Published in final edited form as:

Arch Oral Biol. 2011 January ; 56(1): 79–89. doi:10.1016/j.archoralbio.2010.08.013.

The Impact of Occlusal Function on Structural Adaptation in Alveolar Bone of the Growing Pig, *Sus Scrofa*

Kuang-Dah Yeh¹ and Tracy Ellen Popowics^{2,*}

¹ Hualien Armed Forces General Hospital, Hualien, Taiwan. 4F, 6-3, LN37, Siwei St., Chungho 235, Taipei County, Taiwan, R.O.C

² Department of Oral Biology, School of Dentistry, University of Washington, Seattle, WA

Abstract

Objectives—This study investigated the effects of growth and tooth loading on the structural adaptation of the developing alveolar bone adjacent to the tooth root as the tooth erupted into function. Growth and occlusal function were expected to lead to increased alveolar bone density. Meanwhile, the supporting alveolar bone was expected to develop a dominant trabecular orientation (anisotropy) only after occlusal loading.

Design—Minipigs with erupting and occluding mandibular first molars (M_1 's) were used to study the effects of growth and occlusal function on developing alveolar bone structure through comparison of alveolar bone surrounding M_1 's. A second minipig model with one side upper opponent teeth extracted prior to occlusal contact with the M_1 was raised until the non-extraction side M_1 's developed full occlusal contact. The comparisons between extraction and non-extraction side M_1 alveolar bone were used to emphasize the impact of occlusal loading on alveolar bone structure. Specimens were scanned on a Scanco Medical μ CT 20 at a 22 μ m voxel resolution for structural analysis.

Results—With growth and occlusal function a distinct alveolar bone proper tended to develop immediately adjacent to the tooth root. The cancellous bone had thicker but fewer and more separated trabeculae after growth or occlusal loading. On the other hand, occlusal function did not lead to increased alveolar structural anisotropy.

Conclusion—During tooth eruption, growth and masticatory loads effect structural change in alveolar bone. The impact of occlusal function on cancellous bone anisotropy may need a more extensive period of time to demonstrate.

Keywords

Alveolar bone; Bone growth; Occlusal function; Mechanical adaptation; Trabecular anisotropy; Microcomputed tomography (μ CT)

Introduction

Alveolar bone supports teeth during mastication and is presumed to adapt to occlusal forces; however, the effects of growth and occlusal loading on the structural characteristics of

*Corresponding author: (206)221-5350, Box 357132, University of Washington, Seattle, WA 98195.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

alveolar bone are not clear. Mature alveolar bone is comprised of three components: alveolar bone proper (ABP), outer cortical bone and intermediary cancellous bone, when the tooth has fully erupted.¹ In alveolar bone studies, continuous occlusal load applied by a bite block significantly increased mandibular alveolar cortical bone width in growing rats. On the other hand, reduction of occlusal force through a soft diet led to lower values of alveolar cancellous bone volume fraction and trabecular thickness.² Lacking from this study, however, is an understanding of the roles that growth and occlusal loading play individually and concomitantly in the alveolar bone structural adaptation.

Growth and mechanical usage have been shown to influence both cortical and cancellous bone mass and architecture. In long bones, cortical bone mass is believed to be controlled by mechanical usage (Frost 1987, “mechanostat” hypothesis).³ During functional loading, cortical bone tended to increase bone mass in order to lower peak strain toward a threshold range lower than 1,500 microstrain. In cancellous bone, bone density is found to increase during growth. The fine trabecular patterns of the distal radius in young children coarsens with age⁴ and adolescent lumbar cancellous bone shows higher bone volume fraction and more plate-like trabeculae than in children.⁵ Furthermore, Wolff’s law proposed that trabeculae would orient along the direction of applied load in order to provide better support in that direction. Indeed, biomechanical testing has shown that the stiffness and strength of cancellous bone corresponds with the main orientation of trabeculae with respect to the direction of load.^{6, 7, 8} Additionally, the contributions of trabeculae to the anisotropic elastic moduli have been calculated in a finite element study in which the axial loading of cancellous bone was mainly sustained by the axially aligned cancellous bone.⁹ In pig vertebral and tibial cancellous bone, bone density has been shown to increase from the early phases of growth, whereas trabecular architecture developed a dominate orientation along the loading axis (trabecular anisotropy) much later.⁷ Furthermore, elastic modulus, bone volume fraction and architectural anisotropy were positively correlated with age in ovine tibia cancellous bone.^{10, 11} Therefore, both growth and mechanical loading can lead to increased bone density and anisotropy.

This study examines the impact of age and occlusal contact on alveolar bone structure through comparison of bone distal to mandibular first molars (M_1 ’s) in different pig models. First, a tooth eruption and occlusion model was used. The 13-week pigs that showed M_1 ’s just emerging from gingivae and lacking occlusal contact with opponent teeth, were compared with 23-week old pigs that had full occlusal contact between maxillary and mandibular first molars (M^1/M_1) on both sides. A second tooth extraction model was used to emphasize the impact of occlusal function on the supporting alveolar bone. Our hypothesis was that during growth, alveolar bone will increase in bone density but occlusal loading will further reinforce alveolar bone structure. Specifically, alveolar bone will have increased bone density and will develop a specific trabecular orientation only after occlusal loading, in order to distribute occlusal stresses.

Materials and Methods

Five 13 week old miniature pigs (Sinclair Research Farms, Columbia, MO) and four 23 week old pigs were used in the first tooth eruption and occlusion model. Because alveolar bone growth and tooth occlusion conditions are different between these two age groups, their comparison was expected to reveal the combined effects of growth and occlusal loading on alveolar bone structure.

