Histomorphometric Analysis of Implant Anchorage for 3 Types of Dental Implants Following 6 Months of Healing in Baboon Jaws

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In an effort to better understand the supporting anatomy for unloaded endosseous dental implants, this study focused on the histomorphometric analysis of 3 different types of implants placed into nonhuman primate jaws and allowed to heal for 6 months. This report describes data from 24 screw-type dental implants placed in edentulated (2 months healing time) posterior arches of 4 adult female baboons. Three different implants were placed and allowed to heal for 6 months prior to processing for evaluation: commercially pure titanium (n = 8), titanium alloy (n = 8), and titanium plasma-sprayed (n = 8). Circumferential bone-implant interface sampling from 6 regions along the entire length of each implant was obtained for evaluation of percent bone-implant contact (%BIC) and percent bone area (%BA), within 3 mm of the implant. Data were collected (reliability of 1.6% for both parameters) and analyzed by an observer blinded to implant material using IMAGE analysis software for differences between jaws, implant biomaterials, and jaw/biomaterial (analysis of variance, pairwise comparison using least squares method with Bonferroni adjustment). The results indicated that the overall mean %BIC was 55.8 and mean %BA was 48.1. Maxillary and mandibular differences for both parameters were statistically significantly different: %BIC in maxilla 50.8, in mandible 60.8; %BA in maxilla 43.6, in mandible 52.6 (both significant at the P < .05 level). The biomaterial analyses revealed no significant differences between the different implants for %BIC or %BA. The trend observed—that mandibular values were greater than maxillary values for the overall jaw comparisons—was found to be consistent at the jaw/biomaterial level, although the small sample size limited statistical power. These data, along with data from a previous 3-month study, provide insight into baseline supporting anatomy for dental implants. (Int J Oral Maxillofac Implants 2000;15:785–791)

Key words: *animal study, biomaterials, bone, dental implants, histomorphometry*

The support potential of the remaining oral structures is one of the principal characteristics evaluated when considering the replacement of missing teeth. When clinicians select the use of existing teeth and/or residual ridges to provide the foundation for a conventional prosthesis of choice (ie, fixed or removable prosthesis), this does not alter the quality of the support. However, dental implants do offer a distinctively different quality of support when compared to teeth and/or ridges. Consequently, this decision is a prescriptive opportunity for the clinician to provide support not previously available. Since the primary function of a dental implant is to provide support and functional stability for replacement teeth, it follows that the dynamics of bone healing, which establishes implant support, and bone maintenance, which provides for predictable ongoing implant support, are important features to understand. Brunski has stated that a complete understanding of dental implant prognosis requires a more detailed comprehension of the biology of bone modeling and remodeling as affected by the stresses and strains inherent in the functional implant.[1] The biomechanical issues important to consider for this detailed understanding include the mechanical loading of the implant(s), transfer of the

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load to the interfacial tissues, and the biologic reactions to these loads over time. This report, which provides a description of the healed bone-supporting anatomy 6 months after implant placement and prior to the initiation of loading, is a baseline study necessary for future study of bone biologic reactions to functional load.

Because clinicians are unable to directly observe the biologic bone response to implant placement, decisions are made about healing progress via indirect assessments of this interfacial wound-healing response. Predictive clinical measures that have been used as surrogate measures of these biologic reactions have included noninvasive mobility tests[2–4] and potentially destructive torque testing.[5] While it is tempting to suggest that in the study by Sullivan and coworkers,[5] torque testing eliminated nonosseointegrated implants from an implant population because all implants that survived testing were shown to also survive clinical function, this will never be known without adequate validation using a gold standard. Conducting such a validation study is important, because if the finding is incorrect, this type of test may needlessly remove useful implants. Data to date for both mobility and torque do not convincingly support that either test measures what is actually intended to be measured and therefore may not provide useful diagnostic information.[6]

Mobility, as measured by the Periotest device[2] (Siemens, Bensheim, Germany), has not proven to be predictive of implant support[3]; nor is it able to reliably distinguish the subtle change from clinical stability to initial loss of stability, a critically important diagnostic feature of a device for evaluating implant stability.[4] Torque data have been suggested to provide diagnostic meaning.[5] However, until more definitive data regarding torque sensitivity and specificity[6] are described, this form of biomechanical test prediction should not be routinely used. Results from recent studies illustrate that the histologic data describing the bone supporting certain implant groups do not follow the torque increase, as would be predicted if torque were a measure of the interfacial anatomy.[7] More specifically, percent bone area (%BA) data were found to be no different between implants with significantly greater torque values versus those with lower torque values.[8] These results suggest that the use of torque to predict implant anchorage is very imprecise, as there is no consistent torque value that represents a specific condition of bone adjacent to an implant.

