Evaluation of Osteoclastic Activity in the Canine Supraalveolar Periodontal Defect Model: An Exploratory Study.

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A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Periodontology

Oregon Health & Science University
Portland, Oregon

May 2015

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Acknowledgements

I would like to express the deepest appreciation to my committee chair, Professor Douglas Dickinson, who opened the door for me to start this thesis. His enthusiasm, encouragement, and faith in me throughout have inspired me in my pursuit of knowledge.

I would like to thank my committee members, Professor Ulf Wikesjö and Professor Cristiano Susin who both introduced me to the field of Periodontology, and whose enthusiasm for the complete understanding of the "biological aspects of periodontology" has had a lasting effect.

Also, I would like to thank Professor Ernest Weinberg who has convincingly conveyed a spirit of adventure in regard to clinical research and scholarship, and an excitement in regard to teaching.

In addition, a thank you to Professor James Katancik who has continued to give me guidance and persistent help to enable this thesis to be possible.

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Table of Abbreviations and Acronyms

- AB: Alveolar Bone
- BIC: Bone-to-Implant Contact
- CT: Connective Tissue
- OA: Osteoclastic Activity
- OC: Osteoclasts
- PD: Periodontal Defects
- PID: Peri-implant Defects
- PDL: Periodontal Ligament

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Abstract

Purpose

The aim of this study was to conduct temporal and spatial observations of osteoclastic activity (OA) at periodontal and implant sites following buccal and lingual flap reflection and surgical reduction of the alveolar ridge in a canine periodontal and peri-implant regeneration model to provide baseline information for the design of future studies investigating alveolar OA following injury.

Methods and Materials

Samples for analysis were obtained from a previous study, which evaluated pre-osteoblastic activity.[14] Contralateral routine critical-size, supraalveolar, periodontal (PD) and peri-implant (PID) defects including a space-providing titanium mesh device were created in 12 adult mongrel dogs. Two animals were euthanized at 2, 5, 9 and 14 days, and two at 4 and 8 weeks, to provide tissue samples for histochemical analysis of OA by tartrate-resistant acid phosphatase (TRAP) staining of osteoclasts (OC)**.**

Results

For PD, depending on the location, OA peaked between Days 5-14 in resident tissue, with greater numbers of OC at buccal than at lingual sites. OA in the periodontal ligament and resident trabeculae peaked at 5-9 days with some activity still present at 4 and 8 weeks. For PID, OA also seemed to peak by between Day 5-14; with buccal and lingual sites roughly similar, and osteoclasts still present at 8 weeks. OA at the bone-implant interface apparently peaked by day 14 to gradually attenuate. For PD and PID, OA in new osteoid broadly peaked around one month, but was still present at two months.

Conclusion

Broadly, OA in resident tissues following PD/PID creation peaked by 14 days to gradually return to baseline by 8 weeks. This timing was consistent with the established coupling of osteoblast and osteoclast activity, with an early phase of osteoblast activity during the first two weeks inducing a

transient peak of OA. The data was suggestive of a second peak of OA induced during the 4-8 week period, and likely associated with late bone remodeling and cortication. There were also indications that autologous blood could alter the timing and reduce the extent of peak OA.

Introduction

The periodontium consists of tissues that support the teeth, and includes alveolar bone, cementum, and periodontal ligament.[4] During periodontal surgery, various biological mechanisms are activated that lead to a healing process that initially stabilizes the damaged site and prevents infection. This can be achieved by one of two pathways – repair or regeneration. Healing by repair involves the formation of collagen-rich scar-like tissue. This stabilizes and protects the site, but often does not restore lost function. Ideally, repair would take place *via* the second pathway, regeneration. This process leads to the reconstitution of the lost tissue architecture and function, but does not occur without clinical intervention; healing by repair is dominant.

When comparing the healing pattern after periodontal surgery around teeth with that around implants, there are similarities as well as differences. Healing adjacent to teeth after periodontal surgery involves migration of cells from periodontal tissues including periodontal ligament, cementum, and bone. These cells mediate a process involving a sequence of events including inflammation, granulation tissue formation, maturation, and remodeling.[6] Unlike teeth, dental implants are surgically placed directly into native or regenerated bone, which limits the number of cell types that can migrate to, attach, and differentiate on the implant surface during healing.[56] Nonetheless, the general alveolar bone remodeling process in both models follow a similar sequence of events.[22] This remodeling process involves a dynamic interaction between osteoclasts and osteoblasts to maintain the amount of bone in a level of equilibrium, which plays an important role during both periodontal regeneration and repair.[45] Excessive osteoclastic activity however, may impair periodontal healing after periodontal and dental implant surgeries, which could lead to increased bone/tooth loss and failure of implants.

Although it is known that osteoclasts increase bone resorption in periodontal and dental implant surgeries, the exact behavior of these cells is poorly understood. Prior research has evaluated overall

resorption of alveolar bone following osseous surgery,[22] but no research has evaluated the temporal and spatial activity of osteoclasts following periodontal and dental implant surgery in periodontal and alveolar/peri-implant settings, particularly in a model permissive for tissue regeneration. Therefore, further research is needed to fully understand these processes.

The purpose of this research was to describe trends in OA during wound healing and regeneration at surgically created (sterile) periodontal and peri-implant defect (PD and PID) sites. Characterization of such trends would provide a basis for further studies aimed at improving clinical protocols with enhanced outcomes following reconstructive surgery in periodontal and alveolar/peri-implant settings.

In the present study we hypothesized that –

1) Both the resident and regenerative buccal bone would have higher OA compared to the lingual bone at both tooth and implant sites.

2) OA would be comparable at tooth and implant sites, except at the implant-bone interface, where activity would be higher versus the tooth-bone interface due to additional remodeling.

3) There would be a peak in OA in the two weeks following injury associated with mobilization of regenerative cell populations, and a second peak associated with remodeling between four and eight weeks.

4) The addition of autologous blood prior to wound closure would reduce the amount of OA as it was anticipated that more blood would promote more regeneration.

Materials and Methods

Tissues samples used in the present study were obtained from a previous investigation, which evaluated pre-osteoblastic activity.[14] In brief, twelve adult Hound Labrador mongrel dogs obtained from a USDA licensed vendor were used following a protocol approved by the Georgia Regents University (formerly Georgia Health Sciences University) Institutional Animal Care and Use Committee. Periodontitis-simulating defects were surgically created bilaterally around the second, third, and fourth mandibular premolar teeth.[57] Sulcular incisions were made around teeth and buccal and lingual mucoperiosteal flaps were elevated. Alveolar bone was removed circumferentially around designated premolar teeth, including furcation areas, 5mm apically from the cement-enamel junction with chisels and water-cooled rotating burs. Defect preparations included extraction of the first mandibular premolar and amputation of the first molar level with the crest of the surgically reduced alveolar bone. On the mandibular right premolar teeth, roots were planed with curettes and chisels to remove total cementum for establishment of new attachment.[57] On the left mandibular teeth, extractions were performed; then using 3 – TiUnite, 4.0 x 10 mm, Nobel Biocare implants, immediate implant placements were performed in sites of the distal root of the third and the mesial and distal root of the fourth premolars, and placed halfway apically into the bone to simulate peri-implant defects.[72] A titanium mesh device was placed on bilateral sites to provide space and wound stability for periodontal regeneration.[5,20] The surgical sites were closed for primary intention healing. The animals were divided into two groups of six. In the first group (Group 1), the sites were closed without further intervention. Since the volume of blood accumulating in the defect was, in effect, an uncontrolled variable, in the subsequent second group (Group 2), to control clot volume, 2 mL of freshly drawn autologous blood (without anti-coagulant) was injected under the mesh immediately prior to wound closure. The six animals in each group were scheduled for euthanasia at Day 2, 5, 9, 14, 29, and 56 day post-surgery. Block specimens containing the

defect sites were removed, formalin-fixed, demineralized at 4°C with 10% EDTA, pH 7.4, and processed for light microscopy by paraffin embedding. The mesh and any screws, as well as implants, were removed from the paraffin embedded tissue and 5 µm sections were cut after re-embedding.

The following TRAP staining protocol (Sigma 386A kit) was used to identify osteoclasts. Slides were deparaffinized by two three-minute changes in xylene, followed by rehydration in an alcohol series (100%, 95%, 70% ethanol; four minutes each), and washing in water for two minutes. The staining solution consisted of 44 ml of water pre-warmed to 37°C, 2 ml of acetate solution, 2 ml of Napthol AS-BI phosphoric acid, and 2 ml of tartrate solution. After mixing, the contents of one capsule of Fast Garnet GBC Salt was added, and the solution mixed for one minute on a magnetic stirrer. The solution was transferred to a plastic Coplin jar and placed in a 37°C water bath until the temperature equilibrated. The slides were placed in the Coplin jar and incubated at 37°C for 1 hour in the dark. Staining was confirmed by microscopy, and the slides washed for three minutes in deionized water. Sections were counterstained with acid hematoxylin for five minutes, washed in deionized water for three minutes, then air-dried. Sections were coated with Clearmount, and after drying they were coverslipped using Cytoseal xylene-free mounting medium.

Using Image Pro Plus 6.0 and an Olympus light microscope with 4x objective, individual photographs were taken of each area to be measured, and osteoclasts were counted. The regions delineated for counting are shown in Figures 5A, 5B, 10A, 10B. The buccal and lingual regions were evaluated separately. First, OC counts were measured along the surface of the cortical bone adjacent to the coronal half and then adjacent to the apical half of root/implant length in bone. Next, OC numbers were measured in the periodontal ligament (PDL) from the surgical surface of the defect to the apex of the root. Similarly, for the implant, OC counts were measured in the bone to implant contact (BIC) areas

from the surgical surface of the defect to the apex of the implant. In addition, OC numbers were analyzed on the bone surface within the original trabeculae adjacent to the coronal half and then adjacent to the apical half of root/implant length in bone; trabecular surface lengths were measured and OC counts converted to per 1 cm. Also, new osteoid (cells in the outer, inner, and inside the trabeculae) above the plane of the defect was evaluated. All OC counts were initially expressed as per unit length, and then converted to per cm. Regions were counted by one operator on three separate slides per time point to sample the OC count per region and values were averaged.

Results

Data were missing for some regions in all three sections, such as Group 2 PDL or bone to implant contact, in general due to issues with section quality in that region (often folding or separation of regions during processing). In some other cases, one of the three sections had poor specimen quality in one region. The remaining sections gave good quality histochemical results in regions of interest, allowing osteoclasts to be counted.

Buccal versus lingual trends in OA:

We first sought to determine if resident and regenerative bone showed higher OA in buccal regions in comparison to lingual regions. For PD, in Group 1, mean OA on the alveolar bone (AB) cortical surface (Figure 1A) peaked by Day 14 and numerically was considerably greater on the buccal than the lingual bone surfaces, with the highest numbers in the region nearest to the buccal surgical surface, and with moderate levels of activity persisting through two months. Group 2 showed a modest rise in activity continuing through Day 9, but with the peak at a moderate level of activity occurring around one month before declining to zero (Figure 1B). For Group 1 (no Group 2 data), OA on the periodontal ligament (PDL) attachment surfaces (Figure 2A) peaked by Days 5-9, with lingual appearing to peak earlier, and at perhaps a lower level of OA. There was an indication of a return of OA to a modest level and slightly rising on the buccal side during the 1-2 month period.

In both Groups, mean OA in the original resident bone trabeculae in the upper buccal/lingual regions (Figures 3A, 3B) also peaked by Days 5-9. However, in Group 1 the increase in OA extended to the lower buccal region. In Group 1, but not Group 2, OA returned to a modest level by one month before declining to a presumptive baseline level comparable to the Day 2 OA at two months at both

lingual and buccal sites. Total OA within the new osteoid (Figures 4A, 4B) peaked by Day 29 and then gradually attenuated to a low level by Day 56. In both groups, there were indications that the buccal region contiguous with the resident buccal surface (outer buccal) showed more OA than other regions, including the surface facing the teeth.

OA on the AB surface of PID sites in Group 1 (Figures 6A, 6B) peaked by Day 14, similar to Group 1 PD sites, and was numerically slightly greater on the buccal than the lingual bone. There was an indication of an increase in OA between one and two months, with moderate levels of OA remaining at two months on buccal and lingual surfaces. In Group 2, there was a modest peak at Day 9, declining slightly to levels maintained through Day 56.

In Group 1 (no Group 2 data), OA on the bone to implant contact (BIC) (Figure 7A) rose continuously from low levels at Day 2 to peak broadly at modest levels around Day 14 and then gradually diminish back to low levels at Day 56.

Mean OA in the original trabeculae (Figure 8A, 8B) peaked to moderate levels in Group 1 during Days 5-9 and then attenuated to modest levels through Day 14, in a pattern similar to that seen for PD, but without a modest increase evident during the 28-56 day period. In Group 2, modest levels were maintained throughout, with a small rise centered around Day 9 and ending at a similar level as Group 1 at Day 56.

In Group 1, mean OA within the new osteoid (Figure 9A, 9B) peaked at moderate levels by Day 29 and then with the exception of the contiguous outer buccal surface (that maintained moderate levels through Day 56), gradually diminished to a low level by Day 56, as seen for regenerative bone in PD sites. As seen for PD osteoid, at this time, there appeared to be more OA on the buccal osteoid surface contiguous with the resident bone than in other regions of regenerative bone. In Group 2, much of the

data were missing but seemed to end with a similar number of cells.

Overall, there was a trend for the buccal side to show more OA than the lingual side. This was more pronounced in Group 1 than Group 2, and was most prominent on the resident alveolar bone surface and the outer buccal surface of new osteoid.

PD versus PID trends in OA:

Next, we sought to determine if tooth and implant sites showed comparable activities. In both Group 1 and Group 2, PD and PID sites showed low levels of OA on the alveolar bone surfaces at Day 2, likely reflecting the removal of tissue during surgery (Figures 1A, 1B, 6A, 6B). In Group 1, on the upper buccal surfaces at both types of site, OA rose to a high level by Day 14, declining to moderate levels by Day 29 that were maintained through Day 56 (Figures 1A, 6A). Data from lower buccal regions at most time points were not available. At PD sites, lingual OA rose to only a moderate level by Day 14 that was maintained through Day 56, while at PID sites lingual activity attained a high level at Day 14, declining to moderate levels comparable to PD sites through Day 29 (Figures 1A, 6A). In Group 2, OA at PD sites rose to modest levels by Day 29 in Group 1 before falling to near zero at Day 56, while at PID sites, levels remained broadly low to modest from Day 9 on (Figures 1B, 6B).

Collectively, both types of sites showed high levels of buccal resident bone surface activity through Day 14, but within each Group, PD and PID sites showed several differences with respect to the kinetics of change and the levels at the end of the study at Day 56.

Within original trabeculae of resident bone, both PD and PID sites in both Groups showed similar profiles for OA, although levels were more muted in Group 2 (Figures 3B, 8B). There was an initial rise from low to moderate levels of OA through Days 5-9, declining through Day 14 to

background levels maintained through Day 56, although Group 1 PD showed a slight rise through Day 29.

Similarly, both types of sites showed a similar profile of OA in and on new osteoid (Figure 4A, 9A), and both Groups were broadly the same. Generally, OA rose from zero levels at Day 14 (when substantial amounts of osteoid were present) to peak at moderate levels at Day 29, declining to low levels at Day 56. Much of the data was missing through Day 14 for Group 1 and through Day 29 for Group 2 due to issues with section quality (folding or separation of regions during processing).

