Histologic and histomorphometric analyses of peri-implant bone from loaded dental implants: A case report.

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The histological findings of peri-implant tissues from nine functionally loaded dental implants from an adult cadaver were analyzed, and histomorphometric analyses were performed. Despite the presence of peri-implant bone loss, all implants were found to have a high degree of osseointegration, with the bone-to-implant contact (BIC) ranging from 69% to 88%. The mean value of the BIC was 83.2% ± 4.3% (range: 76.5% to 87.7%) for the maxillary implants and 74.4% ± 7.1% (range: 69.4% to 84.9%) for the mandibular implants. The BIC of the maxillary and mandibular implants was comparable. Relatively prominent bone remodeling and resorption with soft tissue ingrowth were observed in the vertical bony defects compared to the areas without intrabony components, which might represent the sequence of bone loss around the dental implants. *Int J Periodontics Restorative Dent* 2022. doi: 10.11607/prd.5698

Introduction

For decades, dental implants have been used for replacing missing teeth and/or oral rehabilitation in partially and fully edentulous patients. A systematic review demonstrated that the survival rate of dental implants, with a follow-up period of at least 10 years, ranged from 73.4% to 100% with a mean of 94.6% while the mean implant success rate was 89.7% based on the success criteria proposed by Albrektsson et al.1 Compared to conventional fixed prostheses, implants are a promising option for replacement of a missing tooth due to a relatively low and narrow range of failure rates 2 and improvements in masticatory function have been reported in patients who switched from mandibular conventional complete dentures to implant-supported or retained overdentures, particularly in patients with resorbed mandibular ridges.3, 4

Osseointegration, a concept introduced by Brånemark in the 1960s, is a direct contact between living bone tissue and the surface of an implant at the light microscopy level.5 Failure of
osseointegration results in implant failure. One criterion for implant success is clinical immobility.6 On the other hand, primary implant stability, determined by the contact between the bone and implant surface as well as the compressive stresses at the time of implant placement,7 is critical to the success of osseointegration, which in turn, influences the treatment outcomes.8 During the healing process after surgical implant placement, bone formation and remodeling account for secondary implant stability.7 In a case series study, secondary stability, measured with resonance frequency analysis (RFA), was positively associated with implant survival, while primary implant stability did not significantly correlate with implant outcomes.9

Bone-implant contact (BIC), as measured histomorphometrically, is the gold standard for the degree of osseointegration,10 but RFA assessment did not predictably determine BIC in one preclinical study.11 Clinical studies which reported on BIC, have been performed on implants retrieved due to various reasons such as implant fractures, peri-implantitis, prosthetic, psychological reasons, or at autopsy.12, 13 Numerous factors could potentially affect the value of the BIC. Nevins et al. demonstrated that the mean BICs of SLA surface implants were 74.5% for implants placed in healed ridges and 66.2% for immediate implants after a healing period of 6 months.14, 15 In a meta-analysis by Sagirkaya and co-workers, the mean BIC in the mandible was significantly higher than that in the maxilla; in addition, conventionally loaded implants showed the highest mean BIC compared to unloaded implants and immediately loaded implants. The design and/or the surface topography of implants might also have an effect on the BIC.16 Wide variations of the BIC have been reported in clinically stable and successful implants, ranging from 32% to more than 95%.12 Although it remains unclear whether the value of BIC affects the clinical implant stability, the BIC has been considered crucial for long-term implant survival and/or success rates.12, 16 Therefore, the goal of this histomorphometric case report was to measure the bone-to-implant contact ratio from functionally loaded implants from a human cadaver and to compare any differences in BIC between the mandible and maxilla.
Materials and Methods

A fresh frozen cadaveric maxilla and mandible from an 88-year-old at death Caucasian female with nine root form dental implants (five in the maxilla and four in the mandible) were assessed in this report. The cause of death, medical history and dental history were not available. The Indiana University Institutional Review Board reviewed and approved this protocol (#1905922113) prior to the initiation of this study. This study followed the Helsinki Declaration of 1975, with 2013 revisions. First, the mandibular removable prosthesis and retention bars were removed with an implant screwdriver since all implants were utilized for implant retained complete dentures. Secondly, the specimens were sectioned between each implant with diamond discs at approximately 15,000-20,000 rpm with copious irrigation in order to obtain block sections. Once each specimen was detached from the maxilla and mandible, the implant, bone and buccal and lingual soft tissues were maintained as a part of the block section. A total of nine blocks were obtained and stored in 10% neutral-buffered formaldehyde solution to fix the soft and hard tissues for 14 days. Following fixation, those specimens were dehydrated by series of ethanol. After dehydration, each specimen was embedded in polymethylmethacrylate (PMMA) without decalcification. The blocks were sectioned with a diamond saw and ground down to approximately 50-80 μm parallel to the long axis of the dental implants of buccal-lingual (palatal) dimension. The specimens were stained with toluidine blue followed by basic fuchsin. The histomorphometrical images were digitized with the fluorescence microscope (BZ-X800, Keyence, Osaka, Japan) with an objective lens (20X). All images were stitched with BZ-X800 viewer software. All digital images were transferred to ImagePro, Media Cybernetics® (Silver Spring, USA) for further analysis. The BIC was measured by dividing the total surface length of implant surface in bone by the length where actual direct bone-to-implant contact was present. BIC was measured on buccal and lingual side of implant fixture independently. The mean of the buccal and lingual BIC was reported as the percentage of bone to implant contact of each implant. The bone area (BA) was
calculated as the percentage of occupying bone area within each thread. The BA measured the buccal and lingual parts of each implant fixture and the total area was calculated as a percentage of the total bone area.

