Healing and osseointegration of submerged microtextured oral implants

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Abstract: The success of dental implants is primarily dependent upon the degree of osseointegration or bone-to-implant contact (BIC), possibly facilitated by a roughened implant surface. This study was performed to histologically evaluate the nature of osseointegration and bone healing of submerged microtextured implants in eight dogs. Three months following tooth extraction in the posterior mandibles, three microtextured submerged implants were placed in each quadrant. Block biopsies were harvested at 4 and 16 weeks (four dogs each) following surgery, and histologic preparation was performed. Histomorphometric analysis demonstrated that % BIC value increased marginally from 40% at 4 weeks to 48% at 16 weeks, without a statistically significant difference. The first bone-to-implant contact (f-BIC) at 16 weeks was significantly lower than the 4-week f-BIC (0.81 mm vs. 0.56 mm). In conclusion, this study found minimal change in BIC over time (from 4 to 16 weeks) in unloaded microtextured implants, while the mean f-BIC value significantly increased during this same observation period.

Endosseous dental implants have been successfully used for the rehabilitation of fully and partially edentulous patients, with a high predictability [Bränemark et al. 1969; Adell et al. 1981; Schroeder et al. 1981; Albrektsson 1988; Buser et al. 1999; Weber et al. 2000]. Albrektsson et al. [1986] proposed success criteria for submerged dental implants that include that more than 85% of implants should remain in function at 5 years and more than 80% at 10 years. Buser et al. [1997] demonstrated 93.3% of implant success rate in non-submerged implants. It is generally considered that implant success is primarily dependent upon or achieved by osseointegration, a direct contact between the implant surface and ordered, living bone [Bränemark 1985]. Various approaches have been attempted to enhance the level of osseointegration via increasing bone-to-implant contact (BIC). For the purpose, altering the surface and/or shape of the implant has been frequently utilized. An average of 37.2% BIC was observed in the screw-shaped commercially pure titanium implant in rabbits at 3 months following implant placement [Johansson & Albrektsson 1991]. In contrast, it has been shown that rough implant surfaces allow a higher percentage of BIC compared with implants with smooth surfaces [Buser et al. 1991; Ericsson et al. 1994; Gottfredsen et al. 1995; Pebe et al. 1997; Wennerberg et al. 1998; Trisi et al. 1999; Gottfredsen et al. 2001]. The level of osseointegration or BIC is often determined by testing the amount of torque required to remove the implant or by histomorphometric analysis [Wennerberg et al. 1995; Cochran 2000].
Buser et al. (1991) histomorphometrically evaluated BIC of six different implant surfaces [electropolished, sandblasted with medium grit and acid pickling, sandblasted with large grit, sandblasted with large grit and acid etching, titanium plasma-sprayed, and hydroxyapatite plasma-sprayed] in the tibia/femur of miniature pigs. They found significant differences in BIC between the six groups at 3 and 6 weeks following implant placement, with no significant differences in BIC noted between the two healing times in each group. Implant surfaces sand-blasted with a large grit and acid-etched had the highest BIC (52–58%) among the groups, and the BIC remained stable up to 6 weeks. Similarly high levels of BIC were noted in hydroxyapatite (HA)-coated implants, but they often revealed localized resorption during the healing period. Ericsson et al. (1994) evaluated BIC of machined or TiO2-blasted titanium implants in dogs. They found that the mean percentage of BIC was approximately 40% in both implant surfaces at 2 months, whereas TiO2-blasted surface showed greater BIC (65%) than standard machined surface (43%) at 4 months.

Studies suggest that the level of BIC is not significantly influenced by surgical approaches, non-submerged and submerged [Abrahamsson et al. 1999; Kohal et al. 1999]. Kohal et al. (1999) evaluated BIC of HA-coated implants and reported that there was no significant difference in BIC at 6 months between submerged and non-submerged implants (63.4 vs. 70.3%). The study also revealed greater BIC at 6 months (70.3%), compared with 3 months (58.3%) in non-submerged implants.