Next, we sought to compare OA on bone surfaces adjacent to the tooth at PD sites (Figure 2A) versus at the bone-implant interface at PID sites (Figure 7A). (Group 2 data was not available.) In Group 1, both buccal and lingual surfaces in the PDL of PD sites showed a moderate peak at Days 5-9, declining to low levels at Day 14 before increasing modestly through Day 29. In contrast, PID sites showed a rise to modest levels through Day 14 and steadily declined to low levels through Day 56. That is, the implant-bone interface showed less OA than the tooth-bone interface at the early peak, but maintained activity for a longer period of time.

Differences between Group 1 and Group 2 in OA:

Broadly, OA appeared to be reduced at both the PD and PID sites for the second group of animals that had 2 mL autologous blood injected under the mesh immediately prior to closure.

In the upper buccal region of the resident bone surface at PD sites, OA in Group 1, increased progressively from Day 2 to Day 14, attaining the highest level of OA compared to all other regions. Group 2 on Day 14 had only reached 16.7 ± 8.5 (Figures 1A, 1B). This difference was significant ($p=0.008$, t-test, $n=3$ per group). In Group 1, after this peak, OA declined by Day 56 to a level

comparable to the lower buccal region and the lingual upper and lower regions. In Group 2, OA in the upper buccal region climbed progressively through Day 29 to a level comparable to Group 1 at this time, then declined to a low level by Day 56.

OA in the lower buccal region of PD sites showed a pattern similar to the upper and lower lingual regions within each Group, but differed somewhat between Groups. Thus, in Group 1 (Figure 1A), OA remained relatively low during the early and intermediate periods, but rose to a moderate level (18.8 ± 6.6) in all regions by Day 56, suggesting sustained bone remodeling or loss at a moderate level. In Group 2 (Figure 1B), OA paralleled that for the buccal upper region, rising to a moderate level (15-20) by Day 29 and then declining to a low level (2.5 ± 1.7) by Day 56. The difference between the mean OA on AB for Day 56 was significant ($p=0.003$; t-test, n=4 measurements per Group). Thus, significant differences between the Groups in OA on the surfaces of resident bone were observed at PD sites, with lower OA in Group 2.

Addition of autologous blood prior to flap closure showed clear signs of diminished OA on alveolar bone at PID sites during the early stages – the first three weeks of healing. OA peaked in Group 1 at Day 14 with the upper buccal region attaining the highest level of activity. Both Groups continued to have slight levels of OA into Day 56.

Resident trabeculae:

Within the limited number of samples, there was no obvious difference/effect of additional blood on the resident bone trabecular OA at PD sites (Figures 3B, 8B). Both Groups showed a similar general pattern of a broad peak of moderate (~10-20) activity on Days 5-9, declining to modest levels through Day 29 and reaching low levels (~5) on Day 56.

At PID sites, in Group 1, OA increased in all regions during the first week of healing and then diminished. In Group 2, addition of the autologous blood appeared to have slight reduction in OA

particularly during the first two weeks of healing.

New osteoid:

Both Groups showed a similar trend in OA within new osteoid at PD sites (Figure 4A, 4B), with no OA scored in almost all regions through Day 14, reflecting an absence of osteoid or the presence of freshly synthesized osteoid through this period. The exception during this period was Group 2 trabeculae, which showed a modest level of OA as early as 14 days. Activity peaked at moderate levels on Day 29, declining to low levels on Day 56. However, OA on the inner buccal surface in Group 1 seemed to increase slightly by Day 56. Insufficient data were available to allow a comparison of Group 1 and Group 2 for OA in new osteoid at PID sites.

Discussion

This report presents a first, semi-quantitative analysis of early cellular events – involving osteoclastic activity – during periodontal and peri-implant wound healing/regeneration using a surgical adult mongrel dog model.

The first hypothesis proposed was that both the resident and regenerative buccal bone would have higher OA compared to the lingual bone at both tooth and implant sites in several areas including AB, PDL, and new osteoid. Data for OA on AB and new osteoid broadly supported this hypothesis. This was consistent with previous studies that have shown that following tooth extraction, resorption is greater on the buccal than lingual surface. One possible reason is that the buccal bone is predominately comprised of bundle bone, which resorbs completely, possibly due to a lack of supporting function following tooth extraction.[16] Another possible explanation is that generally the buccal bone is thinner than the lingual bone, which may have a greater susceptibility to resorption. Previous studies have also shown that after implant placement, the buccal wall resorbed more than the lingual wall.[37] Several reasons for this include thin tissue biotype, a facial malposition of the implant, and thin or damaged facial wall bone.[58]

Results from this study supported the second hypothesis that OA would be comparable overall at tooth and implant sites, consistent with observations in a previous study that showed both models follow a similar sequence of events in wound healing – first initiated with resorption followed by bone formation. In a comparison of tooth and implant in Group 1 for OA on the bone surface between the tooth or implant (Figure 1A, 1B), the tooth shows the region with the highest peak of OA was the upper buccal; however, healing around the implant (Figure 6A, 6B) had all regions peaking in OA at 2 weeks. During peri-implant healing, there is direct attachment of bone to the implant surface, while in periodontal healing, there is an intervening connective tissue attachment. Perhaps this connective tissue

attachment results in an inhibition of OA in most regions on AB of teeth compared to that of implants.[22] Only data from Group 1 were available for a comparison of PDL and bone-implant OA. These sites appeared to show different OA, with an early spike (Days 5-9) to substantial levels in the PDL, and a decline to modest levels, while the implant-bone interface showed a broad peak to modest levels through Day 14, declining slowly through Day 56. This observation was opposite to that originally hypothesized, where it was thought the bone-implant interface would require extensive remodeling. The delayed return to low levels of OA adjacent to the implant would be consistent with peri-implant bone healing involving an additional remodeling process of *de novo* bone formation around endosseous implants as the formation of a mineralized interfacial matrix is found on the implant surface.[23] OA in original trabeculae between tooth (Figures 3A) and implant (Figures 8A) in Group 1 appear to follow a nearly identical pattern with an early peak around one week that attenuates thereafter. OA in new osteoid between tooth (Figure 4A) and implant (Graph 9A) in Group 1 also appear to follow a nearly identical pattern with a peak around Day 28 that declines through Day 56.

Consistent with the third hypothesis, for both PD and PID, OA within original trabeculae of resident bone peaked during the two-week period following surgical injury. This is possibly associated with mobilization of regenerative cell populations from trabeculae during this period. After endosseous implant placement in humans, the critical period of healing in which an increased OA decreases the initial mechanical stability of the implant prior to the formation of new bone occurs at 2-3 weeks, which is equivalent to the OA peak found at two weeks in our dog study assuming a 1.5-fold difference in healing rate. However, OA on resident bone showed a progressive rise to moderate levels in most regions, peaking around Day 29. Similarly, OA in newly formed osteoid showed a peak around Day 29. This pattern is consistent with a peak in remodeling activity on external surfaces of resident bone and in newly formed woven bone during a roughly two-week period centered about a month after injury.

In a previous human study, OA following osseous surgery on teeth was shown to peak at 2-3 weeks.[13] The present study however, was performed on dogs, which have been suggested to have events of wound healing and bone remodeling occur approximately 1.5 times faster,[35] which is consistent with our study's results.

The second modest peak of OA found between four and eight weeks suggested a late stage of bone remodeling.

Consistent with the fourth hypothesis, the addition of autologous blood prior to wound closure appeared to reduce the amount of OA during healing. Although this initial study did not have a sample size that permitted a statistical analysis of this observation, the consistency of the results from different time-points raises the possibility that the additional blood may influence the regenerative potential, and warrants further exploration of a potential key variable in determining clinical outcome.

A statistical limitation of this study was a sample size of one animal per group at each time point, and just three slides (at most) per animal, which precluded the ability to establish a reliable estimate of the population means and standard deviation. Moreover, it was not clear that the sampling distribution of the osteoclast density was unimodal, indicating the need to use more sophisticated sampling techniques. However, this study was not designed to look into specific time points, but instead, to evaluate trends and help to explain biological phenomena related to the sequence of events of osteoclastic activity following periodontal surgery. The biological processes described in this study appear very similar to those obtained in previous studies evaluating osteoclasts following periodontal surgery,[13] but provide more extensive detail on the events that occur during the early healing period.

These observations may facilitate elaboration of further studies and development of clinical protocols that may enhance outcome following periodontal reconstructive surgery in periodontal defects as well as in peri-implant defects.

For future studies, I would like to take biopsy samples of teeth without surgical defects as controls to evaluate its osteoclastic activity. I would also like to place immediate implants in extraction sites that have not been surgically reduced to see if the current surgical model presents limitations in the normal healing pattern by partial removal of the bundle bone.

Limitations

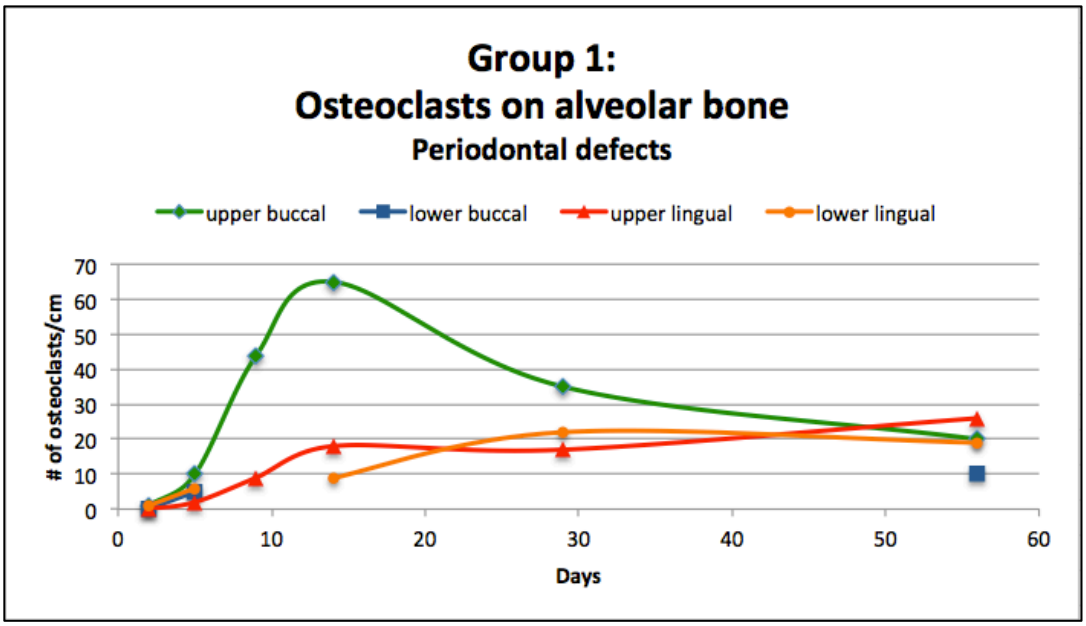
Data for some regions were unavailable due to sample quality.

There were only three slides per animal and only one animal per time point in each group, which precluded the ability to establish statistics.

The surgical preparations created by reduction of alveolar bone to simulate periodontitis defects may have intentionally removed ridge bone that could have otherwise followed a natural resorption process and that would have had the highest amount of osteoclast activity.

Figures and Tables

Figure 1A:



- Upper buccal/lingual refer to consecutive osteoclasts along the surface of the cortical bone adjacent to the coronal half of the root length in bone, converted to osteoclasts per centimeter
- Lower buccal/lingual refer to consecutive osteoclasts along the surface of the cortical bone adjacent to the apical half of the root length in bone, converted to osteoclasts per centimeter.

Figure 1B:

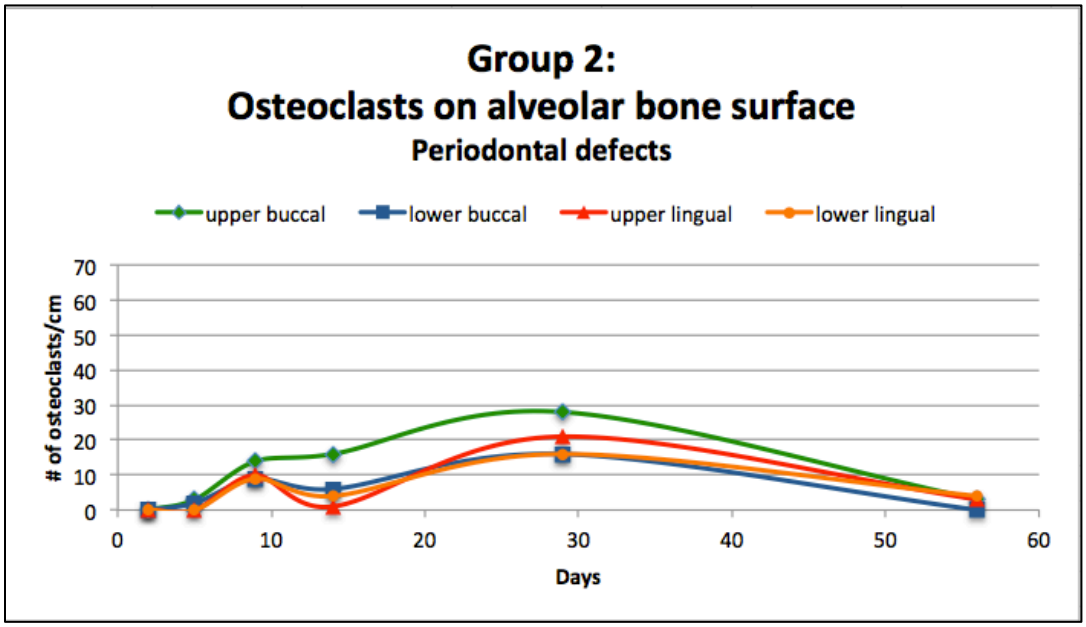
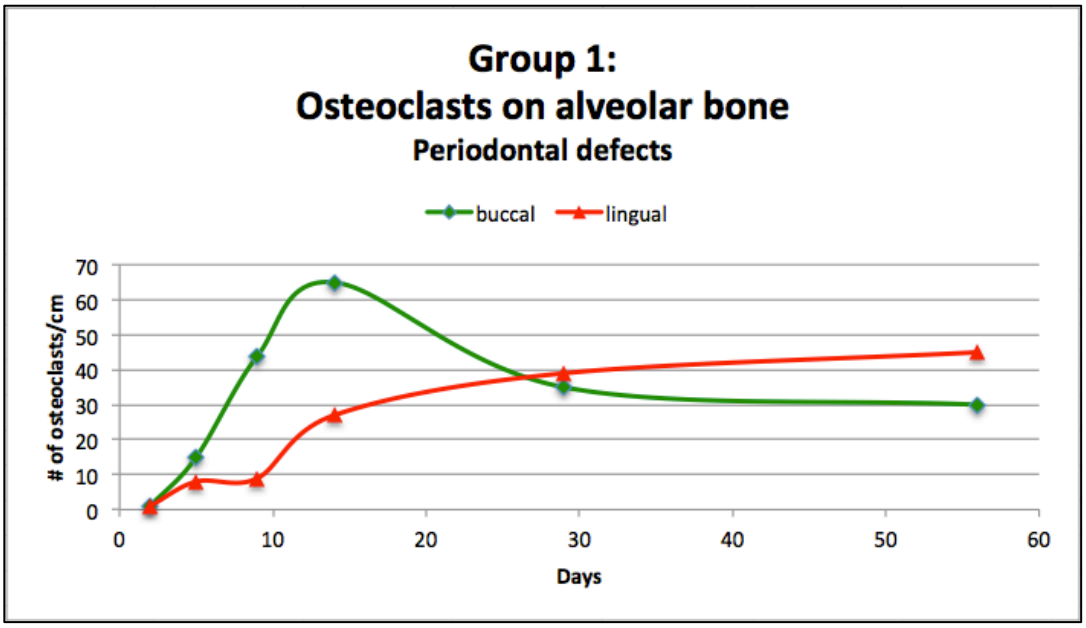