Statistical analysis

The mean values of the BIC and BA were measured and recorded along with the standard deviations for each implant in the maxilla and mandible. The independent-samples t-test was utilized to compare the maxillary and mandibular arches for the BIC and BA. A 5% significance level was used for all tests.

Results

A total of nine dental implants were evaluated. The clinical observation and histological examinations revealed that all implants were machined surface type with an external hex connection, and none of these implants displayed any mobility. All maxillary implants (#4, 5, 6, 8 and 14) served as individual fixed single implant crowns. All mandibular implants were splinted with a single bar and were utilized as an implant-retained complete denture. The implant manufacturer and the duration of the loading time of each implant remained unknown to the investigators, but all nine implants in the study were machined surface implants with external hexagonal connections. All implants showed evidence of marginal bone loss which resulted in one to five threads being exposed, and the amount of peri-implant bone loss varied. The mean bone loss was 2.97 ± 1.45 mm (range: 1.25 - 5.13 mm) in the maxilla and 1.82 ± 0.99 mm (range: 0.76 - 3.13 mm) in the mandible (Table 1).

Regarding the peri-implant bone evaluation, mature lamellar bone surrounded all the retrieved implants, and no epithelium tissue or connective tissue was found on the titanium implant surface except at the areas of bone loss. Therefore, osseointegration was confirmed to some extent. Cortical bone was observed in the crestal area, and cancellous bone and marrow spaces were noted in the apical regions of the retrieved implants. The mean value of the BIC was 83.2 ± 4.3% (range: 76.5 - 87.7%)
with maxillary implants and 74.4 ± 7.1% (range: 69.4 - 84.9%) with mandibular implants. There were no significant differences (p=0.05) in the BIC between the maxillary and mandibular implants. Implant inter-thread areas were filled with lamellar bone for all implants. Bone filled areas within the threads was 84.3 ± 6.2% (range: 77 - 93.9%) for all maxillary implants and 74.5 ± 6.9% (range 69.8 - 84.7%) for all mandibular implants. The BA differences between maxillary and mandibular implants were not statistically significant (p=0.06) (Table 1). All implants were also divided into two groups based on the amount of bone loss. The mean BIC was 82.3 ± 6.2% for implants with >2 mm of crestal bone loss and 75.6 ± 6.9% for implants with <2 mm of bone loss. The mean BA was 82.8 ± 8.7% for implants with >2 mm of bone loss and 76.3 ± 6.1% for implants with <2 mm of bone loss. Statistically significant differences in BIC (p=0.17) and BA (p=0.25) were not detected.

The apex of three implants (#4, 5 and 6) was located within the maxillary sinus; however, there was a layer of thin bone over the apices of #4 and 6 implants (Fig 1). Nonetheless, osteointegration was confirmed with the presence of direct bone contact with the lateral side of the implants.

Bone remodeling and turnover were observed at the interface of the implants with the presence of a larger size of lacunae and osteocytes (Fig 2). Most of the specimens showed that the bone area which was recently or currently undergoing bone remodeling stained slightly stronger compared to the stable areas (Fig 3). This bone remodeling was more prominent with the presence of vertical bone defects in the crestal bone areas compared to the areas without vertical bone loss and also at the lateral sides of the implant bodies (Fig 4). There was epithelial and connective tissue downgrowth noted within the vertical bone lesions, and this might be a consequence of bone loss around the dental implants (Fig 5).

Discussion

To the best of our knowledge, this case is one of only a few reports demonstrating the histological characteristics of functionally loaded implants in a human cadaver. Compared to the specimens from
the retrieved implants, one of the advantages of a cadaveric report is that soft tissues around the implants could be examined. However, in our specimens, the surface epithelium was necrosed and lost during histological processing. Therefore, the supracrestal tissue height\textsuperscript{17} could not be measured in the present case. Additionally, although the influence of implant surface characteristics on surrounding tissues remains inconclusive,\textsuperscript{18, 19} the observations from this report may be different from those of rough-surface dental implants, which are currently the mainstream of dental implants placed today.

