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Alveolar ridge reduction after tooth extraction in adolescents: an animal study

Zongyang Sun, DDS, PhD, Susan W. Herring, PhD, Boon Ching Tee, BS, and Jordan Gales, BS

Abstract

Objective—The mechanism for tooth extraction induced residual alveolar ridge reduction (RRR) during adolescence is poorly understood. This study investigated the alveolar bone morphology, growth, resorption and functional loading at normal and extraction sites using an adolescent pig model.

Design—Sixteen 3-month-old pigs were divided into two groups – immediate post-extraction (IE) and 6-week post-extraction (SE). The IE group received an extraction of one deciduous mandibular molar, immediately followed by a final experiment to record masseter muscle EMGs and strains from the buccal surface of the extraction and contralateral non-extraction sites during function (mastication). The SE group was given the same tooth extraction, then kept for 6 weeks before the same final functional recording as the IE group. Both groups also received baseline (pre-extraction) EMGs and fluorescent vital stains 10 and 3 days before the final functional recording. Immediately after the final functional recording, animals were euthanized and alveolar bone specimens from extraction and contralateral non-extraction sites were collected and used to analyze alveolar bone morphology, apposition and resorption based on fluorescent and hematoxylin and eosin stained histological sections.

Results—At control sites (IE-extraction, IE-non-extraction and SE-non-extraction), the alveolar ridges grew gingivally and buccally. Bone formation characterized the buccal surface and lingual bundle bone, whereas resorption characterized the lingual surface and buccal bundle bone. The SE-extraction sites showed three major alterations: convergence of the buccal and lingual gingival crests, loss of apposition on the lingual bundle bone, and decelerated growth at the entire buccal surface. These alterations likely resulted from redirected crestal growth as part of the socket healing process, loss of tongue pressure to the lingual side of the teeth which normally provides mechanical stimulation for dental arch expansion, and masticatory underloading during the initial post-extraction period, respectively.

Conclusions—These data indicate that the initial phase of RRR in adolescents is a product of modified growth, not resorption, possibly because of decreased mechanical stimulation at the extraction site.

Keywords

Bone remodeling; alveolar bone; tooth extraction; bone strain; pig

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Corresponding author: Zongyang Sun, Rm 4088 Postle Hall, 305 W 12th Ave, Columbus, OH 43210, sun.254@osu.edu, Phone: 614-247-7944; Fax: 614-688-3077.

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INTRODUCTION

A common sequela of tooth extraction is a progressive reduction of the residual alveolar ridge (RRR). In both humans (1–7) and animals (8–11), the reduction tends to be greater in the transverse than in the vertical dimension, and transverse RRR is often more prominent at the buccal (labial) than at the lingual side (12, 13). Typically RRR proceeds rapidly during the first several months after tooth extraction (7, 14), then gradually slows. While most clinical studies on RRR have focused on edentulous alveolar ridges in senior patients, this problem does occur at isolated single-tooth extraction sites in adolescent patients and exhibits similar clinical manifestations (7, 15, 16).

At present, the etiological mechanisms of RRR are not completely clear. Based on radiographic observations of alveolar bone dimensional changes in completely edentulous patients, Atwood (17, 18) proposed that RRR is mainly a biomechanical problem. More specifically, he suggested that functional loading at the edentulous ridge was diminished because of the loss of direct occlusal contact, which subsequently induced bone resorption. This theory of disuse atrophy is now commonly considered responsible for resorption of the residual alveolar process. Alveolar bone resorption does occur reliably after tooth extraction in adult humans (12, 19) and dogs (20). The mechanical aspect of this theory, however, has never been substantiated. Specifically, it remains uninvestigated whether the local alveolar ridge is functionally unloaded after tooth extraction. Recently, it was found that alveolar bone lacking direct occlusal contact still sustains mechanical loading (21), suggesting that the disuse atrophy mechanism of RRR is open to question.

For a single extraction site in adolescent humans or animals, it is even more uncertain whether the disuse atrophy theory is applicable. In this population, not only does the mechanical change after tooth extraction remain unknown, but the question of whether RRR results from bone resorption is also unanswered. During adolescence, the alveolar bone is still growing, vertically to adapt to jaw growth (22, 23) and tooth eruption, and transversely to accommodate dental arch expansion (24). Conceivably, RRR in adolescents could result from growth reduction rather than bone resorption. This conjecture, if confirmed, would suggest that RRR in adolescents has a different biological mechanism than that in adults despite similar clinical manifestations.