Figure 1C (OA on AB: total buccal vs lingual). Group 1: Periodontal Defect



- Buccal/lingual refer to total osteoclasts along the surface of the cortical bone adjacent to the root length in bone, converted to osteoclasts per centimeter

Figure 1D (OA on AB: total buccal vs lingual). Group 2: Periodontal Defect

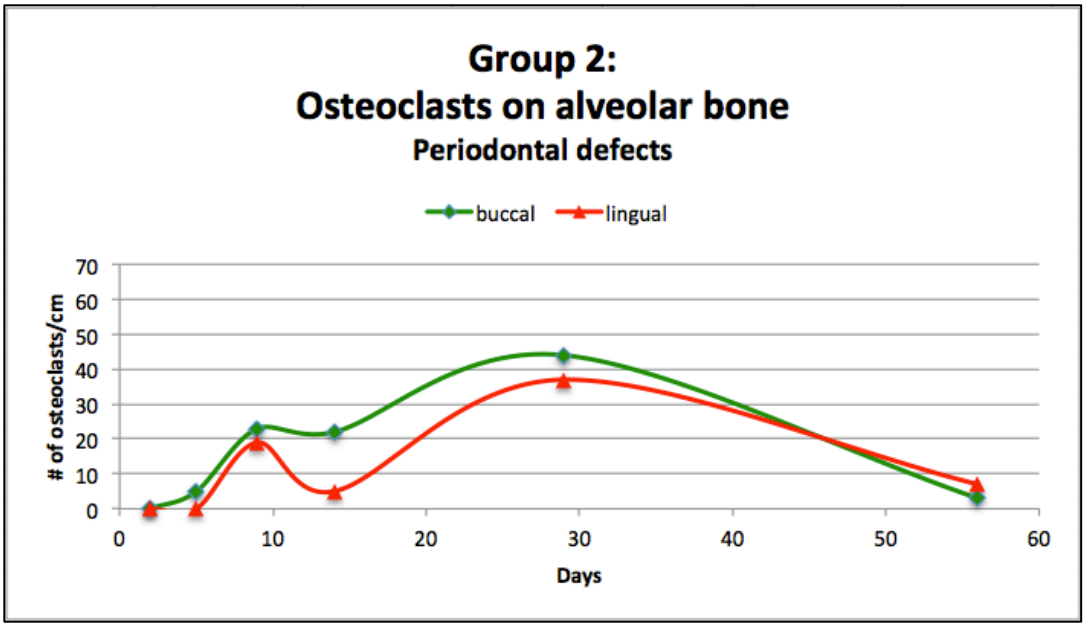
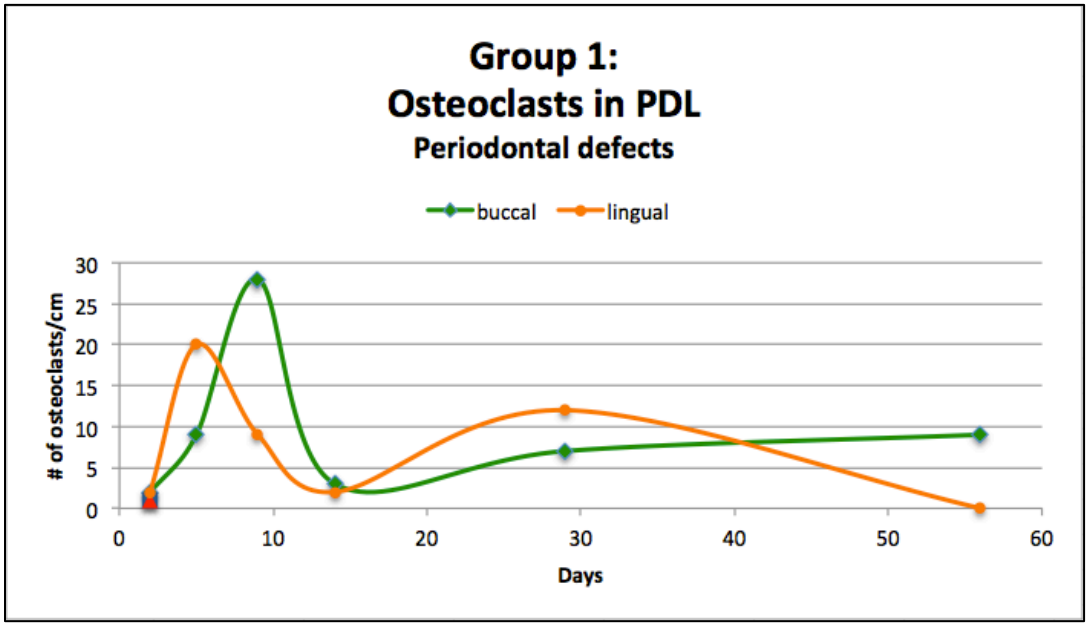
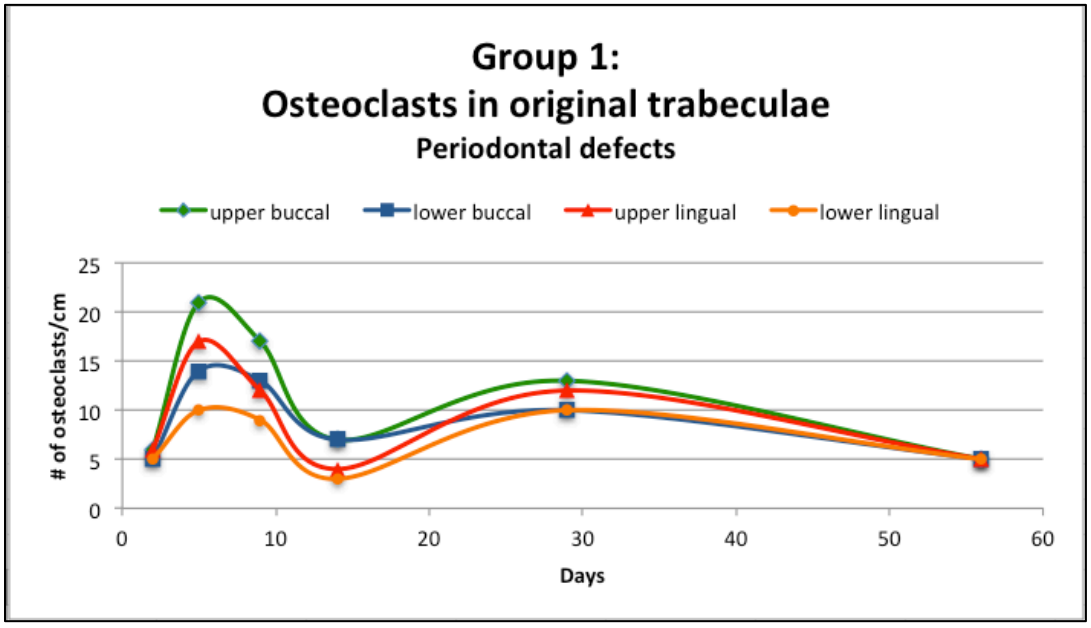


Figure 2A:



- Buccal and lingual refer to consecutive osteoclasts from the surgical surface of the defect in the periodontal ligament adjacent to the root length in bone, converted to osteoclasts per centimeter

Figure 3A:



- Upper buccal/lingual refer to consecutive osteoclasts on the bone surface in the original trabeculae adjacent to the coronal half of the root length in bone, converted to osteoclasts per centimeter
- Lower buccal/lingual refer to consecutive osteoclasts on the bone surface in the original trabeculae adjacent to the apical half of the root length in bone, converted to osteoclasts per centimeter

Figure 3B:

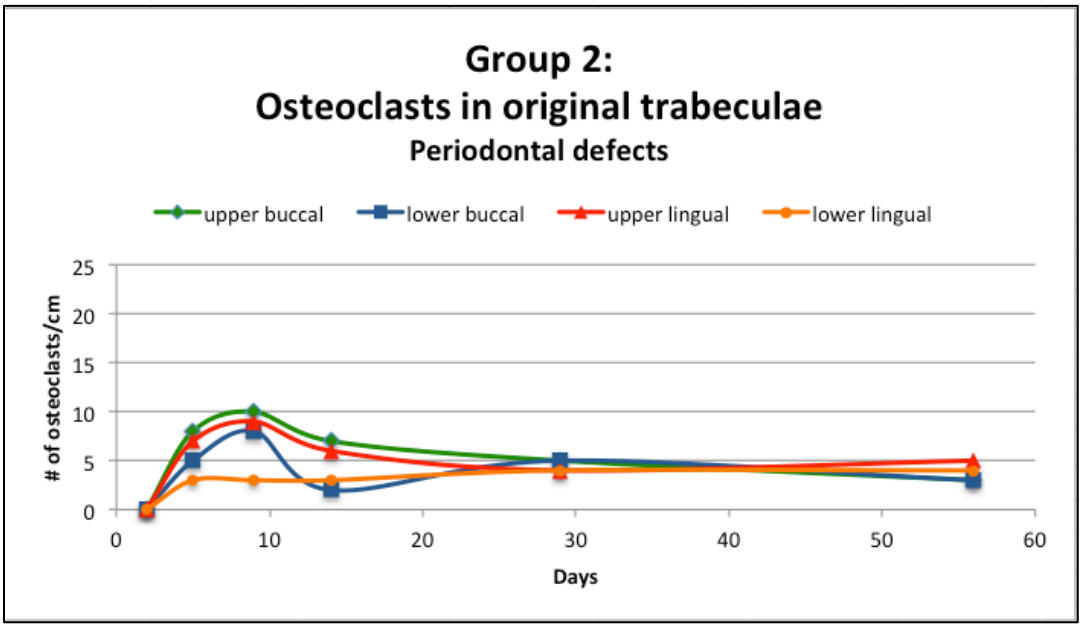
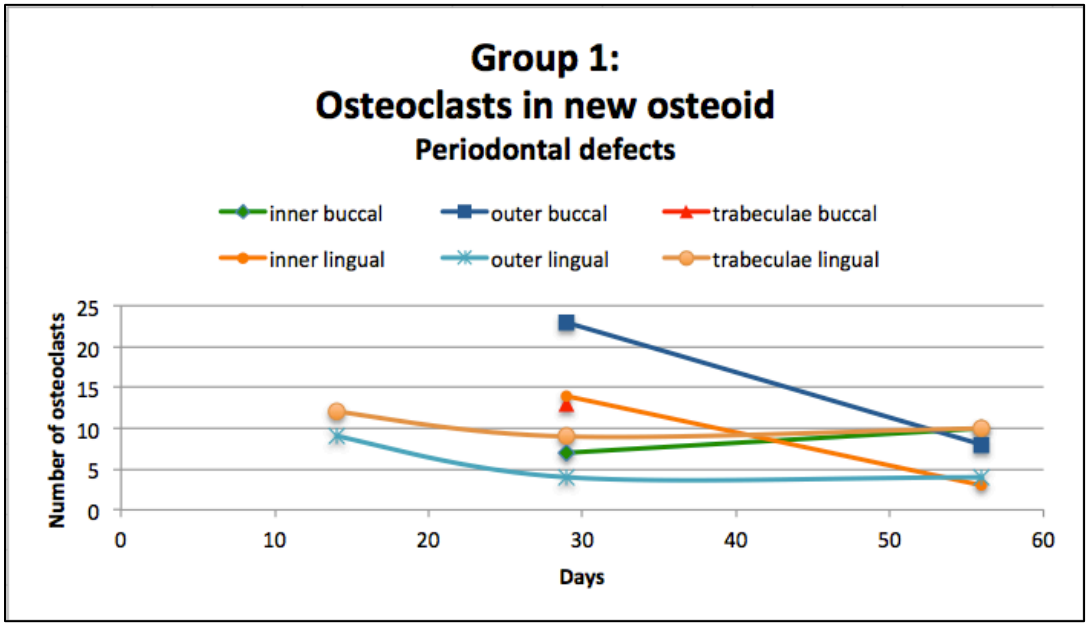


Figure 4A:



- Inner buccal/lingual refer to consecutive osteoclasts on the inner surface of new osteoid adjacent to the tooth, converted to osteoclasts per centimeter
- Outer buccal/lingual refer to consecutive osteoclasts on the outer surface of new osteoid adjacent to the wound space, converted to osteoclasts per centimeter
- Trabeculae buccal/lingual refer to consecutive osteoclasts within the trabeculae of new osteoid, converted to osteoclasts per centimeter

Figure 4B:

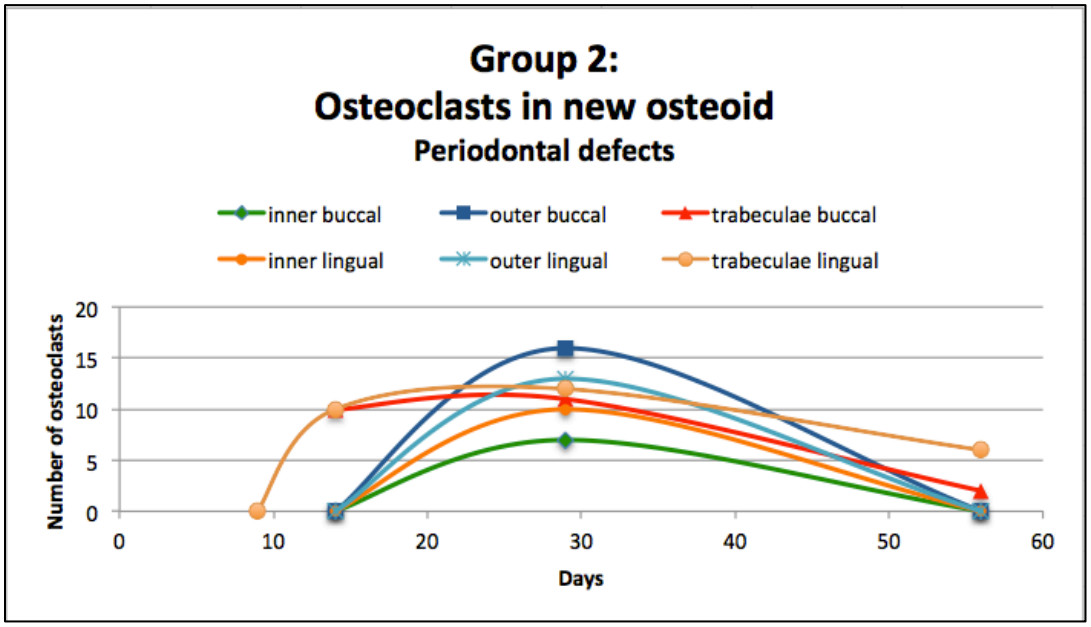


Figure 5A. Osteoclast Quantification for Periodontal Site.