It is generally believed that the degree of BIC varies depending on implant macro/micro structures, surface characteristics, different healing periods, and the presence or absence of loading (Buser et al. 1991; Cochran et al. 1998; Piattelli et al. 1998; Cochran 1999). Furthermore, differences in methodologies used in studies to evaluate the level of BIC may be also attributed to the variance of BIC levels. These include different measurement methods, unequivocal site selections and biologic variation from different study models.

Recently, a screw-type implant with a microtextured surface has been introduced. The microtextured surface is grit-blasted with soluble, ceramic HA powder to increase surface contact areas, then followed by a mild, non-etching, acid (HCl) wash to remove residual particles of the basting medium. Limited data is available on the degree of BIC of the microtextured implant, and information on early bone healing in submerged oral implants has been insufficient. Therefore, the aim of this paper was to histologically evaluate the nature of osseointegration and early bone healing of the microtextured implant at 4 and 16 weeks in dogs.

Material and methods

Eight systemically healthy mongrel dogs, skeletal mature and aged from 1 to 2 years, were used in this study. The dogs chosen had full, well-aligned dentitions without clinical signs of periodontal disease. The study was performed under approval of University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. The experimental protocol was described in the guided bone regeneration (GBR) study previously reported by Oh et al. (2003). Four months of healing was allowed after extraction of the mandibular 2nd through 4th premolars [P2-P4] in each dog, and three microtextured implants [SplinelT™ TWIST MTXTM, Sulzer Dental Inc., Carlsbad, CA, USA] were placed into previously extracted sites. Clinical and histologic evaluations were made at 4 and 16 weeks after implant surgery.

Surgical procedures

Surgical extraction of the mandibular premolars [P2-P4] and the implant surgery were performed under general anaesthesia. The general anaesthesia was induced with 12 mg/kg of sodium thiopental intravenously. For local anaesthesia and haemostasis on the surgical areas, 2% xylcocaine (1:100 000 epinephrine) was employed. To prevent post-surgical infection, antibiotics (amoxicillin 500 mg p.o. b.i.d.) was administered for 2 weeks following surgery. In addition, a long acting opioid, buprenorphine (0.015 mg/kg, i.m.) was given to the animals immediately after surgery and post-surgically every 12 h for up to 96 h as needed.

For implant surgery, mid-crestal incisions were made, followed by full thickness flap reflection. The crestal bone of the implant osteotomy sites was uniformly prepared using a round bur or bone chisel. Following implant site preparation [surgery protocol by Sulzer Dental Inc.] and creation of buccal dehiscence defects [for the GBR study by Oh et al. 2003], three microtextured implants, sized 3.75 mm in diameter and 8 mm in length, were placed in each mandibular quadrant. All osteotomy procedures were carried out under conscious saline irrigation. Primary wound closure was accomplished by flap releasing, followed by suturing with 4–0 Vicryl™ [polyglactin 910] suture material.

Oral hygiene and diet

During the study period, three times of weekly tooth brushing with a soft brush and 0.2% chlorhexidine gel was employed. However, for 2 weeks after each surgery, chlorhexidine swabbing of the surgical wounds was performed instead of tooth brushing to minimise mechanical trauma. In addition, for the first 3 weeks after surgery, the animals received a soft diet in an attempt to reduce mechanical trauma that could negatively influence healing process.

Bone labelling

To assess the pattern of osseointegration in a timely manner, a series of fluorochrome bone labelling materials were used [Giancagle et al. 1998]. Three bone labels were administrated on four dogs to be killed 4 weeks after implant placement in the following sequence: calcein green ([8 mg/kg, i.m.]) 2 days after implant placement, xylcenol orange ([60 mg/kg, i.v.]) 2 weeks after the surgery, and tetracycline HCl ([10 mg/kg, i.m.]) 2 days prior to killing.