Critical analysis of these data reveals that continued refinement of these types of diagnostic tools is needed, and that basic questions regarding typical bone responses to implant placement and function remain to be answered. Two basic questions that need to be answered are: "What is the typical healed bone support of an integrated implant?" and "How does functional loading affect this pattern of healed bone?" The purpose of this study, which focuses on the first question, was to measure the implant supporting anatomy for 3 different biomaterials following 6 months of healing in adult female baboon jaws. The specific aims were to measure and compare percent bone-implant contact (%BIC) and %BA overall, between jaws, and for each implant. The null hypothesis tested states that there is no difference in parameters measured with respect to implant type or jaw.

MATERIALS AND METHODS

Animal Model and Surgical Protocol

Following review and acceptance of a protocol by the Institutional Animal Care and Use Committee, 4 adult female baboons weighing from 12.5 to 18.6 kg were obtained and housed at a fully staffed university laboratory animal research facility (Ohio State University). Each animal had all posterior teeth surgically removed under general anesthesia and, following 10 weeks of uneventful healing, 10-mm screw-type implants were placed in each posterior quadrant following human surgical protocols (Fig 1). The 3 implant biomaterials—commercially pure titanium (cpTi) (originally Nobel Biocare, Chicago, IL; and Steri-Oss, Yorba Linda, CA); titanium-aluminum-vanadium alloy (Ti-6Al-4V) (Steri-Oss, Yorba Linda, CA); and plasma-sprayed titanium (TPS) (Steri-Oss)—were placed in alternating order to assure regional variation for each biomaterial.

Second-stage surgery was accomplished under general anesthesia after 189 days of healing to harvest the implant specimens. This investigation focuses on 24 representative implants of the total population of implants placed and seeks to provide histomorphometric data representing the 3 different biomaterials, for both the mandible and maxilla, following uneventful healing. The number of implants selected for the study was influenced by cost (since the specimen processing method prohibited sampling large numbers), acceptable Periotest values (PTV) (ie, accepted implants exhibited scores of –4 or –5), and equal distribution between jaws (1 implant from each jaw and biomaterial group for each animal). The resulting implant distribution was 12 maxillary and 12 mandibular implants, with 4 maxillary and 4 mandibular implants for each implant group. The data included %BIC and %BA collected from the entire circumference and representative regions along the entire length of the implant as outlined below.

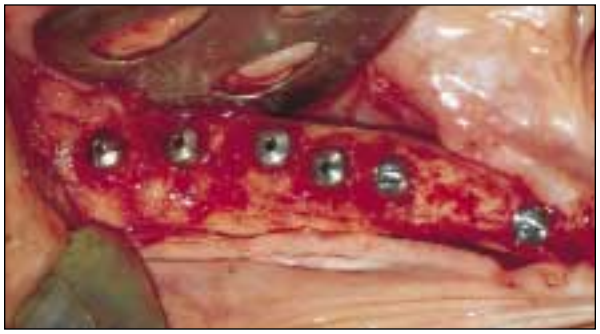

Fig 1 Implant placement in the posterior jaws of female baboons. The edentulous ridge represents a 2-month postextraction recipient site for implant placement, provides adequate height and width for site preparation without ridge modification, and is characterized as type 3 bone, as described by Lekholm and Zarb.[26] Implant stability at placement was adequate. All implants were placed to the level of the smooth collar without countersinking.

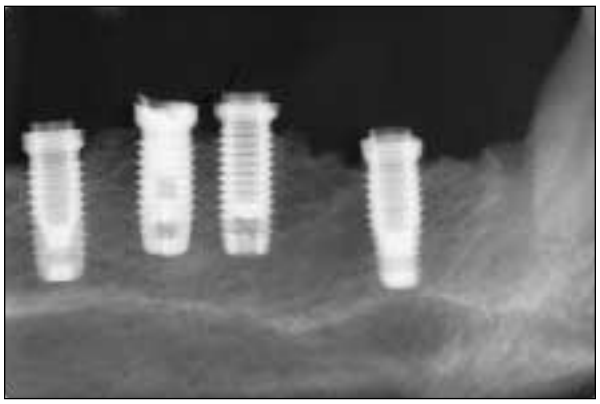

Fig 2 Radiograph exhibiting the trabecular bone supporting the 4 most anterior implants in one maxilla. This trabecular pattern is representative of bone support for both jaws. Implants closest to and farthest from the tooth were used for data collection.