Four tooth extraction pigs were used in the second model, the tooth extraction model (occluding side, n=4; non-occluding side, n=4). In order to clarify the individual role of occlusal loading in alveolar bone adaptation, tooth extraction surgeries were performed to

remove occlusal loading from the mandibular molar on one side. Specifically, the maxillary deciduous fourth premolar (DP⁴) and M¹ of 4 miniature pigs were extracted on one side before these teeth developed occlusal contact with the opposing M₁'s. All four pigs were acquired at 12 weeks of age because at this stage, M₁ has just started to penetrate through the gingiva and no occlusal contact has developed with upper teeth (Fig. 1). Tooth extraction surgery occurred the following week, and the extraction side was chosen randomly each time during surgery to avoid bias induced by side preference. After surgery, M₁'s were allowed to erupt on both sides while only the non-extraction side M₁ was able to develop occlusal contact with opponent teeth.

The effect of tooth extraction surgeries on the masticatory system has been described previously.¹² Electromyography was used to track chewing activity before and after tooth extraction. Additionally, a terminal procedure to measure buccal alveolar strain was carried out once full occlusal contact between non-extraction side M¹/M₁ was achieved (Fig. 2). Immediately following tooth extraction surgeries, pigs favored the non-extraction side during chewing; however, by 5–6 weeks post-extraction, most pigs demonstrated a recovery to the normal alternating chewing pattern. All pigs were 22 or 23 weeks old at termination. Pigs with tooth extractions showed higher *in vivo* buccal shear strains on the occluding side of the mandible relative to the non-occluding side during chewing; however, on both sides strain magnitudes were similar to those recorded in a previous study of mandibular corpus strain below DP₄ in pigs with normal occlusion.¹³

Alveolar bone of left and right sides of the same pig mandible were compared post mortem. Because the tooth extraction pigs were at the same age as the normal 23-week tooth occlusion pigs of the first model at termination, the comparisons between the same age pigs from different models were also expected to emphasize the occlusal function effect on alveolar bone. All experimental procedures were approved by the Animal Ethics and Research Committee and were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of The Health Sciences of University of Washington.

Microcomputed Tomography Scanning

All specimens were cut to fit the microCT cone (with a diameter of up to 34 mm), therefore, each specimen was prepared to a cuboid containing the M₁ distal root and M₂ crypt as well as the alveolar bone between them. Specimens were scanned on a Scanco Medical μ CT 20 (Bassersdorf, Switzerland) at a 22 μ m isotropic voxel resolution and standard filtering and thresholding algorithms¹⁴ were adopted for data analysis. Previous studies have used 22 μ m voxel size for human bone and 28 μ m voxel size for pig bone.^{7, 15} The 22 μ m scanning resolution was chosen to balance the large size of the samples with the greatest accuracy possible. In order to establish that 22 μ m was sufficient, a subset of the same samples (n=2) was imaged at 11 and 22 μ m and showed no difference in any cancellous or cortical bone parameters. Total volume (TV, mm³), bone volume (BV, mm³), bone surface (BS, mm²), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), and trabecular separation (Tb.Sp, mm) were measured. Trabecular number (Tb.N) was calculated as the inverse of the mean distance between the middle axes of the structure. Mean trabecular thickness (Tb.Th) was determined from the local thickness at each voxel representing bone and trabecular separation (Tb.Sp) was calculated by applying the technique used for the direct thickness calculation to the nonbone parts of the 3D image.¹⁶ Structure Model Index (SMI), an estimation of the plate-rod characteristic of the structure, was also directly assessed. Architectural indices were then derived including bone volume fraction (BV/TV, %), the percentage of bone volume in the total volume of a specimen and connectivity density (Conn. D., 1/mm³), the negative Euler number divided by tissue volume. The Euler-Poincaré number represents the extent of connectedness between trabecular elements, where (1-Euler Number) represents the number of branches in the structure that can be removed

without breaking the structure into two parts.¹⁷ Surface density (BS/BV, mm²/mm³), the surface area of the trabeculae in relation to the total bone volume; and the Degree of Anisotropy (D.A.), the eigenvalue of the primary direction divided by the eigenvalue of the tertiary direction¹⁵ were also derived.

The region of focus was the alveolar bone area surrounding the developing roots of M₁ (Fig. 3). The alveolar bone area distal to M₁ was selected as the volume of interest (VOI) because occlusal forces are expected to be highest in the most distal dentition closest to the jaw joint. Therefore the impact of occlusal force on alveolar bone was expected to be easier to identify in this VOI. Specimens were scanned horizontally starting from the tooth crown and moving apically. The first horizontal microCT slice containing the bifurcation of distal roots of M₁ was selected as the starting point for defining the VOI of the bone structure analysis. Extending apically, all slices were included until no distolingual (DL) tooth root structure of M₁ could be seen. The VOI was then defined as the alveolar bone distal to the DL tooth root of M₁ within these selected slices. Definition of the boundaries of the VOI are described as follows. First, the midpoint of the shortest line connecting the disto-lingual and disto-buccal root of M₁ was found. From this midpoint, a line extended distally to define the distolingual alveolar bone VOI, but exclude the M₂ crypt cortical bone layer. Therefore, the buccal boundary of the disto-lingual alveolar bone VOI was midway between the disto-lingual and disto-buccal roots of M₁. The lingual border of this VOI extended to but excluded the lingual cortical bone. For 3D reconstruction of this VOI, a 2D region of interest was first carefully contoured on the first and every 25 slices along the z-axis of the VOI until the last slice; the intermediate slices were interpolated by morphing. This VOI was defined as the combined ABP+cancellous VOI because this VOI was likely to contain alveolar bone proper (ABP) and cancellous bone. This VOI was further separated into individual ABP and cancellous VOI for analysis (Fig. 3). In radiographs, the alveolar bone adjacent to the distal root of erupting M₁ showed a denser structure than the cancellous bone region (Fig. 3A) and in histology studies this denser structure appeared to be about 1.5mm (data not shown). In order to designate a region of interest within the combined ABP + cancellous VOI that would be likely to contain the ABP, if this tissue was developed, the ABP VOI was defined as the immediate 1.5mm bone tissue thickness adjacent to the tooth root. The remaining bone within the combined VOI represented the cancellous bone VOI. In order to have a more detailed inspection of the structure of alveolar bone and its transition status along the developing tooth root, all three VOIs were further divided vertically along the tooth root into cervical, middle and apical thirds. The mean values of VOI structural indices before subdividing into thirds were also reported and defined individually as the combined ABP +cancellous VOI total, ABP VOI total, or cancellous VOI total.