The remaining four dogs to be killed at 16 weeks following implant surgery received a series of four bone labels as follows: calcein green ([8 mg/kg, i.m.]) 2 days after implant placement; xylcenol orange ([60 mg/kg, i.v.]) 8 weeks after the surgery; tetracycline HCl ([10 mg/kg, i.m.]) 12 weeks after the surgery; and alizarin red ([25 mg/kg, i.m.]) 2 days prior to killing.

Histology procedures

Animal sacrifice was carried out at 4 and 16 weeks [four dogs each] with an overdose of pentobarbital ([65 mg/kg, i.v., to effect]).
Immediately after the sacrifice, radiographs were taken to ensure implant positions and osseointegration. Block biopsies including implants and surrounding tissues were obtained, fixed in 10% neutral buffered formalin, dehydrated using ascending grades of alcohol, infiltrated, and embedded in methyl methacrylate (MMA) for non-decalcified sectioning [Sanderson & Kitabayashi 1994]. Each portion of the block biopsy contained three implants, and the orientation of the sections was transverse along the length of the implant. Three transverse serial sections, 500 μm in thickness, were taken along the long axis of the fixture by using a diamond wire saw, representing the mid portion of each implant site [Well Diamond Wire Saws, Inc., Norcross, GA, USA]. Each section was glued to a plastic slide [Wasatch Histology Consultants, Inc., Winnemucca, NV, USA] (Bloebaum et al. 1989), ground to approximately 50-70 μm utilizing an EXAKT Micro Grinder 400 (Exakt Medical Instruments Inc., Oklahoma City, OK, UAS), and polished to an optical finish. One section per each implant site was left unstained for fluorescence analysis, and the remaining two sections were stained with Sanderson’s Rapid Bone Stain™ [Surgipath Medical Industries, Richmond, IL, USA] and an acid fuchsin counterstain for histomorphometric and histologic analyses. Among the stained slides, one slide per implant site demonstrating higher proximity to the mid-portion of the fixture was chosen for histometric analysis.

**Histomorphometry and statistics**

Histometric analyses were performed by a calibrated, masked examiner [JY]. For histometric measurements, microscopic images were transferred to an IBM computer and analyzed with an image analysis software [Image-Pro Plus®, The Imaging Express™, Media Cybernetics Inc., Silver Spring, MD, USA]. Histomorphometric parameters included % BIC (per cent bone-implant contact/entire lingual implant thread length measured from the implant neck to the apical end of the implant) and f-BIC values (distance between the implant neck and first bone-implant contact). Histomorphometric landmarks are illustrated in Fig. 1. In addition to the histomorphometric analysis, patterns of osseointegration and bone healing were observed using histological and fluorescent specimens.

For the statistical analysis of histometric data, student t-test was used to compare differences between 4 and 16 week healing in each parameter. The data were presented as mean ± standard deviation (SD), and the significance level was set at $P < 0.05$.

### Results

#### Clinical and histologic observations

Clinically, of the 48 implants installed, one implant in the 16-week group was lost. No notable radiographic bone loss was found in the remaining 47 implants. In general, the healing was uneventful, and the animals demonstrated normal behaviour during the healing period. Histologically, in contrast to the radiographic findings, fibrous encapsulation down to the apical region was observed around the four implants, two at 4 weeks and two at 16 weeks, revealing 89.6% success rate at the histologic level.

Descriptive histology demonstrated uniformly distributed BIC, which was similar between 4- and 16-week observation periods. However, the level of f-BIC was generally lower at 16 weeks (Figs 2 and 3). Histologic observation under a higher magnification demonstrated active bone modelling with continuous bone deposition to the implant surface at both time points, which was confirmed by the pattern of fluorescent marker distribution.