The main purpose of this study was to elucidate the mechanisms of RRR induced by tooth extraction in adolescents. An adolescent age is targeted because of several reasons. Tooth loss due to trauma (25–27) and dental caries is common during adolescence. A measure to prevent RRR effectively in adolescent patients after tooth extraction has not been established, which is at least partly due to a lack of clear understanding of its mechanism. Furthermore, implant restoration for the residual edentulous site, which may break the progression of RRR, often needs to be deferred for years until jaw growth is complete (22), during which time RRR may have become extensive (28).

A pig model was used to measure bone strain during mastication and to assess alveolar bone morphology, growth, resorption and remodeling. The immediate responses to extraction were compared to the responses after 6 weeks of adaptation. We hypothesized that bone strain would be lower (underloading) at the extraction site than at the contralateral non-extraction site at both time points. Further, although the pattern of bone growth immediately after extraction should be unchanged, we hypothesized that the 6-week sample would show reduced growth compared to the non-extraction site due to chronic underloading.

MATERIALS AND METHODS

Animals

Sixteen 3-month-old domestic pigs (*Sus scrofa*) were obtained from a local farm and divided into two groups (Table 1). Animals were similar in size as well as age, and all were healthy; they were obtained in groups of 2 or 4 representing 5–6 different litters. The immediate post-extraction (IE) group was used to examine the immediate effect of tooth extraction on functional loading, and to assess the baseline status of bone growth/resorption before any bone adaptation to extraction could occur. The six-week post-extraction (SE) group was used to examine the changes of functional loading and bone growth/resorption at a time that RRR is rapid (7, 14). In both groups, masseter muscle electromyography (EMG) was recorded at baseline (pre-extraction) and at the final functional recording, during which strain gage readings from the buccal alveolar bone surface (detailed below) were also collected. The buccal surface was targeted for strain measurements not only because of its accessibility but also because it is more severely affected than the lingual side (12, 13). All pigs also received vital fluorescent dyes to label mineral apposition 10 and 3 days before the final functional recording of EMG and bone strain (Table 1). Immediately after the final functional recording, the pigs were euthanized and specimens were collected to analyze bone morphology, apposition and resorption. All live animal procedures were approved by the Ohio State University Institutional Animal Care and Use Committee.

Pre-study power analysis determined that a sample size of 5/group was required to detect a hypothesized 3-fold masticatory strain difference between the extraction and non-extraction sites with 80% power. Because of strain gage failures, two and four extra animals were added to the IE and SE groups, respectively, to satisfy this requirement. Altogether, 11 pigs were included in bone strain analysis and 16 animals were included in histological analysis.

Extraction surgery and post-surgical care

The pigs were sedated (Ketamine, 1.1 ml/Kg and Dexdomitor, 0.25 ml/Kg, intramuscular injection) and anesthetized (2–3% isoflurane with 2–5% oxygen) by intubation. Either the left or right mandibular second deciduous molar (dm2), was extracted while the contralateral side was left intact. Among the 16 animals, 9 had extractions on the right and 7 on the left side. The dm2 normally completes eruption by 4 weeks of age and exfoliates at 1–1.5 years (29), and therefore its duration in occlusion is reasonably comparable to a deciduous molar in humans. After dm2 was identified in the oral cavity, a slow speed dental handpiece with a carbide fissure bur was used to section it into mesial and distal halves. Then the two halves, one at a time, were slowly loosened from the socket and removed using extraction forceps. Care was taken to minimize trauma to the alveolar bone and to avoid root fracture. After extraction, the socket was cleaned and thoroughly irrigated with sterile normal saline. A gauze pad was pressed over the extraction wound for 10 minutes to stop bleeding.

In the IE group, immediately after tooth extraction, strain gage placement (detailed below) was carried out. In the SE group, after tooth extraction, the pigs recovered from anesthesia and were returned to the housing unit, where they were monitored for pain, bleeding and infection for the first 5 days after the extraction. Oral amoxicillin (10 mg/Kg) was administered twice daily for 3 days for infection control. For pain control, a dose of banamine (2.2 mg/Kg) was injected intramuscularly prior to recovery from anesthesia, then as needed during the post-extraction monitoring period. The pigs were restricted to a soft diet (pig chow softened with water, with the consistency of milk-soaked cereal) for 3 days and then fed standard pig chow (pellets with a texture similar to bran crackers).

