm) \((P < .05)\) and 3 (1.29 ± 0.98 mm) \((P < .01)\). The mean LBT was significantly higher around immediate implants placed in group 2 (1.77 ± 0.34 mm) compared with group 3 (1.29 ± 0.98 mm) \((P < .01)\) (Table 2 and Fig 5).

The mean BIC was significantly higher in group 1 (56.2%) compared with groups 2 (41.23%) \((P < .05)\) and 3 (23.84%) \((P < .01)\).
The mean BIC was significantly higher in group 2 (41.23%) compared with group 3 (23.84%) \((P < .01)\) (Table 2 and Fig 6).

Discussion

To the authors’ knowledge, this is the first experimental study that provides histologic evidence of alveolar bone remodeling occurring around immediate implants placed in accordance with the extraction socket classification.\(^2\) The present histologic results clearly demonstrated that bone thickness (buccal and lingual) and BIC, as well as vertical bone height, were compromised around immediate implants associated with multiple teeth extraction sites (groups 2 and 3) compared with immediate implants associated with single tooth extraction sites (group 1). The present histologic experiment partially agrees with previous studies\(^ {16-19} \) that reported that immediate placement of implants in fresh extraction sites prevents alveolar bone remodeling. However, a recent micro-CT experiment demonstrated that immediate placement of dental implants is unable to halt or delay alveolar bone resorption in multiple contiguous teeth extraction sites.\(^9\)

It is known that the buccal process of alveolar bone is largely composed of bundle bone\(^1,3,4\); however, other studies in baboons have demonstrated that the buccal bone receives an essential portion of its blood supply from interproximal sides.\(^1\) These studies also proposed that buccal bone remodeling was proportional to the number of contiguous teeth extracted and that its vascular supply is compromised to a much greater extent following extraction of multiple contiguous teeth.\(^1\)

There may be a possible biologic mechanism that augments osteoclastic activity, particularly on the buccal aspect of the alveolar bone, compared with the lingual aspect, thereby enhancing buccal bone resorption to a much greater extent around immediate implants placed in groups 2 and 3.

An interesting finding in the present study was that the lingual bone was present at the laser-ablated surface of the implant regardless of socket class. The results demonstrated that connective tissue fibers were running in a perpendicular pattern in proximity to the laser-ablated implant surface. Previous studies\(^ {11,20} \) have shown that laser-ablated microgrooves placed within implant collars are effective means of supporting direct connective tissue attachments to altered implant surfaces. In addition, an interesting finding in the present experiment was that the LJE was absent in all cases, and a direct connective tissue attachment with the implant was observed. Such a direct connective tissue attachment serves as a physiologic barrier to the apical migration of the LJE and prevents crestal bone resorption. It is known that parallel rather than functionally oriented perpendicular connective tissue fibers prevent the formation of the physiologic connective tissue barrier and can cause potential crestal bone resorption. The current results showed healthy peri-implant soft tissue in all experimental groups with absence of the LJE, and the connective tissue fibers were running perpendicular to the implant surface. Around immediate implants placed in group 1, it was evident that the bone was in direct contact with the laser-ablated surface. A biologic seal via connective tissue in direct proximity to the laser-ablated surface and thread was observed in groups 2 and 3, where bone remodeling occurred. A human histologic study demonstrated that there is a possibility of achieving a physical connective tissue attachment to the laser-ablated collar of a dental implant.\(^{21}\)

A limitation of the present experiment is that alveolar bone remodeling around immediate implants was tested in healthy dogs. It is well known that systemic conditions (such as poorly controlled diabetes mellitus and prediabetes) jeopardize periodontal tissues, including alveolar bone.\(^ {22-26} \) It may therefore be argued that the outcomes of the present experiment (BBT, LBT, VBL, and BIC) may be further compromised in each group in immunocompromised individuals and tobacco smokers compared with their respective controls; however, further studies are warranted in this regard. Since this was a 16-week study, it is possible that more bone remodeling may occur in the long term, which requires further long-term experimental studies. Furthermore, this study focused on the posterior region of the mandible. In this regard, the results...
may differ under clinical scenarios where implants are placed in the anterior tooth sites of both arches. This warrants further investigations with emphasis on implant location and study duration.

The present experiment was conducted in the absence of adjunctive periodontal regenerative techniques such as guided bone regeneration (GBR). It may therefore be argued that immediate placement of implants with adjunctive GBR treatment may play a role in minimizing buccal bone remodeling. However, further studies are necessary in this regard. Within the limits of the present histologic experiment, it is concluded that BBT, LBT, BIC, and vertical bone height are compromised around immediate implants placed in multiple contiguous extraction sites compared with those placed in a single tooth extraction site. Immediate implants with laser-ablated abutment promote osseointegration and prevent formation of long junctional epithelium, even in the presence of bony dehiscence defects and thread exposure.

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References