Specimen Preparation and Analysis

Following healing, the jaws were hemisectioned and placed in Carson's fixative for shipment to the specimen-processing laboratory. To assure correct alignment for section orientation, the specimens were radiographed (Fig 2), sectioned using a band saw, and replaced in fixative for 24 hours. Following dehydration and embedding procedures, 15 to 18 serial 100-µm horizontal sections along the whole length of the implant were accomplished using a Leitz 1600 bone saw (Leitz-Wild USA, Rockleigh, NJ). The sections were mounted on 2 × 3-inch plastic slides, and every third section was ground and polished to a 40-µm thickness using a Mark V grinder/polisher (Mark, Salt Lake City, UT). The sections were studied and photographed using a Zeiss microscope (Carl Zeiss, Zurich, Switzerland), and the images of the sections were digitized to a Macintosh II computer equipped with the IMAGE analysis system (National Institutes of Health, Bethesda, MD).

Data collection was from a 3-mm circumferential adjacent region of tissue at 6 levels along the length of the implant to assure apical, middle, and coronal representation of the implant-bone anatomy. For this report, the mean values for %BIC and %BA were computed from the combined 6 sections for the overall population of implants, for each jaw, and for each biomaterial. Analysis of parameter differences was conducted using a mixed-model analysis of variance (SAS Software version 6.10, Cary, NC), with least-squares means pairwise comparison and Bonferroni adjustment for biomaterial differences. This model was chosen to account for the lack of independence of implants within the individual animals.

Surface Characterization

Analysis of surface roughness detail was accomplished by evaluating 4 regions from 1 implant in each group (Vecco Co, Flagstaff, AZ). The surface was characterized by measuring 6 different parameters: Ra (roughness), Rt (highest to lowest point), Rp (midline plane to highest point), Rv (midline plane to lowest point), S area (increase in surface compared to an absolute plane), and Vol (volume that the sample can hold if capacity is determined by highest to lowest peak).

RESULTS

The descriptive data and analysis results are presented in Table 1. The overall mean %BIC was 55.8 (± 5.3 SD). The maxillary %BIC was 50.8 (± 1.4), while the mandibular %BIC was 60.8 (± 1.6), a difference that was significantly different ($P < .05$). The biomaterial-specific %BIC were 56.2 for cpTi, 55.2 for Ti-6Al-4V, and 55.9 for TPS, differences that were statistically insignificant ($P > .05$).

The overall mean %BA was 48.1 (± 4.9). The %BA by jaw mimicked the %BIC finding, showing significantly lower values in the maxilla than in the mandible (43.6 ± 1.7 versus 52.6 ± 1.7, respectively; $P < .05$). As with the biomaterial-specific %BIC data, the %BA was not significantly different for any implant group (cpTi = 47.8, Ti-6Al-4V = 49.2, and TPS = 47.3; $P > .05$).

Comparisons of the biomaterial data for each jaw were not significantly different because of limited sample size within groupings. Table 1 provides

Table 1 Histomorphometric Data (Mean ± SD) for All Samples and by Jaw, Biomaterial, and Jaw/Biomaterial

Sample	n	Percent bone-implant contact	Percent bone area
All	24	55.8 ± 5.3	48.1 ± 4.9
Maxilla	12	50.8 ± 1.4†	43.6 ± 1.7†
Mandible	12	60.8 ± 1.6	52.6 ± 1.7
CpTi*	8	56.2	47.8
Maxilla	4	51.5	43.3
Mandible	4	60.8	52.3
Ti-6Al-4V*	8	55.2	49.2
Maxilla	4	49.8	45.0
Mandible	4	60.6	53.3
TPS*	8	55.9	47.3
Maxilla	4	51.1	42.5
Mandible	4	60.9	52.2

*The biomaterial/jaw sample size restricted statistical comparisons.
†Significantly different from mandible (ANOVA, $P < .05$).
As in the 3-month study, some implants were part of a torque study that focused on biomechanical/histomorphometric correlations. Statistical analysis revealed no significant difference in the histomorphometric data for torqued and un-torqued implants (ANOVA, $P < .05$, total displacement of torqued implants < 30 degrees).

the biomaterial data for each jaw and shows that a trend exists for the mandibular data to be greater than the maxillary data for both parameters studied. Approximations show this difference to be from 16 to 21% greater, as depicted in Figs 3a to 3d.