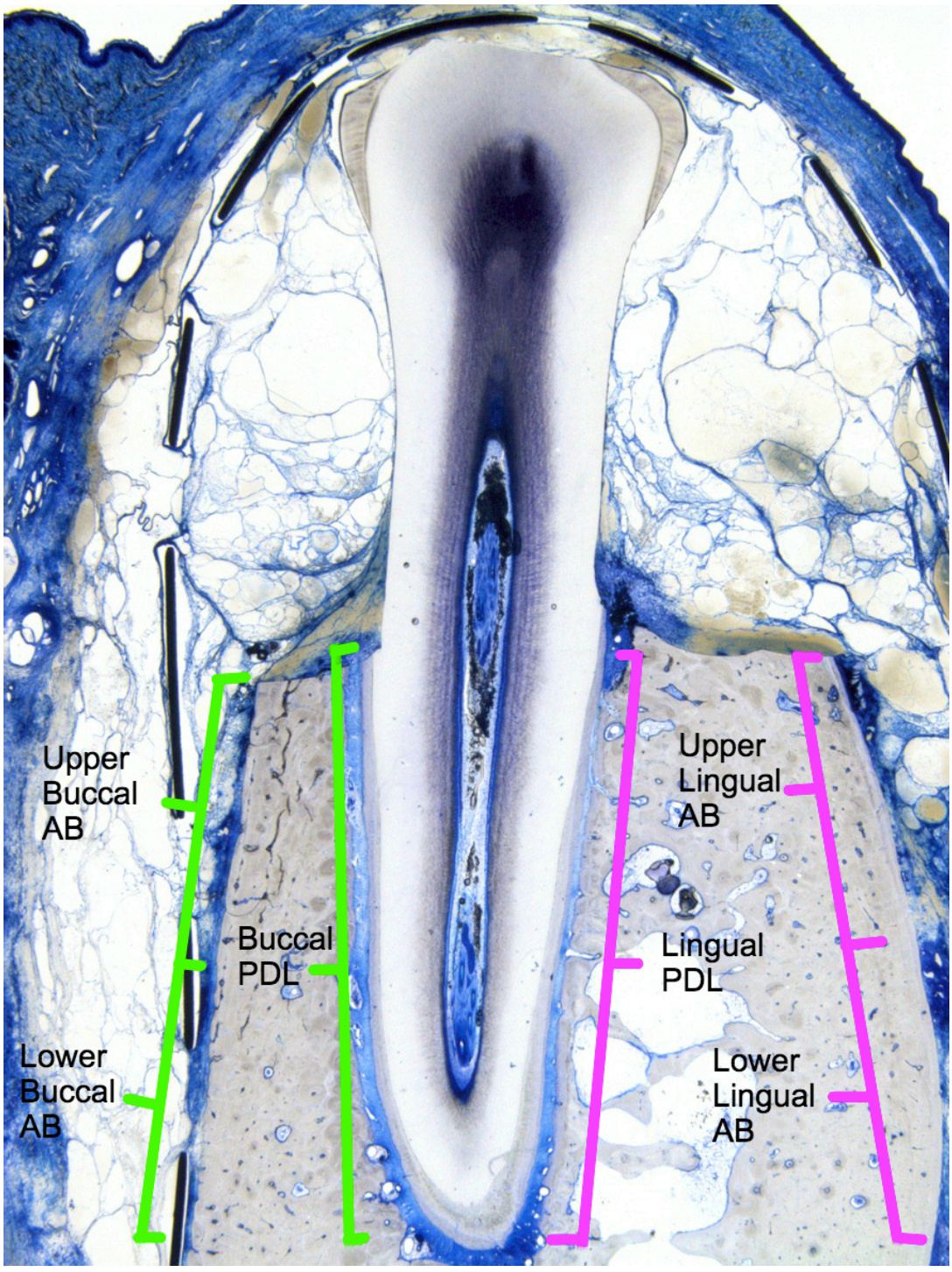


Figure 5B. Osteoclast Quantification for Periodontal Site.

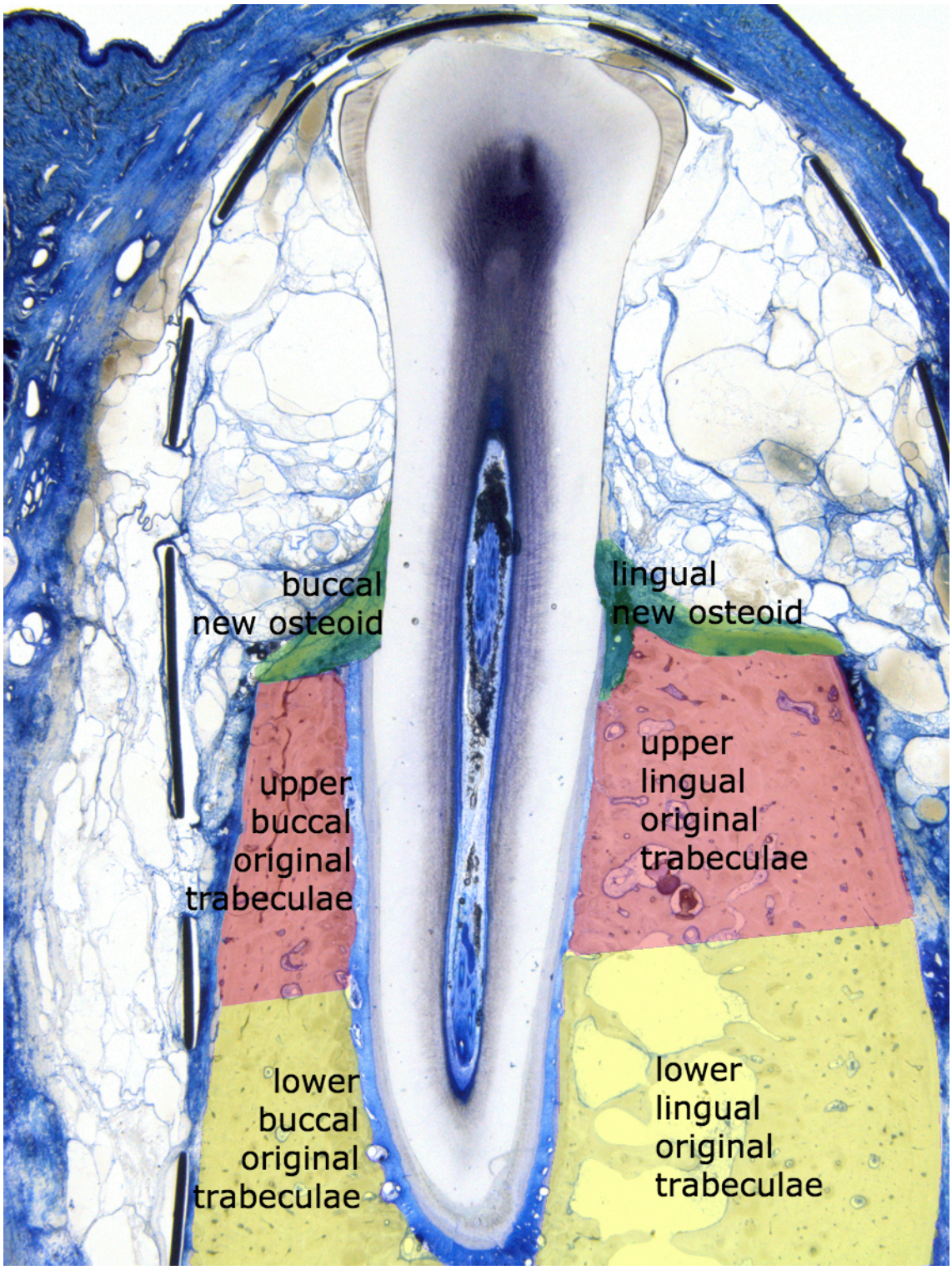


Figure 6A:

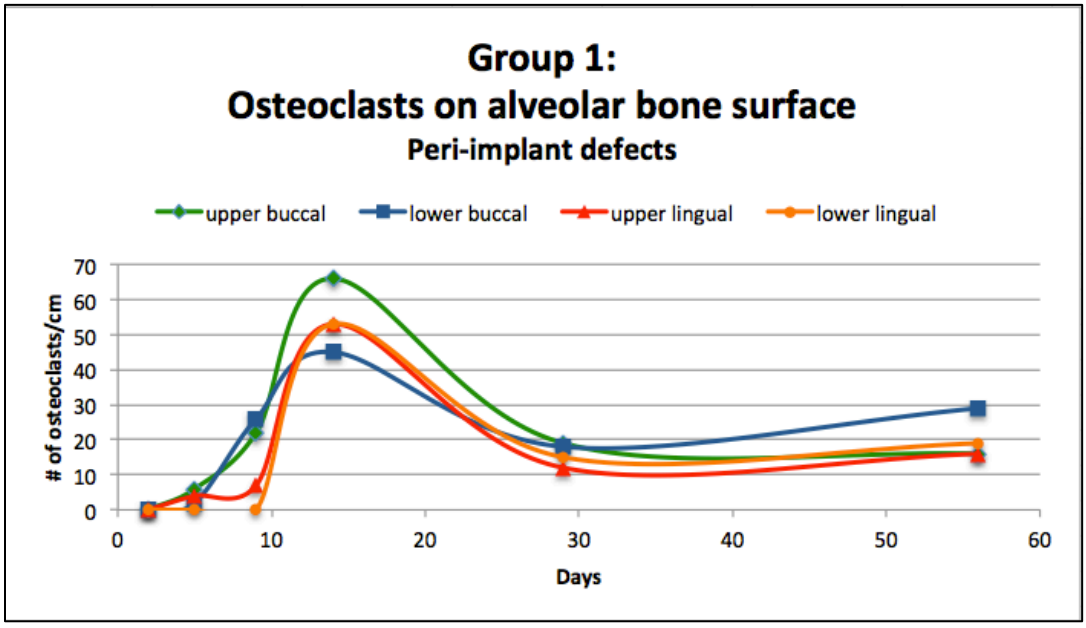


Figure 6B:

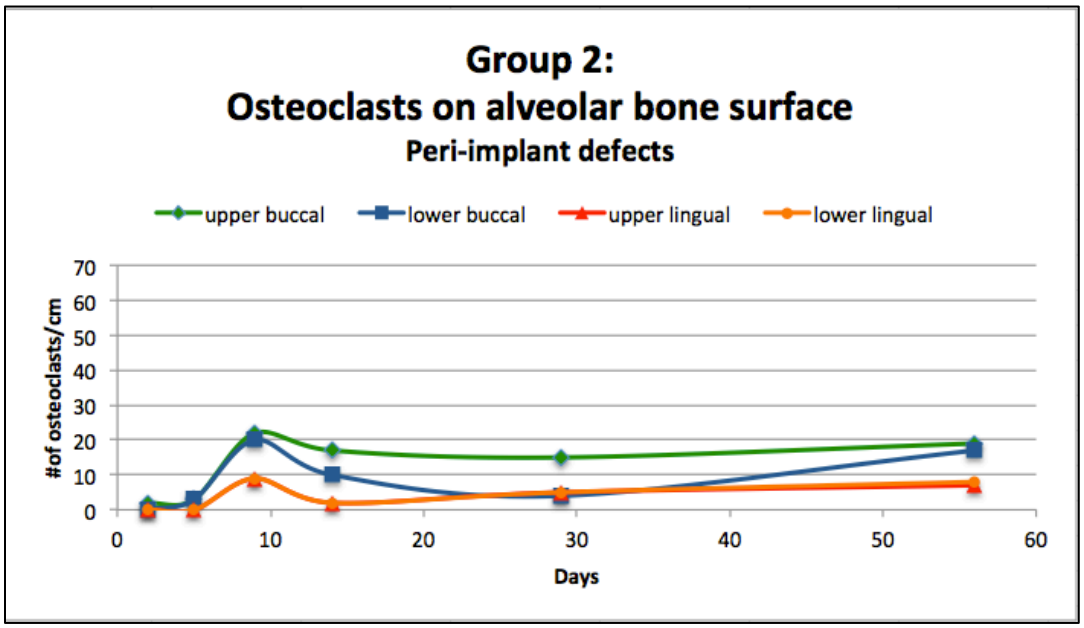


Figure 6C (OA on AB: total buccal vs lingual). Group 1: Peri-implant Defect

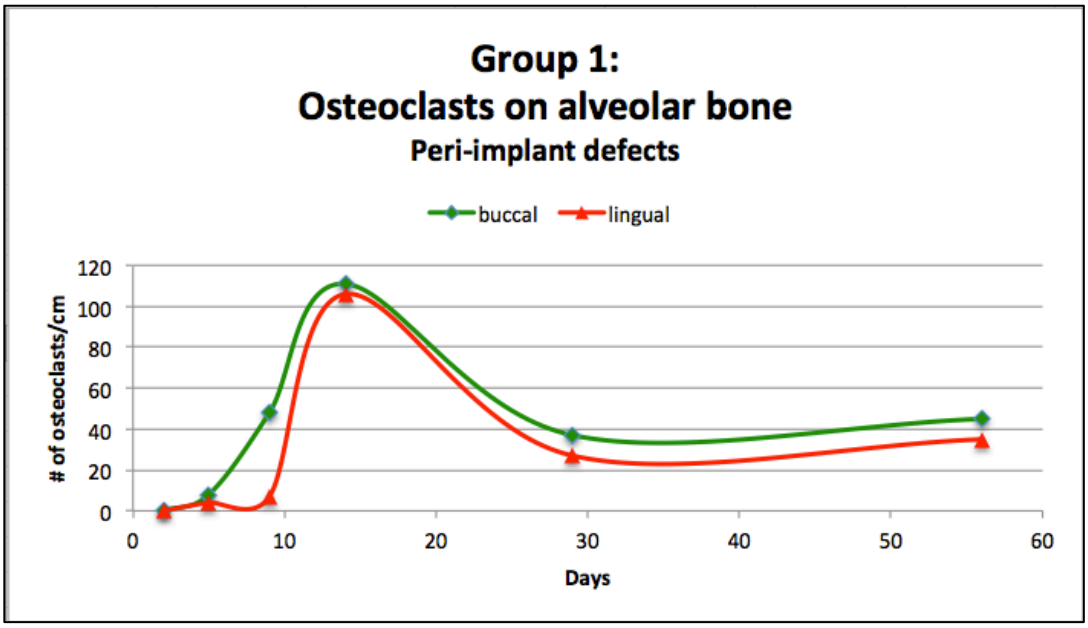


Figure 6D (OA on AB: total buccal vs lingual). Group 2: Peri-implant Defect

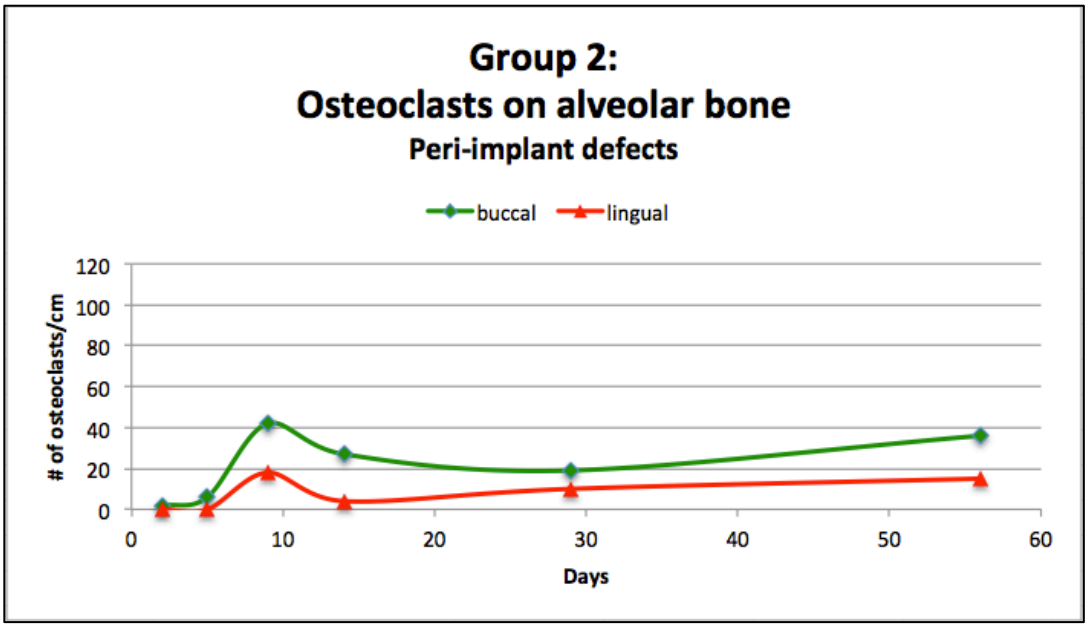


Figure 7A:

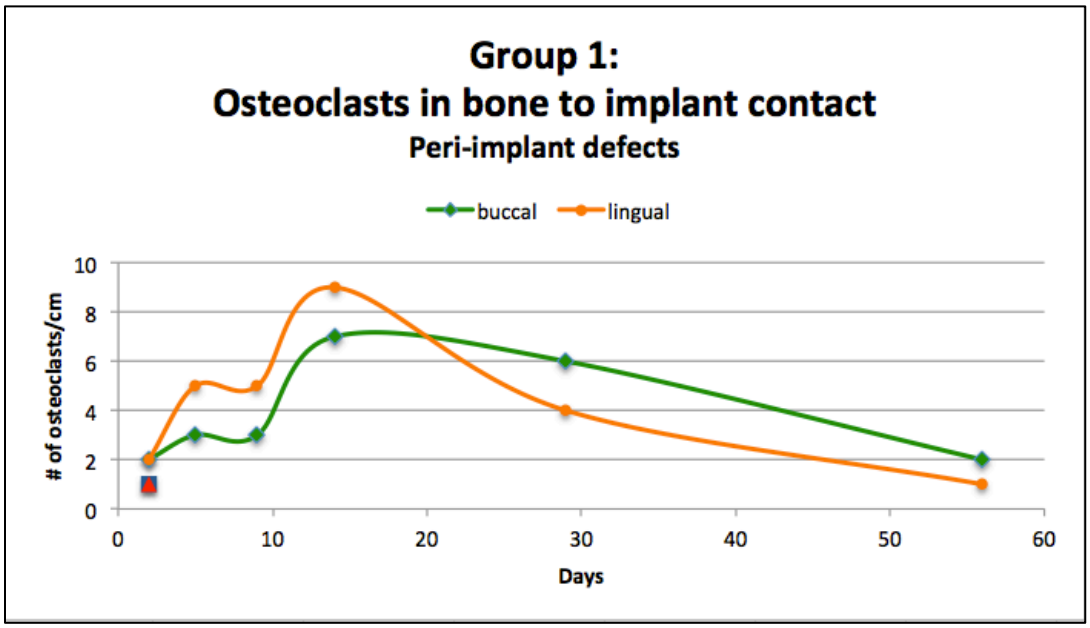

Group 1:
Osteoclasts in bone to implant contact
Peri-implant defects
buccal
lingual
# of osteoclasts/cm
10
8
6
4
2
0
0
10
20
30
40
50
60
Days

Figure 8A:

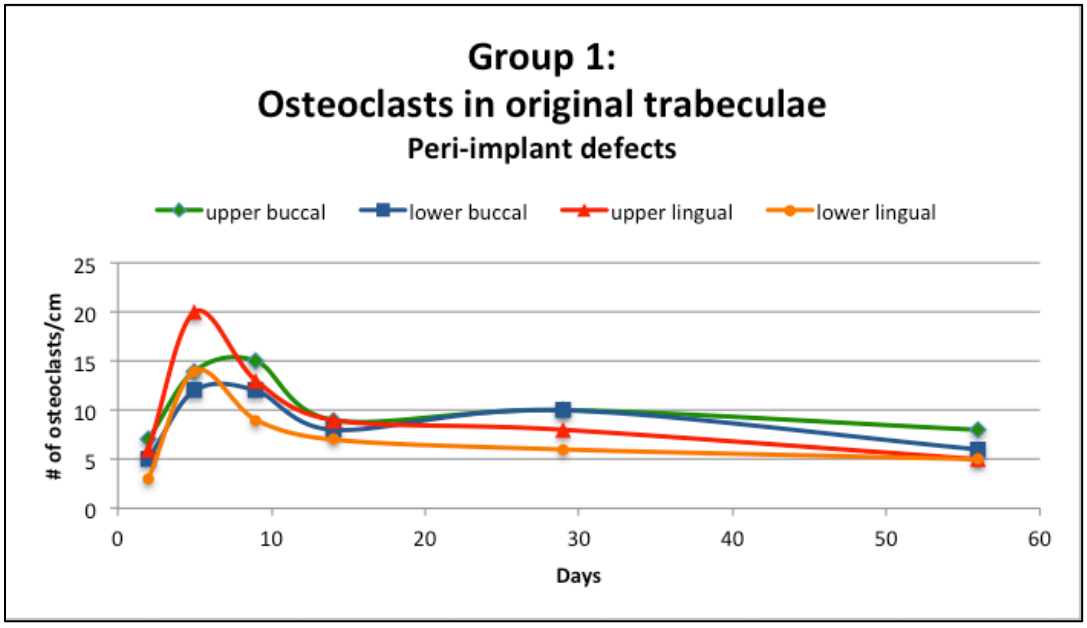


Figure 8B:

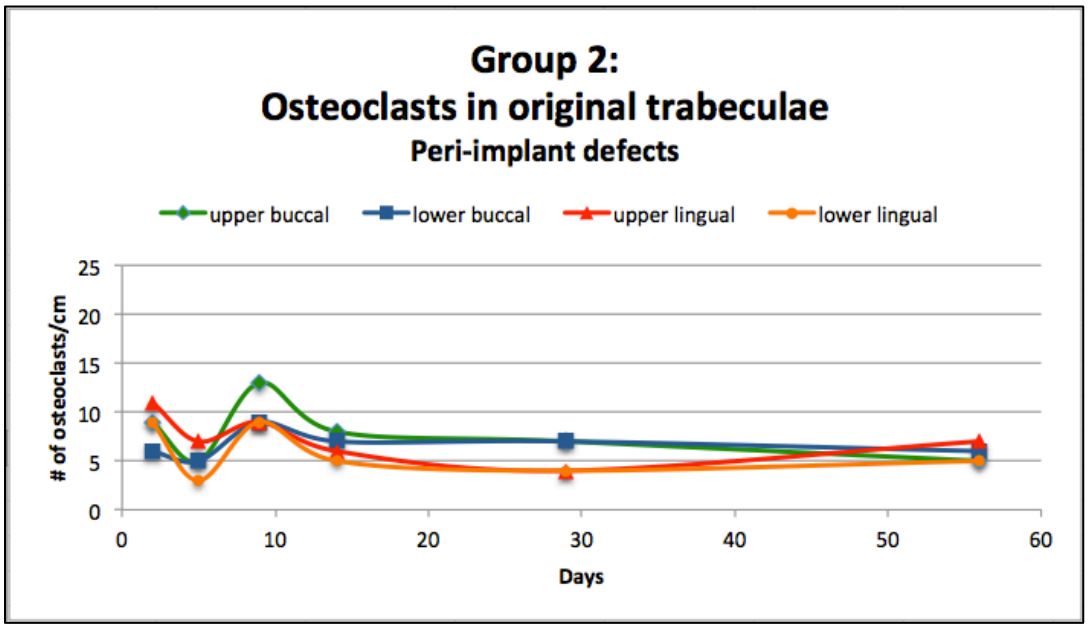


Figure 9A:

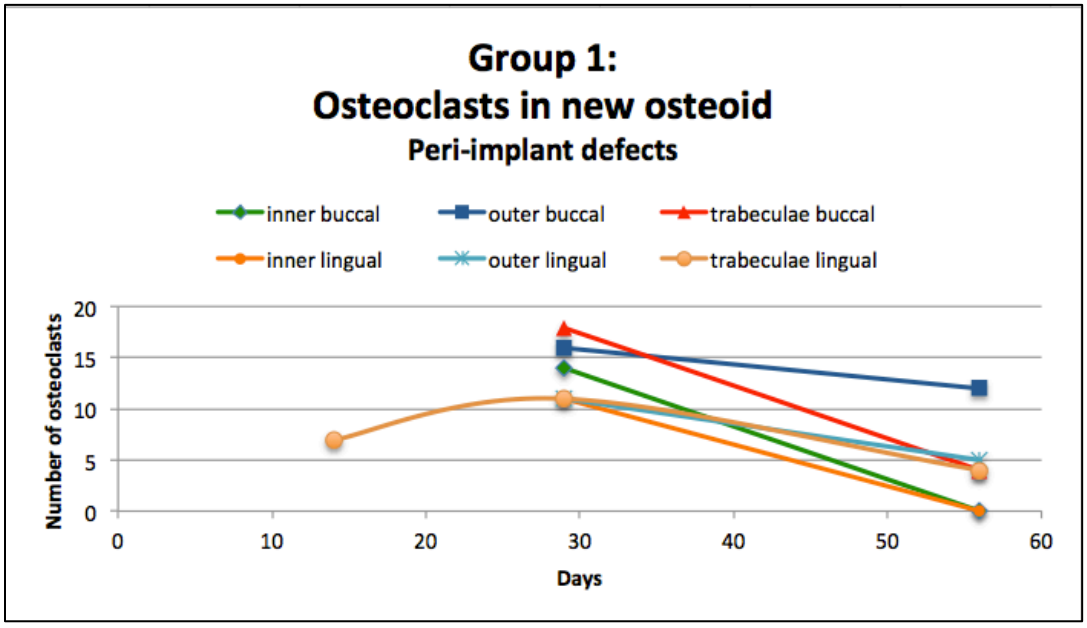


Figure 9B:

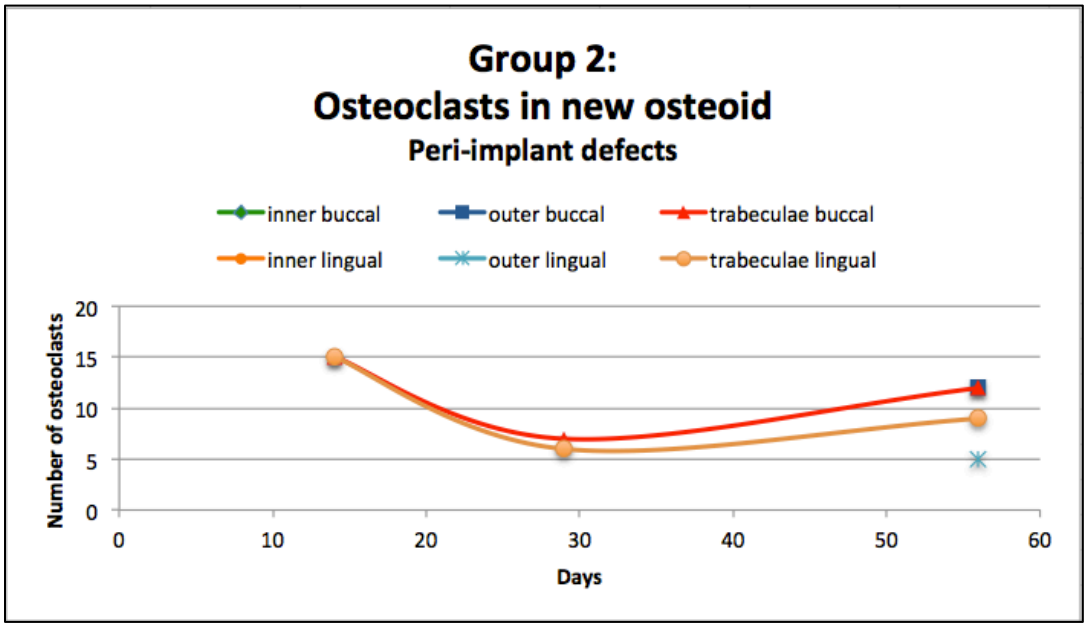


Figure 10A. Osteoclast Quantification for Alveolar Peri-implant Site.

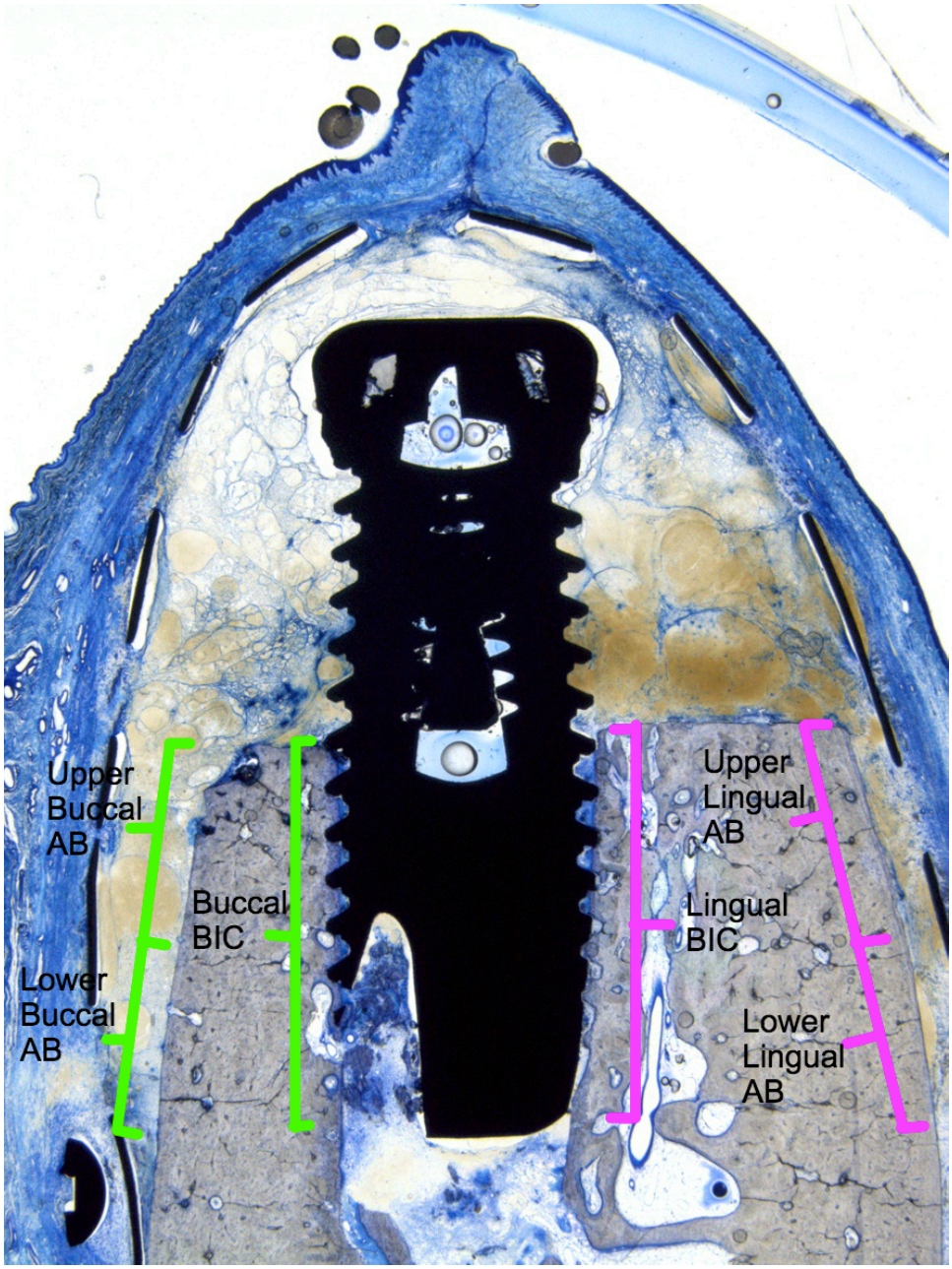


Figure 10B. Osteoclast Quantification for Alveolar Peri-implant Site.