Vital bone labeling

Fluorescent dyes calcein and alizarin complexone (Sigma-Aldrich, St Louis, MO) were prepared (12.5 mg/Kg, in 5 mg/ml normal saline) following procedures detailed previously (30) and injected intravenously 10 and 3 days, respectively, prior to the terminal strain procedure.

Final functional recording: alveolar bone strain and EMG device placement and recording

Alveolar bone strain was measured from the buccal alveolar surface of the extraction and contralateral non-extraction sites. The pig was sedated (Ketamine, 1.1 ml/Kg and Dexdomitor, 0.25 ml/Kg, intramuscular injection) and then anesthetized (2–3% isoflurane with 2–5% oxygen). The mucogingival tissue covering the buccal alveolar surface was reflected by a horizontal incision at the gingival sulcus, and two vertical incisions were made mesial and distal to the dm2 site. The bone surface was exposed after carefully removing the overlying periosteum using a periosteal elevator without damaging the bone. Then the surface was treated with conditioner and neutralizer (Vishay Measurements Group, Raleigh, NC) and dried with air following a standard procedure (30). While keeping the bone surface isolated and dry, a rectangular strain gage (C2A-06-030WW-350, Vishay Measurements Group, Raleigh, NC) was affixed to it using a strain gage adhesive (Vishay). All strain gages were calibrated according to the manufacturer's instructions prior to the procedure. After strain gage placement, the mucogingival flap was sutured to cover the strain gage and the bone surface (Maxon™ 3-0, Covidien, Mansfield, MA). As shown in Fig. 1A, the six lead wires of each strain gage, joined together into 3 cables (one cable per channel), were led through a submucosal-subcutaneous tunnel to exit at the inferior mandibular border, where they were secured to the skin by sutures (Maxon™ 2-0). The strain gage cables were led to a neck collar, further secured by 3 more stitches (Maxon™ 2-0 sutures) to the skin and then tied to the collar. These measures were taken to ensure that no strain gage parts were exposed directly to the oral cavity and to protect the strain gage wires and cables from breakage during live recording. After strain gage placement, the facial hair covering the right and left masseter regions was removed. After cleaning the skin with 70% ethanol, pairs of surface EMG electrodes (Biopac Systems, Inc., Goleta, CA) were attached as detailed previously (30). Tissue adhesive (Vetbond™, 3M™, St. Paul, MN) was applied to the corners of the electrode pads to enhance attachment.

After device placement and analgesic administration, the pig was awakened and fed a soft diet (softened pig chow and apple sauce). All pigs began eating within 20 minutes of the cessation of anesthesia. A soft diet was used to simulate the food choice recommended for human patients after tooth extraction or periodontal surgery.

When the pig was eating, the strain gage and EMG cables were connected to an acquisition system (Biopac MP150 with Acqknowledge III software, Biopac Systems). After balancing the strain gages to confirm their functionality and baseline level, recordings of strain and EMG were collected for 10 minutes. If EMG electrodes became detached, they were reattached, but broken strain gage wires could not be repaired while the pig was eating. In the event of bilateral strain gage failure, the recording was stopped and the pig was sedated for euthanasia. Euthanasia was always conducted within 40 minutes of the completion of strain gage placement.

Euthanasia, specimen harvesting, processing and analysis

After strain and EMG recording, the pigs were sedated, followed by euthanasia by IV injection of pentobarbital. After euthanasia, two alveolar bone specimens were collected from each extraction and non-extraction site for analyses. One specimen (the mesial 1/3 of the site) was fixed in Prefer® (Anatech LTD, Battle Creek, MI), dehydrated and embedded

in Micro-Bed resin (EMS, Hatfield, PA), cut into 30–50 μm thick coronal (buccal-lingual) sections using a diamond microtome (Leica SP 1600, Leica Microsystems, Germany) and mounted on glass slides (VectaMount®, Vector Laboratories, Burlingame, CA). The other specimen (the central 1/3) was fixed, decalcified (15% formic acid and 2.6% sodium formate), dehydrated, cut into 5 μm thick coronal (buccal-lingual) sections and stained with hematoxylin and eosin (HE). Together, the two specimens constituted the buccal surface area covered by the strain gage during strain recording (Fig. 1A).