Table 2 presents data describing the surface characterization for the implants. Six different parameters are provided, which are indices of different physical/geometric surface area expressions. By comparison, a subjective visual evaluation of 5,000× scanning electron photomicrographs by one of the authors ranked the roughness of the implants as: cpTi (Nobel Biocare), cpTi (Steri-Oss), Ti-6Al-4V, and TPS, from smoothest to roughest. The complex nature of judging this seemingly important physical aspect of implantable devices is revealed when this subjective evaluation is compared with the data in Table 2. For all of the 6 parameters measured, the TPS implant ranked as the roughest, while the original Steri-Oss cpTi implant ranked as third roughest. The Ti-6Al-4V implant ranked the smoothest for all parameters, except S area index, where the Nobel Biocare cpTi implant ranked first. The importance of these various surface parameters in long-term endosseous implant performance has yet to be fully described.

DISCUSSION

The use of dental implants to support prostheses allows the clinician greater flexibility than conventional fixed partial prosthodontics. It also permits significant changes in the nature of prosthesis support. Decisions about how to use this unique implant support, which results when a device is placed into a surgical wound in bone, need careful study. The uniqueness stems from the difference between the nature of the support provided for replacement teeth, when compared to a periodontal ligament–suspended natural tooth and mucosa. More information is needed to guide these prescriptive treatment decisions made by clinicians.

As stated previously, an understanding of the clinical prognosis of dental implants requires more complete knowledge of the bone biology associated with the integrated (ie, healed) and functional implant. To that end, the purpose of this study was to provide data regarding the structure of the mineralized tissues adjacent to 3 different implants in primate maxillae and mandibles after 6 months of healing. From this structural understanding, appropriate hypotheses investigating prescriptive and diagnostic decisions regarding the transfer of the load to the interfacial tissues and the biologic reactions to these loads over time can be studied.

Comparison of the findings from this study to previous similar data presents some problems based largely on different animal models or anatomic sites used and sampling differences. Most previous intraoral studies have described mandibular data from dogs[9–12] or rhesus monkeys.[13,14] In general, the dog studies provided unloaded data similar to the cpTi and TPS implants in this study, but with varying healing times. The monkey studies provided data for loaded implants and may be at variance because of load-induced changes in bone support. A

Figs 3a to 3d Photomicrographs of implant sections where a toluidine/alizarin red stain was used. No significant biomaterial-specific bone response difference existed; however, the jaw-specific response for %BA is more easily observed in these representative sections. Bars equal 200 µm.

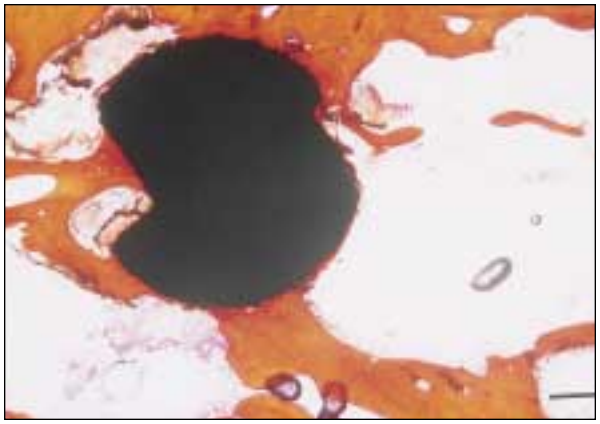

Fig 3a Maxillary TPS implant, apical region.