#### Histomorphometry

Calibration of intra- and inter-examiner errors in histometric measurements demonstrated 97.9% intra-examiner [JY] and 94.2% inter-examiner reliability with the gold standard [TO]. The analysis of the histometric data revealed that the mean %
Fig. 2. Histologic observation at 4 weeks. [A] BIC is relatively uniform along the implant threads. Note the level of f-BIC (arrow) close to the implant neck (original magnification ×20; Sanderson’s Rapid Bone StainTM). [B] Higher magnification demonstrates intimate BIC with abundant bone marrow spaces (original magnification ×100; Sanderson’s Rapid Bone StainTM). [C] Active deposition of the bone labelling material (xylenol orange; arrowheads) is observed near the implant surface, indicating that active osteogenesis occurred at 2 weeks when the fluorochrome was administrated (original magnification ×20; fluorescence). [D] Higher magnification of a 4-week specimen. The deposition of xylenol orange (arrows) toward the implant surface is clearly shown, and active osteogenesis is observed in the bone (asterisks) (original magnification ×100; fluorescence).
Fig. 3. Histologic observation at 16 weeks. (A) The level of f-BIC (arrow) is significantly lower compared with 4-week f-BIC. However, BIC at this time point appears greater than that of 4-week group (original magnification × 20; Sanderson’s Rapid Bone Stain™). (B) Higher magnification of a 16-week specimen. There are numerous fully differentiated osteocytes (arrows) seen around BIC areas, suggesting bone deposition towards the implant surface in an earlier period. More mature form of bony structure is shown farther from the implant site, revealed by an osteon (asterisk) (original magnification × 100; Sanderson’s Rapid Bone Stain™). (C) Continuous deposition of fluorescent markers (arrows) is noted. Also, active bone remodelling is demonstrated by a high number of concentric rings of bone labelling materials in the bone (original magnification × 20; fluorescence). (D) Higher magnification. A timely manner of bone deposition at the implant surface and host bone is evident with different fluorescent markers, including calcein green (asterisk), xylene orange (arrow) and tetracycline yellow (arrowhead), which were administrated at 2 days, 8 and 12 weeks post-implantation, respectively. Alizarin red is not shown in this figure (original magnification × 100; fluorescence).
BIC of the 4-week group was approximately 40.3%, while the corresponding value for the 16-week group was 48.1% [Table 1]. There was no statistically significant difference between the two groups. The mean f-BIC values were 0.56 and 0.81 mm at 4 and 16 weeks, respectively. The difference between the two groups was statistically significant ($P<0.05$).

**Discussion**

This experiment demonstrated that 89.6% of the microtextured implants were successfully osseointegrated, as observed at the histologic level. No statistically significant difference was observed in BIC between 4- and 16-week groups (40.3 vs. 48.1%). However, the f-BIC at 16 weeks was significantly more apically positioned than the 4-week f-BIC.

A number of longitudinal studies have shown successful use of endosseous dental implants [Adell et al. 1981; Albrektsson 1988; Buser et al. 1999; Weber et al. 2000]. This experiment demonstrated a difference between the radiographic finding and histologic analysis in the degree of osseointegration. This is in agreement with the findings of Henry et al. [1997] that clinical or radiological evaluation could overestimate the degree of osseointegration as compared with histometric analysis. Due to absence of loading and insufficient clinical assessment for osseointegration [i.e. no mobility or torque test] in this study, a clinical success rate was not reported.

Various modifications of the implant surface and shape have been utilized to increase BIC. For example, BIC is often increased by roughened implant surfaces [Buser et al. 1991; Ericsson et al. 1994; Gotfredsen et al. 1995; Wennerberg et al. 1995; Pebe et al. 1997; Wennerberg et al. 1998; Trisi et al. 1999; Gotfredsen et al. 2001b]. The present study evaluated BIC of a microtextured implant surface. The microtextured implant surface is prepared by grit-blasting of a microtextured surface and shape have been utilized to increase BIC. For example, BIC is often increased by roughened implant surfaces [Buser et al. 1991; Ericsson et al. 1994; Gotfredsen et al. 1995; Wennerberg et al. 1995; Pebe et al. 1997; Wennerberg et al. 1998; Trisi et al. 1999; Gotfredsen et al.