HE stained sections were viewed under a light microscope (Carl Zeiss MicroImaging, Inc., NY) for general features of bone formation and resorption. Undecalcified sections were kept unstained and viewed under a fluorescent microscope (Carl Zeiss MicroImaging) to quantify mineral apposition. For transverse surface growth, images were captured from the gingival, middle and apical levels for the measurements of two parameters: transverse mineral apposition rate (MAR_T) and transverse mineral apposition zone (MAZ_T) using ImageJ software (National Institutes of Health, Bethesda, MD) (Fig. 1B, D). For vertical growth, images of buccal and lingual crests were captured and used to measure vertical mineral apposition rate (MAR_V) (Fig. 1D). To select the location for measurements objectively, an array of parallel test lines was superimposed (a functional module available in ImageJ). The density of the test lines was determined after a pilot test which confirmed measurement accuracy and efficiency. To assess intra-cortical bone remodeling, images from the lingual plate (remodeling was rare in the buccal plate) were captured for the measurement of two parameters: osteonal mineral apposition rate (MAR_O) and bone formation rate (BFR) (Fig. 1C, D). MAR_O was directly measured, whereas BFR was derived from several preliminary measurements including single-labeled and double-labeled surfaces (sLS and dLS), and bone volume (BV) (Fig. 1D). A test grid, with the density determined by pilot tests, was superimposed on each image for the procurement of all measurements. For each specimen, two sections were analyzed and the average of the two was used for statistical analysis.

EMG and strain data analysis

EMG and strain signals were digitized using Acqknowledge III software (Biopac Systems). Because the position and recording field of the EMG electrodes could not be standardized, the amplitude of the EMG signals could not be used to estimate muscle force. Therefore, the EMG amplitude was not compared between the right and left side masseter muscles, nor between baseline and post-extraction recordings. Rather, EMG recordings were analyzed qualitatively to determine the chewing side, which is characterized by a later and stronger power stroke signal than the balancing side (31).

The three channels from each rosette strain gage were used to calculate the maximum shear strain (sum of the absolute values of principal compressive and tensile strains) to reflect peak masticatory loading applied to the bones (32). For successful strain recordings, 2–3 chewing sequences (11–45 cycles/sequence) were analyzed and the average shear strain magnitude among sequences was obtained.

Statistical analysis

The IE and SE groups were analyzed separately to examine whether the extraction site differed from the contralateral non-extraction site. After verifying that buccal bone strain did not differ between working and balancing sides (paired t-tests), the chewing sides were combined to examine for extraction/non-extraction site differences. Two-sample, rather than paired, t-tests were used because measurements from several animals were unpaired. Quantitative bone growth (mineral apposition) measurements were analyzed by repeated measures analysis of variance (ANOVA) methods, so that measurements from the same animal were treated as repeated rather than independent measurements. Specifically, vertical

crestal growth (MAR_v) was compared by two-way repeated ANOVAs with extraction status (extraction/non-extraction) and alveolar ridge location (buccal/lingual) treated as within-subject factors. The main effects and interaction of these two factors were assessed. After finding that transverse bone surface growth (MAR_T and MAZ_T , buccal only) did not differ among the gingival, middle and apical levels (one-way repeated ANOVA), measurements from all three levels were averaged for repeated measures multivariate ANOVA (MANOVA) with extraction status examined as a within-subject factor, group (SE or IE) as a between-subject factor, and MAR_T and MAZ_T as dependent variables. MANOVA was also used to test the two intra-cortical remodeling measurements (MAR_O and BFR, lingual only).

RESULTS

Chewing pattern and alveolar bone strain

Post-extraction EMGs demonstrated no change in chewing side or frequency from pre-extraction records in either group (Fig. 2A). Overall, pigs chewed equally on the extraction and non-extraction sides (Fig. 2B).