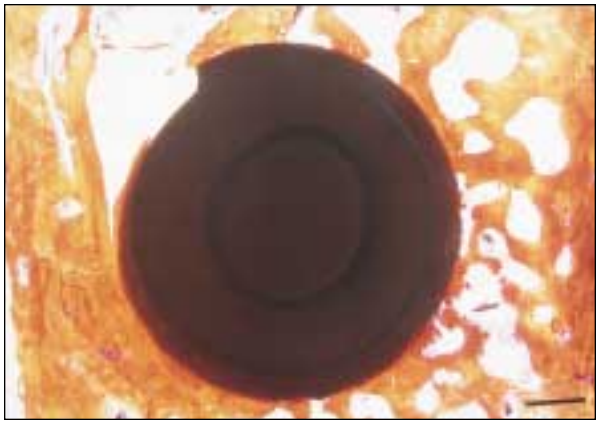

Fig 3b Maxillary Ti-6Al-4V implant, coronal region.

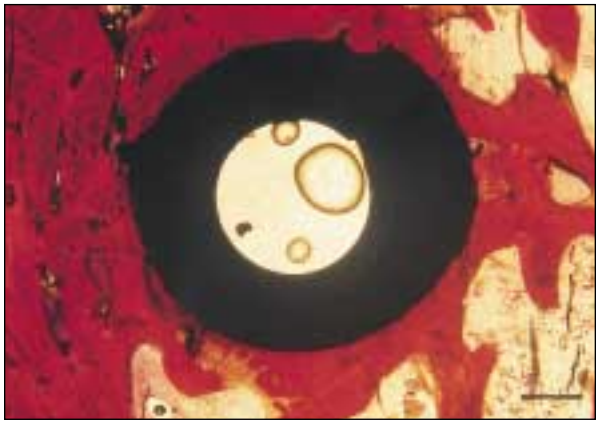

Fig 3c Mandibular cpTi implant, middle region.

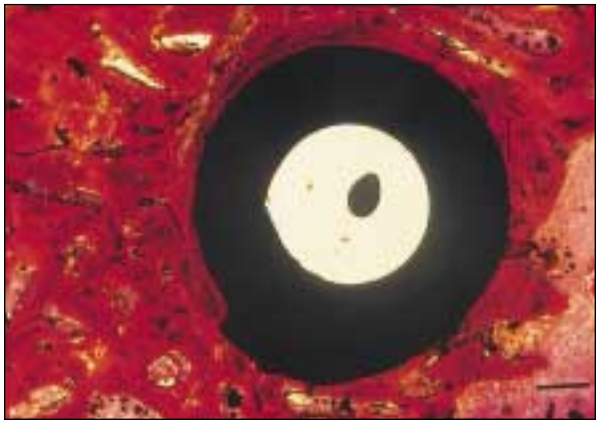

Fig 3d Mandibular Ti-6Al-4V implant, middle region.

Table 2 Characterization of Implant Surface Roughness (Mean ± SD)

Implant type	Ra (nm)	Rt (nm)	Rp (nm)	Rv (nm)	S area index	Vol (µm³)
Plasma-sprayed titanium	7345 ± 1695	93,707 ± 18,237	39,652 ± 15,039	–54,054 ± 6216	3.99 ± 0.62	216,023 ± 55,672
Titanium alloy	350 ± 34	4142 ± 1282	2216 ± 902	–1926 ± 544	1.17 ± 0.08	10,017 ± 1163
Commercially pure titanium (SO)	975 ± 46	20,262 ± 10,555	11,657 ± 3956	–8605 ± 7096	1.26 ± 0.09	34,302 ± 5619
Commercially pure titanium (NB)	632 ± 8.5	7006 ± 508	4522 ± 501	–2483 ± 89	1.13 ± 0.005	29,793 ± 3879

Ra = average roughness; Rt = highest-to-lowest point distance; Rp = midline plane to highest point; Rv = midline plane to lowest point; S area index = increase in surface compared to an absolute plane; Vol = volume sample can hold if capacity is determined by highest and lowest valley peaks. Sampling was performed by Vecco Co, Flagstaff, AZ. On 1 implant from each group, 4 regions provided data with setup parameters of 368 × 240, sampling of 330.98 nm.

study by Arvidson and colleagues[15] revealed that, for cpTi implants placed in dog mandibles for 6 months, histometric analysis revealed that 61.3% of the implant surface was integrated in bone. These data, which represented the 4 most central threads from 24 implants, correlate well with the mandibular %BIC mean value of 60.8 from the 3 implants in this study. No comparative maxillary data are available except from a 3-month healing study.[7]

A great deal of current interest has been focused on the surface roughness effect on bone during healing and maintenance of the integrated interface. Many animal studies have researched this area[16–19] and generally show surface roughness to correlate with better biomechanical and histologic measures of implant integration. However, a recent review of biomaterials and biomechanics of dental implants by Brunski and colleagues[20] stated that "studies as yet do not yield compelling conclusions about the role of surface composition and texture with respect to bone response at the interface." Part of the dilemma may stem from the variety of methods and animal models used to study these phenomena. The use of non-oral sites to study questions of bone healing may be appropriate, but if research questions are specific to oral function, it stands to reason that jawbone should be the site of study. This difference, as well as sampling methods, may account for the differences in the data collected in this study versus that found in other non-jaw studies.