All data are presented as mean and standard deviation (mean±S.D.). Non-parametric Mann-Whitney tests were used to compare indices between the tooth eruption and occlusion groups. Wilcoxon signed ranks tests were used to compare indices between two related samples, the non-occluding (extraction) and occluding (non-extraction) side of tooth extraction models. When comparing indices within the same group, Wilcoxon signed ranks tests were also used. Levene's Test of Homogeneity of Variance was used to compare the standard deviation between different groups. When comparing the structural indices between different models, the Bonferroni correction was used. All statistical analyses were performed using SPSS statistical package (SPSS 15.0, SPSS Inc., Chicago, IL). A p value of less than or equal to 0.05 was considered significant, and marginally significant and significant comparisons are noted in Tables 1–7). G*Power 2 online freeware was used for all power calculations.

Results

1. MicroCT analysis of the tooth eruption and occlusion model

A tendency toward higher ABP bone density was found in the 23-week tooth occlusion specimens versus the 13-week tooth eruption specimens but the cancellous bone density was similar between these two groups (Figs. 4 and 5). In bone density index BV/TV, the index used to represent the percentage of bone volume in the total volume, the 23-week pigs had marginally higher values in the ABP VOI when compared with the 13-week pigs (Table 1). For example, the average BV/TV of the 23-week pig ABP total was 0.66 but was 0.49 of the 13-week pigs (Mann-Whitney test, $p=0.053$). On the other hand, the BV/TV was similar between the cancellous VOIs of the 23-week and 13-week pigs (mean of 0.46 vs. 0.58; cancellous total; Table 2). The 23-week ABP showed a decreasing tendency in BV/TV from cervical to apical (Fig. 6 and Table 1; Wilcoxon signed ranks tests, $p=0.068$). The difference between cancellous bone and ABP VOIs was relatively small in the 13-week pigs but showed a tendency to differ in the 23-week pigs (Tables 1 and 2, Fig. 5). For example, in the 13-week pigs the mean cancellous bone VOI cervical third BV/TV was 0.60 and it was 0.52 in the ABP VOI cervical third, but in the 23-week pigs the mean BV/TV was 0.50 in the cancellous bone VOI cervical third and 0.75 in the 23-week pigs ABP VOI cervical third (Wilcoxon signed ranks test, $p=0.068$).

In some regions, occlusally loaded 23-week pig M_1 alveolar bone had fewer but thicker as well as more separated trabeculae than the 13-week non-occluding pigs. In Connectivity Density (Conn. D.), the index used to investigate the number of connecting trabeculae, the alveolar bone of 13-week pigs showed marginally higher values than the 23-week pigs (Fig. 7). For example, the mean Conn. D. in cancellous bone total VOI was 4.68 ($1/\text{mm}^3$) in the 13-week pigs but it was 2.67 in the 23-week pigs (Mann-Whitney test, $p=0.053$). The mean Conn. D. in ABP total VOI was 5.08 in the 13-weeks but it was 2.31 in the 23-weeks (Mann-Whitney test, $p=0.053$). In BS/BV, the surface area of the trabeculae in relation to the total bone volume, the 13-week pigs had higher average BS/BV in ABP VOI middle third when compared with 23-week pigs. The mean BS/BV of the ABP VOI middle third was 8.42 (mm^2/mm^3) in the 13-week pigs and it was 4.81 in the 23-week pigs (Table 3; Mann-Whitney test, $p=0.014$). Lower trabecular numbers (Tb.N) were found in the 23-week VOIs (Table 4). The average Tb.N was 1.66 ($1/\text{mm}$) in the 23-week pig cancellous bone cervical third but it was 2.62 in the 13-week pigs (Mann-Whitney test, $p=0.014$). The trabecular thickness (Tb.Th) was higher in the 23-week VOI, the cancellous VOI apical third Tb.Th was 0.38 (mm) in the 23-weeks but was 0.30 in the 13-weeks (Table 5; $p=0.014$). The trabecular separation (Tb.Sp) was higher in the 23-week cancellous VOI. The mean Tb.Sp was 0.69 (mm) in the 23-week cancellous VOI middle third but was 0.39 in the 13-weeks (Table 6; $p=0.050$).

No change in trabecular orientation could be found after occlusal function. In Degree of Anisotropy (D.A.), the index used to investigate the principal direction of trabeculae, all the 13-week and 23-week pig alveolar bone VOIs had similar values to one another (Fig. 8); thus, the occlusion specimens did not show higher directionality than the eruption specimens. On the other hand, the 23-week pigs demonstrated a more plate-like bone structure than the 13-week pigs. In Structure Model Index (SMI), an estimation of the plate-rod characteristic of the structure, the 23-week pig alveolar bone showed more negative values in the ABP middle VOI when compared with the 13 weeks, showing a more plate-like structure in some regions of the older pigs. The mean ABP middle VOI SMI was -3.02 (ratio) in the 23-week specimens but was 0.56 in the 13-week specimens (Mann-Whitney test, $p=0.014$).

In most of the indices studied, 23-week pigs showed a lower standard deviation (S.D.) than 13-week pigs. For example, the 13-week ABP cervical BV/TV had a S.D. at 0.17 and the S.D. was 0.03 in 23-week samples (Levene's Test of Homogeneity of Variance, $p=0.004$); thus the 23-week pigs had a more consistent ABP cervical structure. Several other indices also showed the same tendency in the decreasing variability with age. For example, the 13-week cancellous VOI cervical Conn.D. had a S.D. at 2.06 and the S.D. was 0.54 in 23-week specimens ($p=0.005$). The bone appeared to have more consistent structural indices in older pigs than in younger pigs.