An example of masticatory strain recording from one animal is shown in Fig. 2C. Summarized shear strain magnitude is presented in Fig. 2D and Table 2. In the IE group, strain tended to be lower at the extraction site than at the non-extraction site (2-sample t-test, $p = 0.086$, $n=5$). If one extreme value of 126 microstrain from the extraction site is excluded, p becomes 0.029. This extreme value is identified as an outlier from its effect on the coefficient of variation. With this value included, the IE-extraction site measurements showed a coefficient of variation of 1.062, and with it excluded, the coefficient of variation was 0.698 (Table 2). Previous studies on bone surface masticatory strain measurements from the pig report coefficients of variation values in a range of 0.2–0.7 (21, 30, 33). In the SE group, strain was nearly identical between the extraction and non-extraction sites (2-sample t-test, $p = 0.846$) and the coefficient of variation was less than 0.7 at both sites.

Alveolar bone morphology, growth and resorption

Qualitatively, the IE non-extraction, IE extraction and SE non-extraction sites showed some similar features (Figs. 3A and 4A). In all of these control (unadapted to extraction) sites, the contour of the buccal surface had an obtuse deflection angle between the gingival and middle thirds. The lingual surface contour showed more gradual deflection. Combined, the surface contours of the buccal and lingual surfaces at the gingival 1/3 were nearly parallel to each other. A second common feature was prominent vertical crestal growth at the buccal and lingual ridges (mineral apposition, Fig. 3A-a, b). Transversely, however, growth was confined to the buccal surface and lingual bundle bone (mineral apposition, Fig. 3A-a, b, c, d; osteoblastic activity, Fig. 4A-a, d), with the buccal bundle bone and lingual surface showing resorption (resorptive lacunae lacking mineral apposition, Fig. 3A-a, b, c; osteoclastic activity, Fig. 4A-b, c). Third, overall the buccal ridge was thicker than the lingual ridge. The bulk of the buccal ridge was woven bone (Fig. 3A, B) with only a few secondary osteons indicative of intracortical remodeling, while the lingual ridge (except for the appositional bundle bone and crestal bone) was composed of compact bone with abundant secondary osteons (Fig. 3A-d).

Compared to the control sites described above, the SE extraction site showed several distinct differences. First, the buccal deflection angle was nearly flat, and the buccal and lingual surfaces converged gingivally (Fig. 3B, 4B) as vertical growth tilted towards the center of the ridge (Fig. 3B-a, b). Most SE extraction sites (6 out of 9 specimens) had a complete gingival bridge between the buccal and lingual ridges. In addition, although transverse

growth remained active at the buccal surface (Fig. 3B-a, b, Fig. 4B-a), bone apposition at the lingual bundle bone was absent (Fig. 3B-c, d) and the presence of osteoclasts indicated that resorption was taking place here (Fig. 4B-b, d). Finally, trabecular bone loosely filled the entire extraction socket (Fig. 3B), except for 3 specimens (out of 9) which contained a developing tooth, where a larger unfilled space was observed (Fig. 4B).

Quantitative results for vertical apposition (MAR_V) at the alveolar crests are shown in Fig. 5A. In the IE group, neither the main effects of the two factors (extraction status and alveolar ridge location) nor the interaction between the two factors were statistically significant. In the SE group, the interaction between these two factors was of borderline significance (repeated measures ANOVA, $p=0.053$) although their main effects were insignificant. This interaction mainly reflected an increase of MAR_V at the extraction site lingual ridge.

The measurements of transverse growth (MAR_T and MAZ_T) of the buccal surface are shown in Fig. 5B. Compared to the non-extraction site, the extraction site had significantly reduced MAR_T (repeated measures MANOVA, $p=0.048$ for the extraction factor), most of which was from the SE group. For MAZ_T , a significant interaction was detected between extraction and group (repeated measures MANOVA, $p=0.026$), reflecting that MAZ_T at the SE-extraction site was substantially lower than at the SE-non-extraction site, reversing a mild but opposite relationship between the two sites in the IE group. A subsequent repeated measure one-way ANOVA of the SE group found that MAZ_T was significantly lower at the extraction site than the non-extraction site ($F=8.741$, $df_{\text{(extraction factor)}}=1$, $df_{\text{(error)}}=16$, $p=0.009$).

The measurements of intracortical remodeling of the lingual ridge (MAR_O and BFR) are shown in Fig. 5C. Neither parameter was significantly changed by extraction, but varied between groups, with both parameters showing significantly higher values in the SE group than in the IE group (MANOVA, $p<0.05$ for the group factor).