Data from human retrieval studies can provide important insight into the anatomy of bone supporting dental implants. A recent report describing histologic observations on 230 retrieved implants over an 8-year period stated that, for fractured implants (n = 90), %BIC was measured to range from 80 to 100%.[21] These data support the findings of Albrektsson and coworkers,[22] who reported both %BIC and %BA of 82 for loaded implants retrieved from the anterior mandible in humans. The anterior mandible location, the loading history, and the sampling method (ie, data from the "best threads") may explain the difference between these reported data and the data from this study.

This study was designed to complement a previous dental implant study[7] that investigated healing after 3 months. While that study provided maxillary data that may be considered premature, given current loading protocols of 6 months for the maxilla, this study had a healing time of 6 months, which represents a longer period compared to the suggested time for mandibular bone integration. The reason for this is obvious, considering the method of specimen harvesting, which requires the animals to be sacrificed to obtain the specimens. Comparison of the data between the 2 healing studies provides insight into time-dependent bone responses and will be addressed in a separate report.

Considering both parameters measured for this study, the mean values suggest that more than half of the available implant surface and about half the observed area adjacent to the implant was made up of mineralized tissue following 6 months of healing. All implant materials were some form of titanium or its alloy, and there were no observed biomaterial differences for either parameter. This finding is not supported by some research that describes specific bone-implant surface roughness interactions that favor bone apposition.[23] This may be a function of the specific nature of the roughened surface, which may be uniquely distinct from the TPS surface of this study. Given the reported differences in arch structure,[24] it is not surprising that there is consistent evidence that maxillary anatomy around implants is not the same as mandibular anatomy around implants. This, in turn, may impact functional performance when the less-mineralized maxillary arch bone supporting an implant is required to support a dental prosthesis. Whether this difference is clinically significant enough to warrant the assignment of more implants to support similar numbers of replacement teeth in the maxilla versus the mandible needs further clarification. It is interesting to consider that this anatomic bone difference may have some bearing on the difference between root anatomy between arches, and therefore may suggest different implant prescriptions for different posterior arches.

At the time of this study the implants used were from 2 different manufacturers. The Nobel Biocare cpTi implant was used as a reference standard because of previous documentation and its long-term clinical record of success.[25] Parameters of research, such as those measured in this study, are only useful to the extent that they have meaning relative to clinically important outcomes. Including an implant in this study that has been shown to provide long-term functional support for dental prostheses for a variety of clinical applications provides a point of reference for meaningful comparison. Because there was no difference between implants for the 2 parameters studied, it can be stated that interfacial anatomy after 6 months of healing is not likely to be a cause for different performance for these implants.

CONCLUSIONS

The purpose of this study was to measure bone supporting anatomy for 3 different implants following 6 months of healing in adult female baboon jaws. For the parameters %BIC and %BA, approximately half

(55.8% and 48.1%, respectively) of the interfacial tissue for each parameter was mineralized. A significant difference between jaws was observed for %BIC, where the mandible exhibited 10% more linear contact of bone than the maxilla, leading to a rejection of the null hypothesis for jaw differences. Similarly, a significant difference for %BA was seen between jaws, where the mandible exhibited 9% more area of bone than the maxilla. All implant groups were similar in parameter value within the respective jaws, in support of the null hypothesis for biomaterial observation. This baseline data will be used for comparison to similar data following occlusal loading to determine load-induced changes in bone support.

ACKNOWLEDGMENTS

The authors would like to express appreciation to Dr Bob Rashid for his statistical assistance, and to the OSU Wiseman Hall Animal Care Facility faculty and staff for their expertise and help with this project. This project was partially funded by the OSU Maxillofacial Education and Research Fund and Steri-Oss, Inc.

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