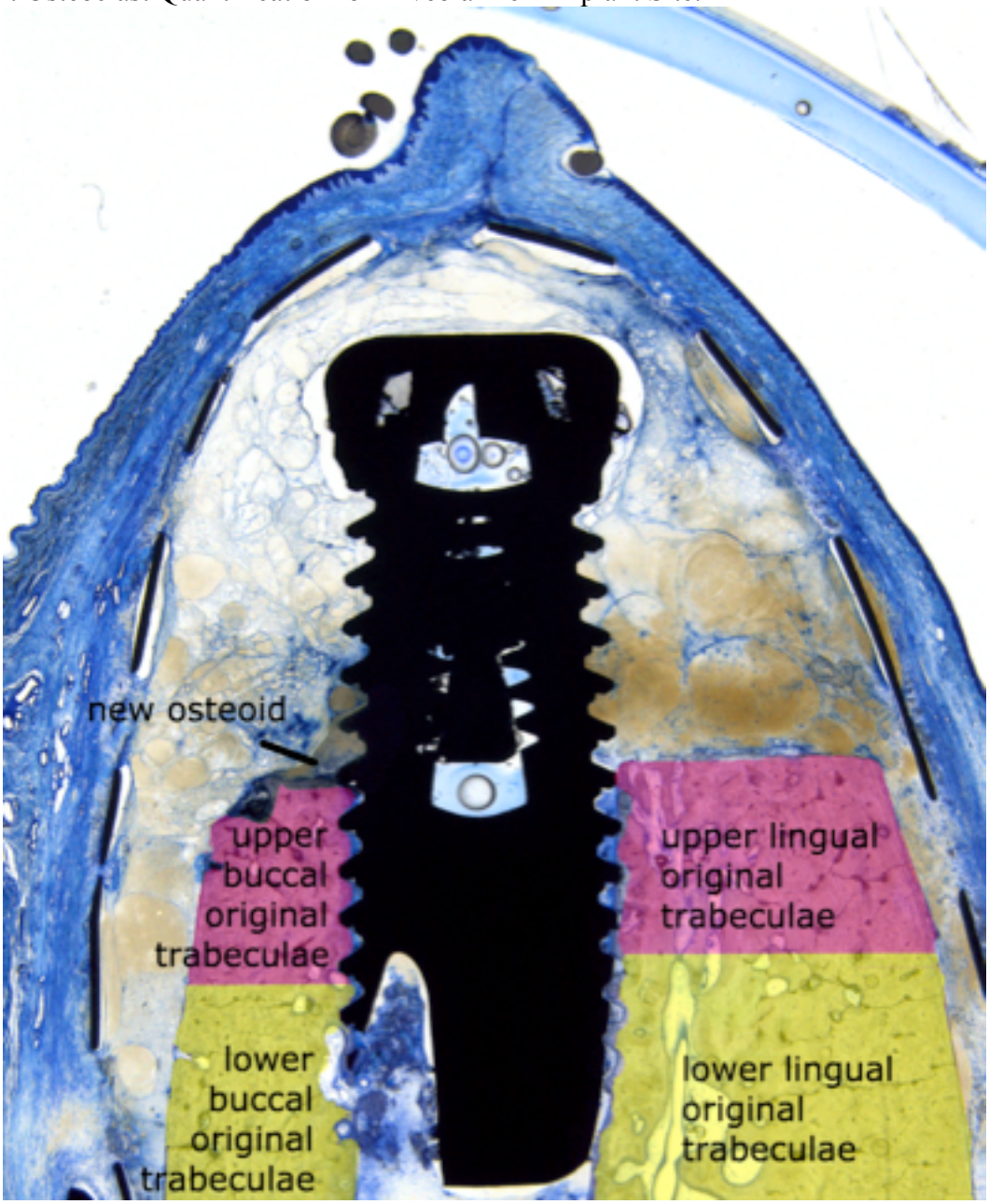


Table 1A (OA on AB). Group 1: Periodontal Defect

Day	Upper buccal	Lower buccal	Upper lingual	Lower lingual
2	1	n/a	0	1
5	10	5	2	6
9	44	n/a	9	n/a
14	65	n/a	18	9
29	35	n/a	17	22
56	20	10	26	19

- Upper buccal/lingual refer to consecutive osteoclasts along the surface of the cortical bone adjacent to the coronal half of the root length in bone, converted to osteoclasts per centimeter
- Lower buccal/lingual refer to consecutive osteoclasts along the surface of the cortical bone adjacent to the apical half of the root length in bone, converted to osteoclasts per centimeter

Table 1B (OA on AB). Group 2: Periodontal Defect

Day	Upper buccal	Lower buccal	Upper lingual	Lower lingual
2	0	0	0	0
5	3	2	0	0
9	14	9	10	9
14	16	6	1	4
29	28	16	21	16
56	3	0	3	4

Table 1C (OA on AB: total buccal vs lingual). Group 1: Periodontal Defect

Day	Buccal	Lingual
2	1	1
5	15	8
9	44	9
14	65	27
29	35	39
56	30	45

- Buccal/lingual refer to total osteoclasts along the surface of the cortical bone adjacent to the root length in bone, converted to osteoclasts per centimeter

Table 1D (OA on AB: total buccal vs lingual). Group 2: Periodontal Defect

Day	Buccal	Lingual
2	0	0
5	5	0
9	23	19
14	22	5
29	44	37
56	3	7

Table 2A (OA in PDL). Group 1: Periodontal Defect

Day	Buccal	Lingual
2	2	2
5	9	20
9	28	9
14	3	2
29	7	12
56	9	0

- Buccal and lingual refer to consecutive osteoclasts from the surgical surface of the defect in the periodontal ligament adjacent to the root length in bone, converted to osteoclasts per centimeter

Table 3A (OA in original trabeculae). Group 1: Periodontal Defect

Day	Upper buccal	Lower buccal	Upper lingual	Lower lingual
2	6	5	6	5
5	21	14	17	10
9	17	13	12	9
14	7	7	4	3
29	13	10	12	10
56	5	5	5	5

- Upper buccal/lingual refer to consecutive osteoclasts on the bone surface in the original trabeculae adjacent to the coronal half of the root length in bone, converted to osteoclasts per centimeter
- Lower buccal/lingual refer to consecutive osteoclasts on the bone surface in the original trabeculae adjacent to the apical half of the root length in bone, converted to osteoclasts per centimeter

Table 3B (OA in original trabeculae). Group 2: Periodontal Defect

Day	Upper buccal	Lower buccal	Upper lingual	Lower lingual
2	0	0	0	0
5	8	5	7	3
9	10	8	9	3
14	7	2	6	3
29	5	5	4	4
56	3	3	5	4

Table 4A (OA in new osteoid). Group 1: Periodontal Defect

Day	Inner buccal	Outer buccal	Trabeculae buccal	Inner lingual	Outer lingual	Trabeculae lingual
2	n/a	n/a	n/a	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	n/a	n/a
9	n/a	n/a	n/a	n/a	n/a	n/a
14	n/a	n/a	n/a	n/a	9	12
29	7	23	13	14	4	9
56	10	8	n/a	3	4	10

- Inner buccal/lingual refer to consecutive osteoclasts on the inner surface of new osteoid adjacent to the tooth, converted to osteoclasts per centimeter
- Outer buccal/lingual refer to consecutive osteoclasts on the outer surface of new osteoid adjacent to the wound space, converted to osteoclasts per centimeter
- Trabeculae buccal/lingual refer to consecutive osteoclasts within trabeculae of new osteoid, converted to osteoclasts per centimeter

Table 4B (OA in new osteoid). Group 2: Periodontal Defect

Day	Inner buccal	Outer buccal	Trabeculae buccal	Inner lingual	Outer lingual	Trabeculae lingual
2	n/a	n/a	n/a	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	n/a	n/a
9	n/a	n/a	n/a	n/a	n/a	n/a
14	0	0	10	0	0	10
29	7	16	11	10	13	12
56	0	0	2	0	0	6

Table 6A (OA on AB). Group 1: Peri-implant Defect

Day	Upper buccal	Lower buccal	Upper lingual	Lower lingual
2	0	0	0	0
5	6	2	4	0
9	22	26	7	0
14	66	45	53	53
29	19	18	12	15
56	16	29	16	19

*All counts for the regions of peri-implant defects measured in similar fashion as for the periodontal defects

Table 6B (OA on AB). Group 2: Peri-implant Defect

Day	Upper buccal	Lower buccal	Upper lingual	Lower lingual
2	2	0	0	0
5	3	3	0	0
9	22	20	9	9
14	17	10	2	2
29	15	4	5	5
56	19	17	7	8

Table 6C (OA on AB: total buccal vs lingual). Group 1: Peri-implant Defect

Day	Buccal	Lingual
2	0	0
5	8	4
9	48	7
14	111	106
29	37	27
56	45	35

Table 6D (OA on AB: total buccal vs lingual). Group 2: Peri-implant Defect

Day	Buccal	Lingual
2	2	0
5	6	0
9	44	18
14	27	4
29	19	10
56	36	15

Table 7A (OA in BIC). Group 1: Peri-implant Defect

Day	Buccal	Lingual
2	2	2
5	3	5
9	3	5
14	7	9
29	6	4
56	2	1

Table 8A (OA in original trabeculae). Group 1: Peri-implant Defect

Day	Upper buccal	Lower buccal	Upper lingual	Lower lingual
2	7	5	6	3
5	14	12	20	14
9	15	12	13	9
14	9	8	9	7
29	10	10	8	6
56	8	6	5	5

Table 8B (OA in original trabeculae). Group 2: Peri-implant Defect

Day	Upper buccal	Lower buccal	Upper lingual	Lower lingual
2	9	6	11	9
5	5	5	7	3
9	13	9	9	9
14	8	7	6	5
29	7	7	4	4
56	5	6	7	5

Table 9A (OA in new osteoid). Group 1: Peri-implant Defect

Day	Inner buccal	Outer buccal	Trabeculae buccal	Inner lingual	Outer lingual	Trabeculae lingual
2	n/a	n/a	n/a	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	n/a	n/a
9	n/a	n/a	n/a	n/a	n/a	n/a
14	n/a	n/a	n/a	n/a	n/a	7
29	14	16	18	11	11	11
56	0	12	4	0	5	4

Table 9B (OA in new osteoid). Group 2: Peri-implant Defect

Day	Inner buccal	Outer buccal	Trabeculae buccal	Inner lingual	Outer lingual	Trabeculae lingual
2	n/a	n/a	n/a	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	n/a	n/a
9	n/a	n/a	n/a	n/a	n/a	n/a
14	n/a	n/a	15	n/a	n/a	15
29	n/a	n/a	7	n/a	n/a	6
56	n/a	12	12	n/a	5	9

Biology of Bone

Introduction:

Bone, a type of dense connective tissue, is a vital component of the vertebrate skeleton. Bones are lightweight, yet strong and hard. They have numerous functions, including enabling mobility and providing support and protection of the organs of the body, the production of red and white blood cells, and storage of minerals. Bones come in many different shapes and sizes, and have a complex internal and external structure. The hard component of bone is primarily a composite material consisting of the inorganic mineral calcium hydroxyapatite (giving bones their rigidity) and an extracellular matrix comprised mainly of organic collagen, an elastic protein that enhances fracture resistance.[40] Mineralized osseous tissue, or bone tissue, is of two structural types – cortical (or compact) and cancellous (Figure 1). Cortical refers to the outer (cortex) layer. The cortical layer gives bone a white, smooth, solid appearance, and constitutes about 80% of the total bone mass of an adult skeleton.[65] The interior of bone is termed cancellous bone, (also known as trabecular or spongy bone), which has an open cell porous network.[39] Trabeculae are made up of a network of rod- and plate-like elements with a three-dimensional internal structure resembling coral, making the organ lighter and providing room for marrow and blood vessels. This structure also gives bone rigidity. Trabecular bone constitutes the other 20% of total bone mass but has approximately ten times the surface area of compact bone.[41] Other types of tissue found in bones include marrow, endosteum, periosteum, nerves, blood vessels and cartilage.

Cortical bone is composed of numerous microscopic columns, called osteons (Figure 2). Each osteon consists of concentric layers, or lamellae, of compact bone tissue surrounding a central canal called the Haversian canal. Volkmann's canals project at right angles, which connect the osteons

together. Osteons are metabolically active, and as bone is formed and remodeled, the location and nature of the cells within the osteon change.[65]

Cortical bone is covered on its outer surfaces by a membrane termed periosteum, and on its inner surfaces by endosteum. Periosteum covers the outer surface of all bones, except at the joints of long bones, while endosteum lines the inner surfaces of all bones.[43]

Within the endosteum, thin sheets of bone-lining cells (one of the terminal fates of osteoblasts) contact and cover the bone surface. Endosteum-covered trabeculae create an irregular network of spaces that provide niches for bone marrow and hematopoietic stem cells that give rise to platelets, red blood cells and white blood cells.[39]

Bone marrow, also termed myeloid tissue, is found in virtually any bone with cancellous tissue. In newborns, all such bones are mainly filled with red marrow, but as the child develops it becomes replaced by yellow, or fatty marrow. In adults, red marrow is found predominately in the bone marrow around the ribs, the femur, the vertebrae and pelvic bones.[66]

Periosteum is a dense irregular connective tissue. It is divided into an outer "fibrous layer" and an inner "cambium layer" (or "osteogenic layer").[67] The outer layer contains fibroblasts, and the cambium layer contains bone lining cells and progenitor cells that can become osteoblasts. The osteoblasts are responsible for increasing the width of a long bone, along with the size of the other bone types, by appositional bone formation.[68] Upon a bone fracture, the progenitor cells differentiate into osteoblasts and chondroblasts, which are vital to healing.