DISCUSSION

While the data generally support our two initial hypotheses that (1) bone strain would be lower (underloading) at the extraction site than at the contralateral non-extraction site and (2) the SE group would show reduced growth compared to the non-extraction site due to chronic underloading, the detailed mechanical and biological processes involved in tooth-extraction induced RRR in adolescent pigs were more complex than we had expected. Specifically, bone growth at the buccal surface was indeed slowed after tooth extraction, but clearly, it is not the only biological mechanism underpinning the RRR. Functional loading at the extraction and non-extraction sites was different immediately after extraction but not at post-extraction week 6. These issues, together with the implications and limitations of our data, are further discussed below.

Normal growth without extraction

The histological observations from control sites (that is, sites that had not adapted to extraction, including both sides of the IE pigs as well as the SE non-extraction side, Figs. 3A, 4A) revealed that normal alveolar growth is gingival and buccal in adolescent pigs. The consistency between the IE animals and the SE non-extraction sites suggests that these directional patterns are present at least between weeks 12 and 20, the age range captured in this study. Growth in the gingival direction clearly accommodates the ongoing tooth eruption, and growth in the buccal direction accommodates dental arch expansion. These phenomena also occur in adolescent humans, as teeth continue to erupt in response to jaw growth in the vertical dimension (23), and intermolar width increases to expand the

perimeter of the dental arch (24). Notably, during dental arch expansion, the buccal-lingual dimension of the alveolar ridge remains relatively constant and the teeth remain relatively centered within the ridge. Our data show that this apparent consistency is achieved by well controlled differential activities at the four surfaces of the alveolar ridge. Specifically, new bone is added to the buccal surface and to the lingual bundle bone, while resorption removes the lingual surface and the buccal bundle bone (Fig. 3A). Combined, these processes allow the posterior teeth to move buccally relative to the mid-sagittal plane of the head while remaining relatively centered between the buccal and lingual alveolar plates.

Growth pattern alterations induced by tooth extraction

In adolescent humans, a single tooth extraction can result in a substantial reduction of the buccal-lingual alveolar dimension, especially at the buccal side (7, 15, 16). Our histological observations demonstrated that in adolescent pigs, buccal alveolar bone reduction in the transverse dimension was accomplished not by resorption, but by an altered growth pattern. This is in striking contrast to adult animals (20) and patients (12, 19), in whom bone resorption is the main cause for the reduced dimensions of the residual ridge.

Specifically, two aspects of buccal alveolar bone growth were altered by extraction and contributed to the manifestation of RRR at the buccal ridge. First, the crests converged gingivally, straightening out the buccal deflection angle (Figs. 3, 4). This morphological change was not caused by a surgical fracture during extraction, because the buccal and lingual ridges were still parallel immediately following extraction, and because healing fracture profiles were never observed in post-mortem specimens. Rather, it appeared to be a true alteration of the direction of vertical crestal growth from upright (parallel to the root) to diagonal (tilted towards the socket). Interestingly, several weeks after extraction, the alveolar bone was still growing vertically at a similar or even a faster pace (the lingual plate) compared to that at the non-extraction site (Fig. 5A). The stimulus that maintains alveolar bone vertical growth at the extraction site is not completely clear, but a wound healing process is very likely playing a role because the changed growth direction was responsible for bridging the buccal and lingual ridges and closing the extraction socket in most specimens, a phenomenon similar to callus formation observed around a healing bone osteotomy (34). Combined, these data suggest that at the gingival portion, the transverse ridge reduction is largely attributable to a redirection of crestal growth, which is needed for healing the extraction socket.

The second alteration in local growth was the deceleration of periosteal apposition over the entire buccal surface 6 weeks after the extraction. The two variables MAR_T and MAZ_T , although correlated (Pearson test of all 30 pairs of measurements, $r=0.720$, $p<0.01$) and therefore partially redundant, captured cross-sectional and accumulative growth, respectively, of the buccal surface. Both variables were substantially smaller at the SE-extraction than the SE-non-extraction site (Fig. 5B). These results thus complement the first alteration (buccal-lingual convergence at the gingival portion) and explain the clinical observation that transverse buccal reduction after tooth extraction is not restricted to the gingival crests only. With a normal apposition rate at adjacent alveoli, the extraction site buccal ridge will appear increasingly displaced to the lingual with time. This corresponds to the clinical appearance of a more severe buccal reduction in the initial phase of RRR (12, 13). Meanwhile, bone resorption on the non-gingival lingual surface continues normally, and intra-cortical remodeling at the extraction site was also unchanged (Fig. 5C), suggesting that the extraction does not accelerate bone loss inside the lingual ridge. Nevertheless, the normal resorption of the lingual surface adds to the progressive narrowing of the edentulous portion of the ridge compared to the adjacent dentate areas, and because the lingual bundle bone is no longer appositional, the lingual plate will become progressively thinner.