In summary, even though the mean BV/TV was very similar between 13-week and 23-week pigs in the combined ABP+cancellous VOIs (0.52 vs. 0.54, 13 vs. 23-week; total), when further divided into separate ABP and cancellous VOIs, the structure showed a tendency to differ. In the cancellous bone VOI, 13-week pigs showed similar average BV/TV with 23-week pigs, but in the ABP VOI, 23-week specimens showed a tendency to have higher BV/TV than the 13-week specimens (mean of 0.49 vs. 0.66; 13 vs. 23-week ABP total, $p=0.053$). The 23-week pigs had fewer trabeculae and marginally less Conn. D. but the trabeculae were thicker and more separated from each other. Corresponding with the trabecular number, 23-week pigs also showed a smaller BS/BV. The structural model index analysis suggested that the pig alveolar bone was more plate-like in 23-week pigs but more rod-like in 13-week pigs. The D.A. demonstrated that the alveolar bone structure in 13-week pigs had similar anisotropy values with the 23-week pigs. However, the 23-week pig alveolar bone structure showed greater consistency than the 13-weeks.

2. MicroCT analysis of the tooth extraction model

Similar bone densities were found between the non-occluding and occluding sides of the tooth extraction model alveolar bone. No differences in average BV/TV were observed in alveolar bone VOIs between non-occluding and occluding sides (Tables 1 and 2). In the cancellous total VOI, however, a marginally significance was found between the two sides of the tooth extraction specimens. The non-occluding side showed marginally lower BV/TV than the occluding side (mean of 0.56 vs. 0.59; non-occluding vs. occluding, Wilcoxon signed ranks test, $p=0.068$, Table 2). In the ABP VOIs, both sides demonstrated marginally higher BV/TV in the cervical and middle thirds than the apical third ($p=0.068$). The same tendency to have a greater density in the ABP as well as a distinction between cancellous bone and ABP VOI total BV/TV in the normal 23-week tooth occlusion pigs was also present on both sides of the tooth extraction model (Wilcoxon signed ranks test, $p=0.068$ for both sides, Tables 1 and 2). However, because of the small sample sizes, the power of analysis was limited (power=0.09, post hoc, G*Power 2). According to the data collected from these four tooth extraction pigs, in order to detect a significant difference in cancellous total bone density between the two sides of the tooth extraction model, 64 animals for each group would be needed to find this difference with a 80% power level in a two-tailed analysis.

Additional structural indices were similar between sides. In Connectivity Density (Conn. D.), the alveolar bone of the occluding side was not significantly different than the non-occluding side in cancellous bone (Fig. 7). The average cancellous total VOI Conn. D. was 2.97 in the occluding side and it was 3.34 in the non-occluding side ($p=0.068$). In BS/BV, there were no significant differences between the two sides (Table 3). But the cancellous bone of the occluding side showed a marginally lower value than the non-occluding side and corresponded with the lower Connectivity Density finding. The mean BS/BV of the cancellous middle VOI was 5.54 in the occluding side and it was 6.03 in the non-occluding side ($p=0.068$). In the cancellous middle VOI, the occluding side also showed a marginally lower Tb.N, higher Tb.Sp than the non-occluding side (Tables 4 and 6; $p=0.068$).

In D.A., the two sides showed similar values to each other. However, in the cancellous middle VOI, the occluding side showed a marginally higher D.A. than the non-occluding side (mean of 1.68 vs. 1.52, $p=0.068$). The SMI of the occluding side cancellous bone showed marginally more negative values when compared with the non-occluding side. For example, the mean SMI of cancellous bone total VOI in the occluding side was -2.24 and it was -1.59 in the non-occluding side (Wilcoxon signed ranks test, $p=0.068$; Table 7). This suggests that the cancellous bone of the occluding side had a tendency to be more plate-like.

The occluding side showed smaller standard deviation than the non-occluding side in several indices. For example, the combined ABP+cancellous VOI apical trabecular number of occluding side had a smaller S.D. than the non-occluding side ($p=0.006$). The cancellous VOI apical trabecular number (Table 4; $p=0.046$), the ABP VOI total D.A. ($p=0.023$), the ABP VOI total SMI ($p<0.005$) and the ABP VOI middle SMI ($p=0.031$) all showed a smaller S.D. in the occluding side when compared to the non-occluding side alveolar bone.

To summarize, no significance could be found in the differences between all the investigated structural indices of the two sides of the tooth extraction specimens. However, a tendency was found to have a more anisotropic, more plate-like, denser alveolar bone with less Conn. D. and lower BS/BV with occlusal function. The occluding side alveolar bone showed evidence of a more consistent structure than the non-occluding side.

3. Comparison of MicroCT bone structural indices on tooth eruption, occlusion and tooth extraction models

The comparisons between the two models in this study showed that the normal 23-week tooth occlusion pigs and the tooth extraction models, terminated at the same age, had similar alveolar bone structure. For example, the 23-week normal occlusion pigs showed a tendency for a distinct alveolar ABP compartment from the cancellous bone in bone density and this tendency was also found in both sides of the tooth extraction specimens. Along the tooth root, the 23-week normal occlusion pigs showed a marginally decreasing ABP bone density from cervical to apical and this difference was also present in the two sides of the tooth extraction specimens.

On the other hand, both the occluding and non-occluding sides of the tooth extraction model showed significant differences to the 13-week tooth eruption model in some indices. First of all, the non-occluding side had higher ABP middle VOI BV/TV than the 13-week eruption specimens (mean of 0.69 vs. 0.43, Bonferroni correction, $p=0.022$; Table 1). Furthermore, the non-occluding side had higher Tb.Sp in cancellous apical VOI ($p=0.022$; Table 6) and lower ABP middle VOI SMI than the 13-week specimens ($p=0.012$). The occluding side also showed some differences to the 13-week tooth eruption specimens, such as higher Tb.Th in cancellous apical VOI ($p=0.011$; Table 5), lower BS/BV ($p=0.036$; Table 3) and lower cancellous apical SMI than the 13-week specimens ($p=0.044$; Table 7).