Comparing the BIC and BA of implants in the maxilla with that of the mandible, no statistically significant differences were found in this case, which is in agreement with the previous investigations.\textsuperscript{20, 21} Romanos et al. reported mean BICs of 48\% and 44\% for the grit-blasted-acid-etched titanium implants placed in the maxilla and mandible, respectively.\textsuperscript{20} However, these values were somewhat lower than those found in the present report. The implants in the previously cited paper were placed in a heavy smoker with an immediate loading protocol and loading for seven months. In addition to the potential effects from the history of smoking and the loading protocol, the relatively short loading period might account for the differences between those from our specimens. In contrast, with a longer functionally loading period, higher BIC values were demonstrated in this case with machine-surface implants. A cadaveric case report reported that four titanium plasma sprayed implants, which supported a bar overdenture, had a mean BIC of 76.4\% after twelve years of loading.\textsuperscript{22} This value is comparable to the results of our specimens. One limitation of the current case is the unknown loading time of the implants as the medical/dental history of the donor was not known.

The location of the maxillary sinus is one of the major concerns for implant placed in the maxillary arch. The apex of three implants were located within the sinus in this case report. While the bone was not visible on the CBCT images, histological observation revealed a thin layer of bone in two implants. This could possibly be the new bone formation on the maxillary sinus floor, which has been demonstrated with histologic evidence in clinical studies.\textsuperscript{23, 24}
Bone level changes around dental implants could result from physiological and/or pathological processes. A long-term retrospective study by Simion et al. showed that the mean marginal bone loss around machine-surface implants was 1.9 mm and the progression of peri-implantitis was fairly slow. In our specimens, more than 2 mm of bone height was lost in five implants; accordingly, pathologic bone loss was assumed to occur around these implants although bone remodeling may also account for the bone loss noted in these specimens. A few local factors that might play a role in the bone loss were observed. Although the role of keratinized tissue in crestal bone loss and/or peri-implantitis is still controversial, significant bone loss, amounted to 5.1 mm, was noted around the implant with a lack of keratinized mucosa and thin mucosal tissues. In this case, thin buccal bone was observed in the implants placed in the maxillary anterior region, which were found to have more than 2 mm of vertical bone loss. This finding is in agreement with a recent preclinical study, which revealed that thin buccal bone wall was more prone to physiological and pathological bone loss.

Wehner et al. showed that circumferential bone loss was more frequently observed in implants placed in a position where there is sufficient distance to the facial plate. In contrast, dehiscence-type defects were more dominant in implants near the lateral aspect of the alveolar ridge. The authors assumed that this might be because the thin bony wall would be completely resorbed as the disease progresses to a later stage. In line with previous reports, more prominent bony remodeling and resorption were observed in vertical bone loss areas with soft tissue down-growth in the present case. Lastly, more stable conditions were noted in horizontal bone level areas in our specimens, which might represent the later stage of the disease following bone remodeling.

Conclusions

The percentages of bone-implant contact were high in all implants after functionally loading in the present report. In the same individual, there were no statistically significant differences in either the BIC or BA between the implants located in the maxilla and those in the mandible. Bone remodeling
and resorption were more prominent within the intrabony defects with soft tissue ingrowth compared to the suprabony defects. This finding might represent the sequence of bone loss around the dental implants and might be correlated to the patterns of bone destruction that are observed clinically.

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References


Figure Legends

Fig 1. An implant from the maxilla presented with a small intrabony defect at the palatal aspect and a horizontal defect at the facial aspect. Bone loss was down to the bottom of the 6th thread on the
facial aspect and to the 4th thread on the palatal aspect. Thin bone (arrow) was present on the apex of the implant, which was located within the maxillary sinus. (toluidine blue/basic fuchsin stain; original magnification ×4)

Fig 2. The relatively dark-staining at the peak of the implant thread, where the bone was undergoing remodeling and turnover, was present with a larger size of the lacunae and osteocytes (arrow). (toluidine blue/basic fuchsin stain; original magnification ×20)

Fig 3. Beneath the soft tissue, which covered one and a half threads of the implant, recent or current bone remodeling was seen at the bone-implant interface, which stained slightly darker (arrows). (toluidine blue/basic fuchsin stain; original magnification ×20)

Fig 4. Prominent bone remodeling, represented by the relatively dark-staining, was seen within the intrabony defect (the left side of the implant). In contrast, the homogenous and relatively light staining bony area appeared to be less amount of remodeling (the right side of the implant). (toluidine blue/basic fuchsin stain; original magnification ×4)

Fig 5. (a) (b) (c) Soft tissue ingrowth along with the darker-stained bony surface was present in the intrabony component. (d) Bone surface where no intrabony component was present was stained homogenously and this might be interpreted as the later stage of peri-implantitis. (toluidine blue/basic fuchsin stain; original magnification ×20)
### Tables

Table 1 Histomorphometric Measurements of the BIC, BA, and Bone Loss

<table>
<thead>
<tr>
<th>Arch</th>
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<td>Range</td>
<td>Mean (%)</td>
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<td>Mean (mm)</td>
<td>Range</td>
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<td>69.8 – 84.7</td>
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Figures #5698

Fig 1

Fig 2

Fig 3

Fig 4