Relationship between histological and mechanical changes after tooth extraction

The finding that RRR at the buccal ridge in adolescent pigs was primarily due to alteration of buccal alveolar bone growth, rather than bone resorption, indicates that the disuse atrophy theory is not applicable to adolescent RRR. Instead, the findings seem to better fit the functional matrix theory, which postulates that bone growth and modeling change proportionally to functional demands (35).

For the changes in the bundle bone, a loss of tongue pressure could be responsible. Currently, it is agreed by many that tooth and alveolar bone positions are governed by a force equilibrium between the lips, cheeks and tongue (36). During adolescence, the dental arch normally expands, exhibiting an increase of intermolar and interpremolar width (24), which suggests that the pressure from the tongue outbalances that from the cheeks, perhaps because of tongue growth. Pressure from the tongue is exerted on the lingual surface of the alveolar bone and the tooth crowns. Pressure on the alveolar bone could explain the typical bone resorption at the lingual surface (Fig. 4A-c). When applied to the tooth crowns, the force would be transmitted to the roots and induce their buccal movement. Buccal movement of the roots would produce tension on the lingual periodontal ligament and bundle bone and compression on the buccal ligament and bundle bone, subsequently resulting in apposition and resorption of those surfaces, respectively (Fig. 3A-a, b, c, d). Tooth extraction would eliminate pressure delivery to the tooth but not to the alveolar bone. As a result, there would no longer be tensile stimulation applied to the lingual bundle bone, accounting for the cessation of apposition there (Fig. 3B-c, d). Resorption on the lingual surface would remain because of the continuation of tongue pressure on the bone (Fig. 4B-c).

Decreased masticatory loading could be responsible for growth deceleration at the buccal surface (37). We used strain gages to measure alveolar bone deformation in response to masticatory force and indirectly reveal the magnitude of loading. Our strain data suggested a disequilibrium of masticatory strain at the buccal surface between the extraction site and non-extraction site immediately after extraction (IE group), showing relatively smaller values at the extraction site (Fig. 2D, Table 2). Four of the five animals in this group showed underloading of the extraction site relative to the contralateral extraction site. The single outlier in the IE group (Table 2) could have been a product of either measurement error or individual variation, which we could not unambiguously distinguish. Even with this outlier included, however, the data suggest that a decrease of functional loading from mastication may account for at least some of the deceleration of mineralization observed at the buccal surface.

Nevertheless, another interpretation of the strain data in Fig. 2D and Table 2 is possible; rather than the extraction side being underloaded, the non-extraction side of the IE animals might have been overloaded. Based on indirect evidence, however, we reason that a functional overloading at the non-extraction site is less likely. The tooth extracted in this study (dm2) is relatively small (indicated by low strain values in Table 2 compared to those from a molar location (21)) and likely not so functionally critical as to require compensatory overactivity of the non-extraction side. Lack of compensation is supported by the fact that pigs did not favor the non-extraction side during chewing (Fig. 2A, B), as would be expected if the non-extraction site were overloaded (21). Moreover, it is reasonable to expect that the loss of occlusion should disturb force transmission to the buccal bone. Thus, the lower strain on the IE extraction site probably reflects a true underloading that occurs immediately after occlusion is lost. Six weeks after extraction (SE group), the difference between the extraction and non-extraction sites had disappeared, primarily because strain at the non-extraction site decreased (Fig. 2D). This decrease is likely age-related (the SE group being 6 weeks older than the IE group), because the buccal alveolar ridge was thicker at the

SE non-extraction site, and hence would be stiffer and show less strain for a given load. Meanwhile, strain at the SE extraction site remained low because it was still underloaded (lack of occlusion) and its buccal growth was less than the non-extraction site (Fig. 5B), so it was not as stiffened with age as the non-extraction site.