Discussion

In all of the older pigs, bone density of the ABP VOI showed a tendency to increase and to become more distinct from the cancellous bone VOI, regardless of occlusal status. All 23-week pigs showed higher ABP density than the 13-week specimens and marginally lower cancellous bone density than the corresponding ABP. In addition, the bone densities were similar between the two sides of the tooth extraction specimens. This is in contrast to previous studies in mouse in which after removal of antagonist lower molars, there was lower average bone density on the non-occluding side maxillary molar alveolar bone when compared to the occluding side.¹⁸ In the present study, bone growth in the absence of

occlusal load apparently lead to the development of a denser and more distinct ABP in the non-occluding side of the 23-week pigs.

The presence of a well-developed ABP compartment in older pigs lacking occlusion suggests that ABP development was independent of occlusal function. Instead, other sources of loading may contribute to the development of alveolar bone proper compartments. In the monkey mandible, both the working and balancing sides experienced bending or torsion during mastication.^{19, 20} Mandibular torsion or bending during mastication may promote adaptive change of alveolar bone in pigs, and occlusal function may be only a minor factor in determining alveolar bone structure. Although maxillary tooth extractions resulted in greater alveolar strains on occluding side jaws, there were still substantial alveolar strains in the non-occluding side during chewing.¹² Masseter muscle activity in conjunction with occlusion of anterior teeth may result in torsional deformation of the mandible during chewing and could account for the strain levels in alveolar bone supporting non-occluding teeth.¹³ The absence of first molar occlusal loads in 13 week pigs, in addition to lower level masseter forces during mastication and mandibular twisting, could also explain the indistinct ABP compartment in younger pigs.

The tendency to develop a distinct ABP compartment in all of the older pigs may also represent the different roles the ABP and cancellous bone play in alveolar bone load resistance and distribution. The cortical bone is believed to resist deformation while the role of cancellous bone is more stress-distributing.^{3, 6, 8} However, the ABP also functions in periodontal ligament attachment of the tooth. In this study, the ABP VOIs of all of the 23-week pigs showed marginally higher BV/TV in several regions than the corresponding VOIs of 13-week pigs. Also a decreasing tendency in BV/TV from cervical to apical was found in all of the 23-week pig ABPs whereas the corresponding regions in the 13-week pigs showed no regional difference. A numerical model of loading at the human first maxillary molar suggested that occlusal stress would concentrate mainly on cervical cortical bone around the tooth root.²¹ The tendency for a denser and apically decreasing ABP density in 23-week pigs could indicate that the ABP responded to occlusal loading with increased bone apposition in the 23-week pigs, especially in the cervical ABP. In the absence of occlusion, fortification of the ABP may promote tooth attachment and resistance to torsional forces during mastication.

The main function of cancellous bone is different from cortical bone, in that cancellous bone distributes applied load to adjacent cortical bone; thus, the structural fortification of cancellous bone could be expressed in ways other than increase of bone density. For example, a tendency toward structural consolidation was found in the cancellous bone of all of the 23-week specimens in that the cancellous bone VOIs showed marginally less connectivity, less trabecular number and higher trabecular thickness and separation in several regions when compared with the 13 week pigs. This finding matches previous studies that the fine trabecular pattern of the distal radius in young children coarsens with age, and the trabeculae increased in thickness and decreased in numbers when maturing.^{1, 2, 7, 10, 11} The alveolar cancellous bone structure tended to develop into principal struts to replace the initial fine trabeculae after growth or occlusal loading. This arrangement could indicate that the cancellous bone responded to growth and occlusal function with structural consolidation in the 23-week pigs for fortification. In the absence of occlusion, structural consolidation of the cancellous bone may promote resistance to torsional forces within the mandible.

In contrast to expectations, alveolar bone did not show changes in anisotropy distal to M₁ in specimens with occlusal function. In cancellous bone, trabeculae are proposed to reduce stress concentrations by distributing strain within the trabecular network.^{6, 22} The trabeculae

are thought to adapt their orientation to be mainly along the direction of applied load in order to provide better support in this direction.⁶ In a study using microCT, pig vertebral and proximal tibial cancellous bone was found to have increased anisotropy with age. The pig's increasing body weight during growth was suggested to increase load to the vertebrae and tibia and to induce the change in anisotropy.⁷ In this study, cancellous bone from 13-week tooth eruption pigs showed similar D.A. with all of the 23-week pigs; thus anisotropy did not increase in the older pigs with or without occlusal function. One possibility is that the experimental period in this study may not be long enough for a change in alveolar bone to be identified. In the previous microCT study, the anisotropy of pig vertebral and proximal tibial cancellous bone only started to increase after 23 weeks old.⁷ Thus, anisotropic change of alveolar bone in the tooth occlusion model may have become more accentuated after a longer period of function.

After growth, the pig alveolar bone showed a tendency to change from a more rod-like into a more plate-like structure. The SMI was lower (more plate-like) in 23-week normal occlusion ABP middle VOI than 13-week specimens, and both sides of the tooth extraction model showed the same tendency when compared with the 13-week specimens. This matches previous findings that with aging, human lumbar bone transitions from a rod-like to a more plate-like structure.⁴ The negative values of SMI, which could come from a very dense and concave plate-like structure,²³ were common in all of the 23-week alveolar bone. These findings indicate that the alveolar bone structure consolidated from the initial fine rod-like structure into thicker, more plate-like structure after growth. The plate-like structure is found to provide enhanced bone strength relative to the rod-like structure.⁹ Therefore, the alveolar bone transformed into a more plate-like structure that can provide better strength after growth, with or without occlusion.

The decreased standard deviations found in the average BV/TV of 23-week normal occlusion specimens demonstrated that the alveolar bone structure developed into a more consistent framework when compared with 13-week bone that had a wider range of BV/TV values. Structural consolidation into a more consistent bone structure was a clear tendency for older bones with occlusal function. This may indicate that there is a minimum requirement in bone structure needed to resist the applied loads during mastication in older bones. Therefore, the alveolar bone indices appeared at a similar range after growth and occlusal function. Before the introduction of occlusal loading and at a younger age, the alveolar bone structure is of less importance so the range of structure patterns can differ without causing significant consequences. This consolidation tendency also matches the concept of 'load predictability' in which bone is hypothesized to develop into a form with a more predictable load pattern.²⁴ Distinct locations of bone are reinforced in structure to guide future bone strain in predictable pathways. Therefore the chance of developing dangerous strain levels in unfortified structural regions can be minimized. The development of a more consistent and consolidated structure of alveolar bone after growth and occlusal loading may indicate that during mastication the alveolar bone experienced significant loading and required reinforcement. After growth, the ABP developed into a denser and more plate-like structure while the cancellous bone developed into a more consolidated structure, regardless of occlusal status. After growth and occlusal loading, the ABP and cancellous bone developed into a more consistent structure. These changes may provide better support to the tooth during occlusal loading and mandibular torsion.