Unlike osseous tissue, periosteum contains nociceptive nerve endings, causing it to be very sensitive to manipulation. In addition, it gives nourishment by connecting the blood supply from the body to the marrow. Periosteum attaches to bone by strong collagen fibers called Sharpey's fibres that

extend to the outer circumferential and interstitial lamellae. It also serves as an attachment for muscles and tendons.[69]

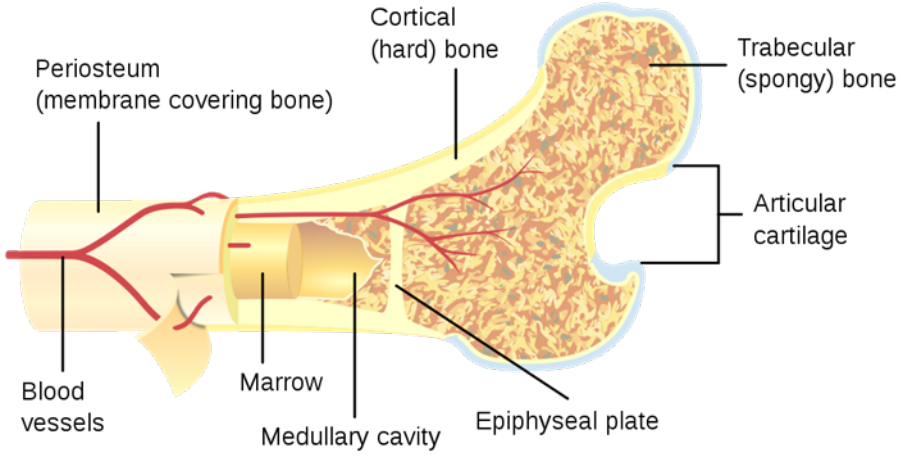


Figure 1. Cross-section of bone[51]

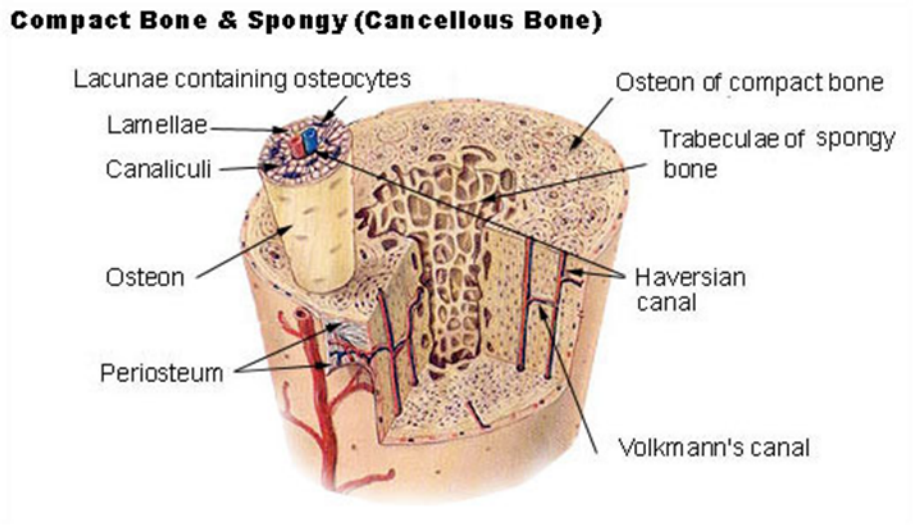


Figure 2. Cross-section details of long bone[52]

Cells:

Bone is a metabolically active tissue containing numerous types of cells, with three main types: osteoblasts, which are involved in the creation and mineralization of bone tissue; osteoclasts, which are

involved in the reabsorption of bone tissue; and osteocytes, with a regulatory function.[61] Osteocytes and osteoblasts are derived from osteoprogenitor cells, while osteoclasts are derived from the same progenitor cell lineage that differentiates to form monocytes and macrophages.[39] Within the marrow of the bone there are also hematopoietic stem cells that give rise to other cells, such as red blood cells, white blood cells, and platelets.[39]

Osteoblasts are mononucleate bone-forming cells. They are found on the surface of osteoid seams and secrete a protein called osteoid that mineralizes to form bone. The osteoid seam is a thin region of newly produced organic matrix, not yet mineralized, found on the bone surface. Osteoid is composed predominately of Type I collagen.[62] Osteoblasts also produce hormones, including prostaglandins, to act on the bone itself. They robustly produce alkaline phosphatase, an enzyme involved in the mineralization of bone, in addition to many matrix proteins such as osteocalcin.[63] During bone growth, the width of the bone increases as osteoblasts lay new bone tissue at the periosteum. To prevent the bone from becoming unnecessarily thick, osteoclasts resorb the bone from the endosteal side.

Osteocytes are star-shaped cells that are the most commonly found cell in mature bone.[61] They are derived from osteoprogenitors that differentiate into active osteoblasts, which then become encased in osteoid. They occupy spaces called lacunae, and have several processes that reach out to contact osteoblasts and other osteocytes, likely for communication.[64]

Trabecular bone that encloses the bone marrow is covered by a single layer of thin, sometimes inconspicuous, flat, elongated (spindle-shaped) endothelium-like cells with a round or oval nucleus. At least some of these "bone lining" cells, or endosteal cells, are derived from osteoblasts. They form a continuous single layer membrane over the trabecular bone surfaces termed endosteum. This lies

adjacent to hematopoietic tissue or a zone of tightly packed or loosely arranged mononuclear (hematopoietic) cells, some apparently originating from the endosteum. During a reparative process following injury in which bony trabeculae are mechanically fractured, endosteal cells give rise to osteoprogenitor cells.[42]

Osteoclasts are the cells involved in bone resorption; breaking down bone. Afterwards, osteoblasts form new bone. Bone is remodeled continuously through a balance of resorption and apposition. Osteoclasts are large, multinucleate cells found on the surface of bone in Howship's lacunae (or resorption pits). These lacunae are the result of bone tissue that has been resorbed.[61] Since the osteoclasts are derived from a monocyte stem-cell lineage, they have phagocytic-like mechanisms similar to macrophages.[39] Osteoclasts mature and migrate to specific surfaces of bone. On arrival, active enzymes, including tartrate resistant acid phosphatase (TRAP), are secreted against the mineral substrate.[61] TRAP is a widely-used histochemical marker for osteoclasts on bone. Bone reabsorption by osteoclasts also plays a role in calcium homeostasis.[39]

Development:

Bone formation is termed "ossification". During the fetal stage of development this occurs by one of two processes, intramembranous ossification or endochondral ossification, depending on the bone. Intramembranous ossification is the formation of bone from connective tissue by direct mineralization, while in endochondral ossification, bone is created from cartilage.[70]

Intramembranous ossification occurs primarily during formation of the flat bones of the skull but also the maxilla, mandible, and clavicles. Here, the bone is produced from connective tissue like mesenchyme tissue rather than from cartilage. There are four main steps in the process of intramembranous ossification.[71] First, an ossification center develops as osteoblasts secrete organic

extracellular matrix. Calcification of this matrix occurs directly as calcium and other mineral salts are deposited. Next, the extracellular matrix develops into trabeculae that fuse to form spongy bone, and finally, mesenchyme at the periphery of the bone develops into periosteum.

Endochondral ossification occurs in long bones and most of the remaining bones in the body. It is initiated by the formation of hyaline cartilage that continues to grow. Like intramembranous ossification, there are several steps in the process of endochondral ossification.[70] First, a mineralized bone collar forms by mineralization around the hyaline cartilage matrix. Next, cavitation of the hyaline cartilage within the cartilage model occurs, followed by invasion of internal cavities by the periosteal bud, and then spongy bone formation. Formation of the medullary cavity occurs as ossification continues, in addition to the appearance of secondary ossification centers in the epiphyses. Finally, the epiphyses ossify; when completed, hyaline cartilage remains only in the epiphyseal plates and articular cartilages.

Bone Remodeling:

Bone is being formed continuously and replaced in the process of remodeling. This constant turnover of bone involves resorption followed by replacement of the bone. This is the result of the interactions of osteoblasts and osteoclasts in response to stimulation from various signals. The coordinated activity of these cells is together referred to as a remodeling unit. About one-tenth of the skeletal mass of an adult is remodeled each year.[44] The remodeling process regulates calcium homeostasis, repairs micro-damage to bones resulting from everyday stress, and also shapes and sculpts the skeleton during growth.[44] Repeated stress from weight-bearing exercise or during bone healing causes the bone to thicken at the points of maximum stress (Wolff's law). This may be the result of bone's piezoelectric properties, which cause bone to generate small electrical potentials under stress.[45]

The interaction between osteoblasts and osteoclasts is regulated by several signaling systems that either promote or inhibit the activity of the bone remodeling cells, controlling the rate at which bone is formed or destroyed. In addition, the cells use paracrine signaling to control the activity of each other.[45] For example, the rate at which osteoclasts resorb bone is decreased by osteoprotegerin and calcitonin. Calcitonin is produced by parafollicular cells in the thyroid gland, and binds receptors on osteoclasts to directly inhibit osteoclast activity. Osteoprotegerin is secreted by osteoblasts and is able to bind RANK-L (receptor activation of nuclear factor kappa-B ligand), a key activating protein, inhibiting osteoclast stimulation.[46] Osteoblasts can be stimulated to increase bone mass both through increased secretion of osteoid and by inhibiting the stimulation of osteoclasts, thereby reducing the break-down of osseous tissue.[46] Increased osteoblast secretion of osteoid is stimulated by the secretion of growth hormone by the pituitary, and by thyroid hormone and sex hormones (estrogens and androgens). These hormones also promote increased secretion of osteoprotegerin.[46] Osteoblasts can also be induced to secrete a number of cytokines that promote reabsorption of bone by stimulating osteoclast activity and their differentiation from progenitor cells. Vitamin D, parathyroid hormone and stimulation by signals from osteocytes induce osteoblasts to increase secretion of RANKL and interleukin 6, which then stimulate increased reabsorption of bone by osteoclasts. These same signaling molecules also increase secretion of macrophage colony-stimulating factor by osteoblasts, which promotes the differentiation of progenitor cells into additional osteoclasts, and decrease osteoblast secretion of osteoprotegerin.[46]

RANKL-RANK, OPG:

As introduced above, the molecular interactions between the RANK receptor, its ligand RANKL, and the decoy receptor osteoprotegerin (OPG) have a crucial role in the regulation of bone remodeling. RANKL, a member of the TNF (tumor necrosis factor) superfamily, is a potent stimulator of both osteoclast formation and their bone-resorbing activity.[1] Upon binding to RANK (receptor activator of

nuclear factor kappa B), its receptor located on osteoclasts and their progenitors, increased osteoclast differentiation and activation occurs, resulting in expression of osteoclast-specific molecules and resultant bone resorption (Figure 3). Until recently, it was thought that the principle sources of RANKL are stromal and osteoblastic cells; however, a recent study by Nakashima et al. proved that the main RANKL production site resides within osteocytes.[2] OPG is a secreted TNF receptor superfamily member acting as a decoy receptor molecule for RANKL, thereby counteracting its osteoclastogenic activity by reducing the amount of RANKL available for binding to RANK. OPG is produced by a variety of cells, including stromal cells, B-lymphocytes and dendritic cells.[3] The ratio of RANKL to OPG is therefore a major determinant of osteoclast activity.

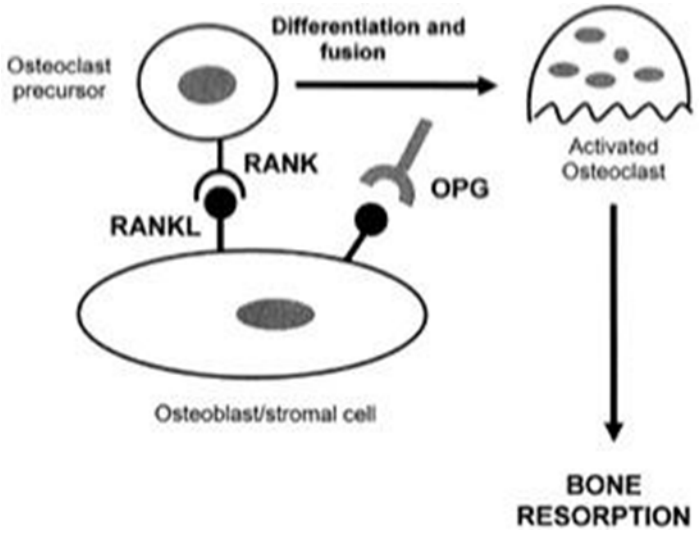


Figure 3. RANKL-RANK/osteoprotegerin molecular complex in bone remodeling[54]

Healing in Response to Injury – Repair versus Regeneration

If an animal survives the immediate trauma of an injury, biological mechanisms are activated leading to a healing process that initially stabilizes the damaged site and prevents infection. This can be achieved by one of two pathways. Healing by repair involves the formation of collagen-rich scar-like tissue. This stabilizes and protects the site, but often does not restore lost function. Ideally, repair would take place via the second pathway, regeneration. This process leads to the reconstitution of the lost tissue architecture and function. However, in the absence of clinical intervention, regeneration in mammalian tissues, if it occurs at all, is a relatively limited process.

Soft tissue healing after dental implant surgery is similar to healing of soft tissue surrounding a tooth after periodontal flap surgery. A fibrin clot serves as a provisional matrix allowing epithelial and fibroblast migration towards the implant surface. Epithelial and connective tissue contact to the implant or abutment surface is established within one to two weeks and further maturation follows for up to at least twelve weeks. In the established interphase, the peri-implant epithelium is similar to, but longer than, the junctional epithelium against the tooth. Orientation of collagen fiber bundles also differs from the natural tooth. Instead of attaching to the neck of the implant, collagen fibers run parallel and circumferentially around the implant.[56]

There are also similarities in hard tissue healing around teeth compared to that of implants, as well as unique differences. Healing adjacent to teeth after periodontal surgery involves migration of cells from periodontal tissues including periodontal ligament, cementum, and bone. These cells mediate a process involving a sequence of events including inflammation, granulation tissue formation, maturation, and remodeling.[6] Unlike teeth, dental implants are surgically placed directly into native or regenerated bone, which limits the number of cell types that can migrate to, attach, and differentiate on

the implant surface during healing.[56] Nonetheless, the general bone healing pattern in both models follow a similar sequence of events.

The present study used a surgically-created, supraalveolar defect model in the mandibular premolar region of dogs to assess patterns of healing after periodontal and peri-implant surgery.[57]

Guided Tissue Regeneration

The periodontium consists of tissues that support the teeth; alveolar bone, root cementum, and periodontal ligament.[4] The replacement or reconstitution of this lost or injured part so that form and function of lost structures are restored is termed periodontal regeneration.[5] By definition, periodontal regeneration must include regeneration of alveolar bone, cementum, and periodontal ligament (PDL).

The ideal goal of therapy of periodontal defects includes regeneration of the lost supporting tissues. Studies of periodontal wound healing have resulted in the development of a treatment modality known as guided tissue regeneration (GTR).[47] This modality is based on the principle of selecting for bone, cementum, and periodontal ligament regeneration following periodontal surgery. GTR involves the placement of a barrier covering the periodontal defect so that the gingival tissues (epithelium and connective tissue) are prevented from contacting the root surface during healing. The first generation of GTR barriers employed non-resorbable materials, requiring a second procedure for surgical removal. The introduction of resorbable barriers eliminates the need for surgical removal; however, the barrier must remain intact long enough for tissue guidance and for the bioresorption process to not interfere with regeneration. This allows for space maintenance between the barrier membrane and the periodontal defect; this permits PDL progenitor cells to produce new bone, cementum, and PDL.

Histologic evidence for new connective tissue attachment has been reported in animal studies[48] in addition to human case reports[49] following GTR therapy, and gain of clinical attachment level and probing bone level following GTR treatment has been reported in both short-term clinical studies[49] and long-term clinical studies.[50] Thus, it can be concluded that regeneration of the periodontium – the formation of new bone, cementum, and PDL – can be accomplished using the biological principles of GTR, and the results obtained through GTR can be maintained on a long-term basis.