**Table 1. The percentage of bone-to-implant contact (BIC) and first bone-to-implant contact (f-BIC) at 4 and 16 weeks**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>BIC (%)</th>
<th>f-BIC (mm)</th>
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<tbody>
<tr>
<td>4 weeks</td>
<td>22</td>
<td>40.3 ± 16.4</td>
<td><em>0.56 ± 0.35</em></td>
</tr>
<tr>
<td>16 weeks</td>
<td>21</td>
<td>48.1 ± 16.0</td>
<td>0.81 ± 0.43</td>
</tr>
</tbody>
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Data were presented as mean ± standard deviation. *$P<0.05$ (statistically significant difference between 4 and 16 weeks).

Recently, Gotfredsen et al. [2001b] investigated bone reactions adjacent to titanium implants with a titanium plasma-sprayed (TPS) or a machined surface subjected to a 24-week period of static load in three dogs. The mean histometric values of BIC after the loading period were 60% for the TPS group and 53% for the machined group, higher than the mean BIC value found in the present study. This might be explained by the employment of loading in their study. This is in agreement with the finding that the presence of loading may affect the degree of BIC [Piattelli et al. 1998, Gotfredsen et al. 2001]. Also, when comparing degrees of BIC reported in the literature, it should be noted that each study used different measurement methodologies, healing periods, and study models. Especially, it is critical whether the measurements included surfaces along the entire implant threads or a few best threads in evaluating percentage of BIC. Furthermore, and most importantly, it remains unclear how much BIC is required to achieve clinically successful osseointegration, and whether or not implants with a rough surface are always beneficial regardless of host bone quality.

A growing body of literature indicates that BIC remains steady or slightly increased when a longer healing time is allowed [Buser et al. 1991; Hale et al. 1991; Ericsson et al. 1994; Gotfredsen et al. 1995; Kohal et al. 1999]. The microtextured implant used in the present study revealed 40% BIC at 4-week healing and 48% BIC at 16 weeks. Although there was a trend that BIC increased when more healing time was allowed, the difference was not statistically significant. This might have been partly due to the difference between the two time points in the level of the f-BIC [0.56 mm at 4 weeks and 0.81 mm at 16 weeks]. The level of the f-BIC found in the present study corresponds to the mean f-BIC value of submerged implants reported in Kohal et al. [1999]. Histometric data of their study demonstrated that the average distance from the implant neck to the f-BIC was 0.58 mm at 6 months of healing in HA-

TiO$_2$, when compared with machined implants [29.7 vs. 19.9% for all threads and 40.9 vs. 34.5% for three consecutive best threads].

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coated submerged implants. The f-BIC after healing is considered as the reference from which future implant crestal bone loss is evaluated [Adell et al. 1981]. Furthermore, initial f-BIC levels may have a predictive value for early implant bone loss, related with different peri-implant environments.

Although there have been a number of studies that have hypothesized and explored the causes of early implant bone loss [Oh et al. 2002], little data are available with regard to whether or not there is naturally occurring implant crestal bone resorption during early bone healing. Further studies are needed to understand the nature of bone healing and osseo-integration.

Several limitations were associated with this study. This is a descriptive type of study that lacks a comparison (or control) group, such as machined or other roughened surfaces. Another limitation was the limited healing periods without loading. Future investigations will be needed to determine the effects of loading on BIC and f-BIC using microtextured implants.

Under the constraints of the present study, it can be summarized that: [1] 89.6% of the microtextured implants revealed osseointegration at the histologic level, [2] there was a trend of increasing BIC from 4 weeks (40%) to 16 weeks (48%), but not statistically significant; and [3] the mean f-BIC value was significantly higher at 16 weeks compared with 4 weeks.

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References