Although the differences were not significant between the two sides of the tooth extraction model, when occlusal function was emphasized, a tendency was found for occlusal force to demonstrate an impact on the alveolar bone structure. For example, the occluding side showed a tendency toward denser cancellous bone (higher cancellous total VOI BV/TV), fewer trabeculae (lower cancellous total connectivity, Conn. D.); and lower bone surface

(BS/BV), lower trabecular number (Tb.N) and higher trabecular separation (Tb.Sp) in the middle cancellous VOI. The occluding side also showed a tendency toward a more anisotropic (higher degree of anisotropy (D.A.), middle cancellous VOI), and more plate-like cancellous bone structure (lower cancellous total SMI) than the non-occluding side ($p=0.068$ for all). The occluding side also showed significantly lower standard deviations of some indices than the non-occluding side. Occlusal force alone caused the alveolar bone to develop toward a more consolidated, more anisotropic and more consistent structure. Occlusal function may have effects on the supporting alveolar bone, but the sample sizes are too small in this study to demonstrate significance. An additional possibility is that the experimental period in this study may not be long enough for a change in alveolar bone to be identified.

This study provides new insight into how different regions of alveolar bone respond to occlusal loading. Alveolar bone change along the developing tooth root was compared relative to a stable reference point, i.e. the root bifurcation. For example, when comparing the cervical third cancellous bone of 13-week to the cervical third cancellous bone of 23-week specimens, the structural change before and after occlusal function of alveolar cancellous bone closest to the root bifurcation was analyzed. This root bifurcation reference point also helped with the following comparisons of the middle and apical thirds; thus changes in the alveolar bone along the whole tooth root before and after occlusal loading could be revealed. This method provides a more detailed site-specific description of alveolar bone adaptation under occlusal loading rather than the general description of the change of whole alveolar bone surrounding the tooth root in other studies.^{2, 18}

In summary, this study demonstrated that both growth and occlusal loading could promote a distinct ABP compartment formation surrounding the tooth root; however, other sources of mandibular loading may also be involved. In the comparisons of 23 week normal occlusion specimens and the two sides of the tooth extraction specimens to the 13 week tooth eruption specimens, bone growth and mandibular twisting during chewing were suggested to lead to a tendency for a denser and distinct ABP development. Comparisons between tooth eruption 13 week and normal occlusion 23 week pigs showed that the anisotropy of alveolar cancellous bone did not increase with occlusion and may need a longer period of time to become more accentuated. When occlusal loading was emphasized in the tooth extraction model, the occluding side alveolar bone showed a trend toward a more consolidated and consistent structure than the non-occluding side. In cancellous middle VOI, the D.A. of the occluding side showed a tendency to be higher than the non-occluding side; however a similar increase was not noted in the cancellous VOI of the normal occlusion 23 week vs. 13 week pigs. The observed bias toward chewing, as well as the significantly higher alveolar strains on the occluding side suggest greater usage of the occluding side of the tooth extraction model, and may account for the tendency toward anisotropic cancellous bone.

Limitations of the study include the small size of the sample, the specificity of the alveolar region examined and the time period for alveolar bone growth following tooth extractions. The small sample sizes studied limit the interpretation of the specific roles of growth and occlusal loading to alveolar structure and additional studies are required to elucidate the relative contributions of each. Over-generalization of these study results to other alveolar bone region should be avoided since mandibular bone density is different from that of the maxilla, and even in the same jaw, the bone density is not consistent in all areas.²⁵ Also a pig model older than 23 weeks could further clarify the impact of occlusal loading on the anisotropic change of alveolar cancellous bone.

Acknowledgments

The authors wish to thank the University of Washington School of Dentistry Dept. of Orthodontics, Dr. Susan W. Herring, Dr. Katherine L. Rafferty for their contributions to this research and the lab of Dr. Ted Gross, Dept. of Orthopedics and Sports Medicine, University of Washington for MicroCT analysis. This work was supported by NIH/NIDCR DE 015815.

Abbreviations

M₁	mandibular first molar
ABP	alveolar bone proper
microCT	microcomputed tomography
M¹	maxillary first molar
DP⁴	the maxillary deciduous fourth premolar