The dynamic interaction between osteoclasts and osteoblasts must play a role in periodontal regeneration in order to increase the amount of bone and to maintain equilibrium afterwards. Current techniques in GTR allow for somewhat predictable outcomes to occur in sites amenable to regeneration. However, further research is needed to fully understand these processes to provide a basis for the rational development of improvements.

Periodontal Wound Healing

Wound healing in regenerative therapy is divided into three sequential phases: inflammation (early and late), granulation tissue formation, and matrix formation and remodeling.[6] Healing of the attachment on a root surface that has had all cementum and residual CT fibers removed occurs by a specific sequence of events. Initially, a fibrin clot fills the space between root and flap.[7] Within one hour, the early inflammation stage of healing is initiated by neutrophils infiltrating the clot from the mucogingival flap. Within six hours, the root surface becomes lined by neutrophils, which decontaminate the wound by phagocytosing injured and necrotic tissue. Within three days, the inflammatory reaction moves into its late phase as the neutrophil infiltrate gradually decreases while the numbers of macrophages increases.[8] Within seven days, the phase of granulation tissue formation gradually enters into the third phase of wound healing in which the newly formed cell-rich tissue undergoes maturation and remodeling.[9] While healing of the tooth-mucogingival flap interface occurs by biological processes similar to healing of a soft tissue wound, further maturation and functional adaptation require a mechanism by which collagen fibers become attached to the instrumented cementum or root dentin.[4] First, the healing connective tissue recognizes the instrumented root as an inert foreign body. Collagen fiber bundles align parallel to the root surface in a vertical orientation termed “collagen adhesion”.[10] Next, the presence of the denuded root stimulates differentiation of cementoblasts, which will deposit hard tissue onto the root surface into which new collagen fibers may be anchored.[4] This process does not begin until the third week of healing.[11] Third, a resorptive activity is initiated by osteoclasts. Demineralization of the mineral components result from acids released by osteoclasts, which also degrade the organic matrix, thus creating a suitable substrate for anchorage of new collagen fibers. Reparative cementum may be deposited in the resorbed areas in addition to bone formation, thus completing the new attachment.[12] Osteoclasts play a major role in periodontal wound

healing, as their numbers and activity during the process of alveolar bone healing influence the final outcome.

Peri-implant Wound Healing

Introduction:

There are both differences and similarities when comparing periodontal and peri-implant wound healing. One of the main differences between the two systems is that during peri-implant healing, there is direct attachment of bone to the implant surface, while in periodontal healing, there is an intervening connective tissue attachment. The major similarity between the two forms of healing is that they follow a similar sequence of events that is initiated with bone resorption followed by bone formation.[22] Implant-to-bone interactions are characterized as specific, protein-mediated, dynamic, signal-generating events. Following the placement of a dental implant, hemostasis and fibrin clot formation occur immediately. The static blood volume around the implant will vary considerably as a function of the implant design and site.[29] Next, fibrinolysis and osteogenic cell migration occur with the formation of a loose connective tissue stroma that supports angiogenesis.[22] The surface of the implant is surrounded by serum proteins, mineral ions, polysaccharides, and lipids, as well as cytokines produced by immune cells.[26] Vascular ingrowth or angiogenesis is mediated by extracellular matrix components and growth factors.[33] The relative absence of serum proteins such as albumin indicates a selective accumulation or deposition of molecules at the interface.[34] Because they contain arginine-glycine-aspartic acid (RGD) and polyaspartic sequences, osteopontin and bone sialoprotein appear to be involved in cell adhesion and mineral binding.[25,34] Also, the implant surface gives anchorage for the fibrin clot to withstand detachment forces during cell migration and thus maintain a migratory pathway for the differentiating osteogenic cells to reach the implant surface.[18]

Osteoinduction and Osteoconduction:

The induction of bone formation at the site of a surgically created wound (such as an implant osteotomy site) reflects a significant alteration in the cellular environment.[22] Peri-implant bone healing involves three distinct cellular mechanisms: osteoconduction, de novo bone formation, and bone remodeling.[23] The terms osteoinduction and osteoconduction refer to interrelated but not identical phenomena that occur during bone wound healing.[20] Osteoinduction is the phenotypic conversion of mesenchymal stem cells into bone-forming cells.[18] Primitive, undifferentiated, pluripotent mesenchymal stem cells are stimulated to develop into the bone-forming cell lineage, producing osteoblasts and osteocytes.[20] Osteoconduction has been defined as appositional bone growth, permitting bone formation on a surface or down into channels, pipes, or pores.[20] There is evidence for de novo bone formation around endosseous implants as there is formation of a mineralized interfacial matrix, similar to that found in natural bone tissue, on the implant surface.[18] Osteoconduction relies on the migration of differentiating osteogenic cells to the implant surface.[23] Undifferentiated mesenchymal stem cells migrate to the implant surface, attach to it, and then proliferate. Environmental factors such as oxygen tension help determine whether mesenchymal stem cells will differentiate into chondrocytes, fibroblasts, or osteoblasts.[24] Adherence can occur when a cell binds directly to the surface or when it binds to RGD – containing proteins that adhere to the surface.[25] At this time, the mesenchymal stem cells produce their own extracellular matrix, including cytokines and growth factors, and modify the implant surface.[26] Then mesenchymal cells undergo osteoblastic differentiation.[26] Cells of mesenchymal origin are very sensitive to surface properties such as surface roughness, energy and topography.[27] New osteoblasts produce osteoid, including matrix vesicles and growth factors. Matrix calcification occurs, which leads to the formation of woven bone that is subsequently remodeled with osteoclast recruitment.[28]
These two processes lead to two mechanisms by which bone can become juxtaposed to an implant surface: contact osteogenesis and distance osteogenesis.[30] Contact osteogenesis involves de novo bone

formation directly on the implant surface. Distance osteogenesis involves new bone formation on the surfaces of existing peri-implant bone. Concomitant with new bone formation, the placement of implants in the alveolar process elicits a sequence of healing events, including necrosis and subsequent resorption of traumatized bone around the implant body.[31]

Timeline of Osseointegration:

Osseointegration was defined by Branemark as a direct structural and functional connection between living bone and the surface of a load-carrying implant.[19] Histologically, it is defined as direct anchorage of an implant through the formation of bone directly on the surface of an implant, without an intervening layer of fibrous tissue.[20,21] Clinically, this is analogous with ankylosis of the implant-bone interface.[21] This ankylotic interface is created during the healing period immediately post-surgery and is maintained in dynamic equilibrium throughout the postintegration period.

The events involved in bone apposition following implant therapy occur in specific stages.[26] Immediately after implantation, serum proteins adhere to the implant. Within the first three days, mesenchymal cells attach to the implant and proliferate. At six days, osteoid is produced, and at the end of two weeks, matrix calcification is complete. At three weeks, remodeling is under way.

A critical factor in successful osseointegration of an implant is achieving stability in the bone at the time of placement. Relative motion between the dental implant and the surrounding bone during the early healing phase is a high risk factor for early implant loss due to failure of osseointegration. Following the placement of an endosseous implant, primary mechanical stability begins to be replaced by biologic stability. The transition from primary mechanical stability (provided by the implant design) to biologic stability (provided by newly formed bone as osseointegration occurs) takes place during early wound healing.[31] There is a period of time during healing in which osteoclastic activity has

decreased the initial mechanical stability of the implant but the formation of new bone has not yet occurred to the level required to maintain implant stability. During this critical period, a loaded implant is at greatest risk of relative motion and would theoretically be most susceptible to failure of osseointegration. However, only by bone remodeling is there a gradual replacement of peri-implant bone, with the possibility of de novo bone formation at the implant surface.[29]

A series of cellular events are responsible for this transition. Osteogenesis *in vivo* must find a balance between achieving adequate coverage of an implant with osteogenic cells and the ability of those cells to differentiate into osteoblasts in a timely manner.[28] A previous dog study investigated different temporal phases of wound healing that result in osseointegration.[31] Within two hours the wound chamber was occupied by a coagulum of neutrophils, erythrocytes, and macrophages in a fibrin network. At four days, along the surgical surface, osteoclasts were observed and mesenchymal cells (fibroblast-like cells), vascular structures, and densely packed connective tissue cells were found within the wound chamber. At one week, woven bone was first seen along the implant surface and along vascular units. Trabeculae were lined with osteoblasts and a provisional matrix, which had collagen fibrils and vasculature. At two weeks, there was appearance of new bone formation. At one month, there was marked formation of woven bone combined with lamellar bone. At two and three months, there were clear signs of remodeling. In this study, osteoclastic activity was seen as early as four days following implant placement, and new bone formation was noted at one week post-surgery. Replacement of the original bone that was responsible for the initial stability of the implant at placement was well underway at two weeks post-surgery.

Comparing the results from this dog study with the timeline of the same events in human bone formation is difficult. A rough estimate of comparative healing rates between dogs and humans would suggest that the events of wound healing and bone remodeling happen approximately 1.5 times faster in

dogs than in the human.[35] This would make the critical time frame for implant healing in humans to be 2 to 3 weeks post-surgery.

Role of the Blood Clot

The formation of a blood clot is the initial response to trauma. It serves two main functions: it temporarily protects the denuded tissues, and it serves as a provisional matrix for cell migration.[60] This clot contains all cellular components of blood, such as red and white blood cells and platelets, in a matrix of thrombospondin, vitronectin, fibrin, and plasma fibronectin. Clot formation initiates an early stage of inflammation. Within hours of trauma, inflammatory cells (mainly monocytes and neutrophils) fill the clot. These cells cleanse the wound of necrotic tissue and bacteria through phagocytosis and release of enzymes and toxic oxygen products. At three days, inflammation goes into its late phase. Macrophages migrate into the wound and secrete polypeptide mediators targeting cells involved in wound healing.[60] The macrophage is involved in formation of granulation tissue. Maturation of the granulation tissue will result in regeneration or repair of the injured tissues.[60]

The blood clot plays a significant, but incompletely understood, role in modulating the relationship between repair and regeneration. A red blood cell-rich avascular fibrin clot serves as a protective barrier between the newly formed osteoid, granulation tissue and inflammatory cells. Two weeks following periodontal surgery, the clot material is physically displaced by the newly forming bone rather than replaced.

In the wound space, reparative and regenerative tissue compete for the same space, and once they meet following dissolution of the clot, regeneration stops.[14] While it might be anticipated that more blood would promote more regeneration, as yet there is only limited evidence to support this idea, and the roles of the blood clot in modulating regeneration, including the balance between osteoblast and osteoclast activity, remain to be fully delineated.

Bone Resorption following Implant Placement: Buccal vs Lingual Activity

In a clinical study comparing bone healing following implant placement immediately after tooth extraction versus 6–8 weeks following tooth extraction, it was observed that a marked 20-35% reduction of the buccal–lingual width of the bone ridge had occurred 4–6 months after implant placement and independent of the timing of implant placement.[36]

Another study demonstrated there was markedly reduced dimension of the buccal bone wall while there was stimulated bone formation on the lingual wall, suggesting that the new bone that formed at the lingual surface compensated for bone loss that occurred at the buccal surface.[37]

Buccal bone resorbs by approximately 0.6 mm in the horizontal direction over 1 year after implant surgery, and the vertical height of the buccal bone decreases by approximately 1 mm during a period of 6 months to 1 year after surgery. Thick buccal bone at baseline tends to persist at 1 year, albeit with a decreased thickness. The buccal mucosa recedes by approximately 0.4 mm over 6 months after prosthetic treatment, but it exhibits no correlation with buccal bone resorption.[38] After four months of healing following alveolar ridge augmentation using guided bone regeneration (GBR), it has been reported that the loss in supplemental bone width ranged from 39 – 67% and 60 – 76% in height.[59]

Risk indicators for soft and hard tissue recession of the buccal surface following implant placement include thin tissue biotype, a facial malposition of the implant, and a thin or damaged facial bone wall.[58] The amount of existing buccal bone volume prior to implant placement is assessed through clinical bone sounding or obtaining a 3-dimensional cone beam computed tomography (CBCT) scan. Current strategies to minimize buccal bone resorption following implant placement include bone/soft tissue grafting prior to or in conjunction with implant placement, and placement of the dental implant in a palatal or lingual position of the proposed prosthetic tooth. Limitations of these strategies include

errors in clinical detection of buccal bone fractures during implant surgery, and the lack of understanding of the exact dimensions of gingival biotype that allow for the preservation or recession of buccal bone.

Bone Resorption following Tooth Extraction: Buccal vs Lingual

Following tooth extraction, typically there is a reduction of both the height (2.5–7 mm) and width (3–7 mm) of the alveolar process. Most change occurs in the first months, while minor additional resorption of the ridge continues over periods ranging between 10 and 20 weeks.[15] The width of the alveolar ridge is reduced approximately 50% following a non-grafted extraction site; with the most change occurring during the first 3 months of healing.[17] The amount of residual ridge resorption is typically greater on the buccal surface than along the lingual or palatal surface, although the absolute amounts and differences vary widely.[16] A reason for this may be that the buccal bone is composed predominately of bundle bone, which resorbs completely, possibly due to lack of a supporting function provided by the tooth following extraction. Another possible explanation for greater resorption of the buccal surface as compared to the lingual surface, is that generally the buccal bone is thinner and more easily damaged during extraction compared to the lingual bone, and consequently may have a greater susceptibility to resorption.

Strategies that may be employed to prevent buccal bone resorption following tooth extraction include both hard and soft tissue grafting to thicken the biotype to resist recession. However, with the current level of knowledge, these techniques do not give completely predictable outcomes. A more detailed understanding of the osteoclast-mediated resorptive process is critical for the clinical success of ridge preservation following extractions.

Summary

The remodeling process mediated by osteoclasts and osteoblasts is essential for the formation and replacement of structurally robust bone in periodontal and implant procedures. Following osseous periodontal surgery, it is known that osteoclastic activity peaks at 2-3 weeks followed by an increase of osteoblastic activity.[13] After dental implant surgery, there is a sequence of healing events, including necrosis and subsequent resorption of traumatized bone around the implant body by osteoclasts concomitant with new bone formation.[31] However, a complete description of how osteoclasts function during the events of alveolar bone regeneration and resorption is lacking. Inadequate bone formation following periodontal regenerative therapy or buccal bone resorption following tooth extraction may dramatically affect treatment outcomes. A better understanding of how osteoclasts function during alveolar bone regeneration will contribute to the development of rational strategies to enhance therapeutic outcomes.

Our present knowledge in this area is insufficient to permit the design of an ethical large animal study with sufficient statistical power. The purpose of this preliminary project was to conduct spatial and temporal observations of osteoclastic activity at periodontal and implant sites following periodontal and dental implant surgery in a canine periodontal and peri-implant regeneration model using a limited number of subjects to provide baseline information for the design of future studies.

In the present study we hypothesized that –

1) Both the resident and regenerative buccal bone would have higher OA compared to the lingual bone at both tooth and implant sites.

2) OA would be comparable at tooth and implant sites, except at the implant-bone interface, where activity would be higher versus the tooth-bone interface due to additional remodeling.

3) There would be a peak in OA in the two weeks following injury associated with mobilization of regenerative cell populations, and a second peak associated with remodeling between four and eight weeks.

4) The addition of autologous blood prior to wound closure would reduce the amount of OA as it may enhance the formation of a blood clot.

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