References

1. Saffar JL, Lasfargues JJ, Cherruau M. Alveolar bone and the alveolar process: the socket that is never stable. *Periodontology* 2000 1997;13:76–90. [PubMed: 9567924]
2. Mavropoulos A, Kiliaridis S, Bresin A, Ammann P. Effect of different masticatory functional and mechanical demands on the structural adaptation of the mandibular alveolar bone in young growing rats. *Bone* 2004;35(1):191–197. [PubMed: 15207756]
3. Frost HM. Bone “Mass” and the “Mechanostat”: a proposal. *The Anatomical Record* 1987;219:1–9. [PubMed: 3688455]
4. Kneissel M, Roschger P, Steiner W, Schamall D, Kalchauer GA, Boyde A, et al. Cancellous bone structure in the growing and aging lumbar spine in a historic nubian population. *Calcif Tissue Int* 1997;61:95–100. [PubMed: 9312401]
5. Korstjens CM, Geraets WGM, Van Ginkel FC, Prahl-Andersen BL, Van Der Stelt PF, Burger EH. Longitudinal analysis of radiographic trabecular pattern by image processing. *Bone* 1995;17:527–532. [PubMed: 8835306]
6. Wolff, J. The law of bone remodeling. Springer-Verlag; Berlin: 1892.
7. Tanck E, Homminga J, Van Lenthe GH, Huiskes R. Increase in bone volume fraction precedes architectural adaptation in growing bone. *Bone* 2001;28(6):650–654. [PubMed: 11425654]
8. Currey, JD. Bones, structure and mechanics. Princeton University Press; Princeton: 2002. p. 436
9. Liu XS, Sajda p, Saha PK, Wehrli FW, Bevil G, Keaveny TM, et al. Complete volumetric decomposition of individual trabecular plates and rods and Its morphological correlations with anisotropic elastic moduli in human trabecular bone. *Journal of Bone and Mineral Research* 2008;23(2):223–235. [PubMed: 17907921]
10. Nafei A, Danielsen CC, Linde F, Hvid I. Properties of growing trabecular ovine bone Part 1: Mechanical and physical properties. *J Bone Joint Surg (Br)* 2000a;82-B:910–20.
11. Nafei A, Kabel J, Odgaard A, Linde F, Hvid I. Properties of growing trabecular ovine bone Part 2: Architectural and mechanical properties. *J Bone Joint Surg (Br)* 2000b;82-B:921–7.
12. Yeh K, Popowics T, Rafferty K, Herring S, Egbert M. The effects of tooth extraction on alveolar bone biomechanics in the miniature pig, *Sus scrofa*. *Arch Oral Biol*. 2010 in press. 10.1016/j.archoralbio.2010.05.014
13. Liu ZJ, Herring SW. Masticatory strains on osseous and ligamentous components of the jaw joint in miniature pigs. *J Orofacial Pain* 2000;14:265–278. [PubMed: 11203760]
14. Laib A, Rueggsegger P. Calibration of trabecular bone structure measurements of in vivo three-dimensional peripheral quantitative computed tomography with 28-μm-resolution microcomputed tomography. *Bone* 1999;24(1):35–39. [PubMed: 9916782]

15. Ding M, Odgaard A, Christian Danielsen C, Hvid I. Mutual associations among microstructural, physical and mechanical properties of human cancellous bone. *J Bone Joint Surg (Br)* 2002;84-B: 900–7. [PubMed: 12211688]
16. Hildebrand T, Ruegsegger P. A new method for the model-independent assessment of thickness in three-dimensional images. *Journal of Microscopy* 1997;185(Pt 1):67–75.
17. Ciarelli TE, Fyhrie DP, Schaffler MB, Goldstein SA. Variations in three-dimensional cancellous bone architecture of the proximal femur in female hip fractures and controls. *Journal of Bone and Mineral Research* 2000;15(1):32–40. [PubMed: 10646112]
18. Ejiri S, Toyooka E, Tanaka M, Anwar RB, Kohno S. Histological and histomorphometrical changes in rat alveolar bone following antagonistic tooth extraction and/or ovariectomy. *Arch Oral Biol* 2006;51(11):941–950. [PubMed: 16814741]
19. Hylander WL. Mandibular function in *Galago crassicaudatus* and *Macaca fascicularis*: an in vivo approach to stress analysis of the mandible. *J Morph* 1979;159:253–296. [PubMed: 105147]
20. Hylander, WL. Craniofacial biology. Center for Human Growth and Development; Ann Arbor, Mich: 1981. Patterns of stress and strain in the macaque mandible.
21. Nomoto S, Matsunaga S, Ide Y, Abe S, Takahashi T, Saito F, et al. Stress distribution in maxillary alveolar ridge according to finite element analysis using micro-CT. *Bull Tokyo Dent Coll* 2006;47(4):149–156. [PubMed: 17510544]
22. Hayes, WC.; Snyder, B. Toward a quantitative formulation of Wolff's law in trabecular bone. In: Cowen, SC., editor. Mechanical properties of bone. The American Society of Mechanical Engineers; New York: 1981. p. 43–68.
23. Hildebrand T, Laib A, Müller R, Dequeker J, Rüegsegger P. Direct three-dimensional morphometric analysis of human cancellous bone: microstructural data from spine, femur, iliac crest, and calcaneus. *J Bone Miner Res* 1999;14(7):1167–1174. [PubMed: 10404017]
24. Bertram JEA, Biewener AA. Bone curvature: Sacrificing strength for load predictability? *Journal of Theoretical Biology* 1988;131(1):75–92. [PubMed: 3419194]
25. Park HS, Lee YJ, Jeong SH, Kwon TG. Density of the alveolar and basal bones of the maxilla and the mandible. *Am J Orthod Dentofacial Orthop* 2008;133(1):30–37. [PubMed: 18174068]

Biographies

Kuang-Dah Yeh, DDS, MDS, PhD, Kuang-Dah Yeh graduated from the PhD program of Department of Oral Biology, School of Dentistry, University of Washington in August 2009. Yeh now serves in the Hualien Armed Forces General Hospital, Hualien, Taiwan.

Tracy Ellen Popowics, PhD, Tracy Ellen Popowics is an Associate Professor in the Department of Oral Biology, School of Dentistry, University of Washington.

Figure 1.

Lateral cephalogram showing the development of first molar occlusion on the non-extraction side. Orientation: from left to right: posterior teeth to anterior teeth. A: The mesial cusps of M^1 and M_1 have begun to penetrate the overlying mucosa (13 weeks of age). B and C: Drawings of tooth contours. Solid line: ipsilateral M^1 and M_1 . Dashed line: M^1 and M_1 of the other side.

Figure 2. Lateral cephalogram showing first molar occlusion. Orientation: from left to right: posterior teeth to anterior teeth. A: Non-extraction side distal cusps of M¹ were almost fully occluded with M₁ (6 weeks after tooth extraction, 23 weeks old). B and C: Drawings of tooth contours.

Figure 3. Lateral cephalogram of an erupting M₁ showing VOI's of study. Arrow: alveolar bone proper. *: cancellous bone. B: Outline of the VOI's between the M₁ distal root and M₂ crypt (Not drawn to scale). C: Individual VOI's. 1: ABP cervical, 2: ABP middle, 3: ABP apical, 4: Cancellous cervical, 5: cancellous middle, 6: cancellous apical, 7: cortical layer of M₂ crypt, which was excluded from analysis.

Figure 4. Alveolar bone proper (ABP) and cancellous bone total VOI's showing the alveolar bone distal to the distolingual root in an erupting M₁ (A), an occluding M₁ (B), and in a pig with a unilateral maxillary DP⁴ and M¹ extractions, the occluding side M₁ (C) and non-occluding side M₁ (D).

Figure 5. BV/TV (%) of ABP total vs. cancellous bone total VOI. The 23-week ABP total VOI showed marginally higher BV/TV than 13 weeks (mean 0.66 vs. 0.49, $p=0.053$).

Figure 6. BV/TV (%) of ABP from cervical to apical. Bone volume density tended to decrease from cervical toward apical in older pigs.

Figure 7. Connectivity density (1/mm³) of cancellous bone total VOI's. Marginally higher connectivity density in 13-week vs. 23-week (Mean 4.68 vs. 2.67, $p=0.053$). Connectivity density was similar between occluding and non-occluding side alveolar bone of the tooth extraction model.

Figure 8.
Degree of Anisotropy of cancellous bone. No significant differences were observed in degree of anisotropy between different groups.

Table 1

Means and standard deviations of ABP VOI bone density (BV/TV, %).

VOI	13-week	23-week	Non-occluding side	Occluding side
ABP total	0.49±0.14 ¹	0.66±0.03 ¹	0.65±0.09	0.64±0.04
ABP cervical	0.52±0.17	0.75±0.03 ^{2, 3}	0.75±0.10 ⁴	0.73±0.10 ⁶
ABP middle	0.43±0.17 ^{8*}	0.64±0.04 ²	0.69±0.11 ^{5, 8}	0.63±0.07 ⁷
ABP apical	0.51±0.21	0.58±0.09 ³	0.51±0.10 ^{4, 5}	0.55±0.02 ^{6, 7}

¹
p=0.053,²⁻⁷
p=0.068,⁸
Bonferroni correction,

p=0.022*

Table 2

Means and standard deviations of cancellous bone VOI bone density (BV/TV, %).

VOI	13-week	23-week	Non-occluding side	Occluding side
Cancellous bone total	0.58±0.17	0.46±0.03	0.56±0.06 ^I	0.59±0.06 ^I
Cancellous bone cervical	0.60±0.17	0.50±0.13	0.62±0.06	0.63±0.07
Cancellous bone middle	0.57±0.17	0.46±0.07	0.57±0.05	0.56±0.07
Cancellous bone apical	0.65±0.25	0.51±0.07	0.51±0.09	0.60±0.10

^I
p=0.068

Table 3Means and standard deviations of ABP VOI BS/BV (mm²/mm³).

VOI	13-week	23-week	Non-occluding side	Occluding side
ABP total	7.14±2.34	4.56±0.15	5.04±1.47	5.19±1.02
ABP cervical	6.19±2.39	3.93±0.34	4.16±1.53	4.36±1.28
ABP middle	8.42±2.69 ¹	4.81±0.25 ^{1*}	4.94±1.66	5.62±1.47
ABP apical	8.11±3.63 ²	5.07±0.82	6.46±1.69	6.11±0.76 ^{2*}

¹ p=0.014*,² Bonferroni correction,

* p=0.036

Table 4

Means and standard deviations of cancellous bone VOI trabecular numbers (Tb.N, 1/mm).

VOI	13-week	23-week	Non-occluding side	Occluding side
Cancellous bone total	2.21±0.98	1.37±0.20	1.69±0.13	1.68±0.08
Cancellous bone cervical	2.62±0.76 ¹	1.66±0.36 ^{1*}	2.00±0.22	2.00±0.19
Cancellous bone middle	2.46±1.05	1.41±0.33	1.87±0.13 ²	1.71±0.16 ²
Cancellous bone apical	3.42±2.07	1.49±0.22	1.55±0.20	1.70±0.07

¹
p=0.014*,

²
p= 0.068

Table 5

Means and standard deviations of cancellous bone VOI trabecular thickness (Tb.Th, mm).

VOI	13-week	23-week	Non-occluding side	Occluding side
Cancellous bone total	0.33±0.03	0.37±0.00	0.39±0.06	0.41±0.06
Cancellous bone cervical	0.31±0.07	0.34±0.05	0.40±0.08	0.39±0.06
Cancellous bone middle	0.30±0.06	0.36±0.00	0.36±0.04	0.39±0.07
Cancellous bone apical	0.30±0.05 ^{1, 2}	0.38±0.01 ^{1*}	0.38±0.06	0.43±0.07 ^{2*}

¹
p=0.014*,

²
p=0.011*

Table 6

Means and standard deviations of cancellous bone VOI trabecular separation (Tb.Sp, mm).

VOI	13-week	23-week	Non-occluding side	Occluding side
Cancellous bone total	0.46±0.19	0.66±0.10	0.52±0.05	0.54±0.04
Cancellous bone cervical	0.35±0.10	0.58±0.17	0.40±0.06	0.44±0.09
Cancellous bone middle	0.39±0.18 ¹	0.69±0.17 ^{1*}	0.47±0.05 ²	0.54±0.08 ²
Cancellous bone apical	0.32±0.18 ³	0.57±0.06	0.59±0.08 ^{3*}	0.53±0.07

¹
p=0.050*,²
p=0.068,³
p=0.022*

Table 7

Means and standard deviations of cancellous bone VOI SMI.

VOI	13-week	23-week	Non-occluding side	Occluding side
Cancellous bone total	-0.58±1.55	-0.54±0.07	-1.59±0.83 ¹	-2.24±0.93 ¹
Cancellous bone cervical	-1.09±1.83	-1.27±1.53	-2.19±1.39	-2.89±1.43
Cancellous bone middle	-0.17±1.26	-0.62±0.71	-1.55±0.73	-1.69±1.02
Cancellous bone apical	0.26±1.26 ²	-0.79±0.74	-0.88±0.98	-2.09±1.28 ²

¹
p=0.068²
p=0.044*