Thus, the extraction and non-extraction sites of IE pigs differed in strain but not in growth, whereas those of SE pigs showed similar strain but different growth patterns. The growth measurements probably reflect the impact of strain history rather than concurrent strain because the strain recordings followed the vital staining by several days. Further, the bone response necessarily lags behind the mechanical stimulus, so concurrent strain and growth measurements cannot reflect the relationship directly. Very likely, the extraction/non-extraction difference in buccal surface growth in the SE group reflects the disequilibrium of strains between the two sites in the IE group. More specifically, immediately after extraction, the extraction site was underloaded (IE group); as a result, buccal surface growth was decelerated and these changes became significant several weeks later (SE group).

Implications and limitations

The pig has a masticatory apparatus and dentition generally comparable to the human (29, 38–42), and the age of pigs used in this study was comparable to adolescent humans (40, 42, 43). Therefore, the results can tentatively be extrapolated to adolescent humans. The post-extraction alteration of growth pattern (redirected crestal growth, cessation of apposition on lingual bundle bone and decelerated buccal surface growth) explain the clinical manifestations of RRR in the rapid phase. While redirected crestal growth is part of the socket healing process, the latter two alterations are very likely induced by post-extraction mechanical changes, i.e., loss of tongue pressure through the tooth and underloading from mastication.

RRR is a chronic problem. The present study only captured its initial rapid phase and provided detailed information about the alterations of growth and mechanics. These data may be relevant for prevention or early intervention, but cannot be used to address why and how RRR transitions into a relatively stable phase. Another potential limitation is the strain gage technique. This technique is often used on limb and craniofacial bones and despite technical challenges (44–46), is currently the best technique available to assess functional loading in live animals. The intraoral environment of the gages in the present study was especially difficult, which resulted in a relatively high strain gage failure rate. However, animals were added to meet the predetermined sample size requirement, which ensures the strength of our data.

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Abbreviations

RRR	residual ridge reduction
IE	immediately post-extraction
SE	six weeks post-extraction
dm2	second deciduous molar

MAR_T	transverse mineral apposition rate
MAR_v	vertical mineral apposition rate
MAZ_T	transverse mineral apposition zone
BV	bone volume
dLS	double-labeled surface
sLS	single-labeled surface
MARo	osteonal mineral apposition rate
BFR	bone formation rate
df	degrees of freedom

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Fig. 1. Illustration of strain gage placement and appositional measurements

(A) Strain gage placement. A rectangular strain gage was attached to the mesial-middle portion of the buccal alveolar surface of dm2 or its extraction site. The wires and cables of the strain gage were brought extraorally through a submucosal-subcutaneous tunnel. **(B) Surface mineral apposition.** The buccal outer surface is to the right side of the image. A test array of 6 equally spaced ($167 \mu\text{m}$) horizontal lines was superimposed perpendicular to the buccal surface. At each intersection of the test line and bone surface, the distance (double-arrow line segment, $d1$) between red (alizarin complexone, broken line a) and green label fronts (calcein, b) was measured for transverse mineral apposition rate (MAR_T) calculations (formula in D). Apposition in the gingival direction (MAR_V) was measured

similarly. The distance (double-arrow broken line segment, d2) between the red label front (a) and the start of the continuous and intense green label (broken line c) was measured to calculate transverse mineral apposition zone (MAZ_T , formula in D). **(C) Osteonal apposition.** The lingual surface is to the left side of the image. A test grid was superimposed. Total hits (intersections) on bone and on labeled osteons were manually counted. Interlabel distances were taken from each osteon (exemplified as short white segments). Osteonal MAR (MAR_O) and BFR values were calculated as shown in D. **(D) Description of histomorphometric parameters.** Calibration bars, 500 μm .

Fig. 2. Physiological data

(A) An example of EMG recordings at baseline and immediately after extraction (IE#5). R, right chew; L, left chew; RMa, right masseter; LMa, left masseter. Chewing pattern and frequency were unchanged after extraction. The amplitude of the EMG signals was not standardized and could not be compared between muscles or between time points.

(B) Summary of chewing side distribution. No preference to chewing side was found either immediately or 6 weeks after the extraction. **(C) An example of strain recording during mastication (IE#2).** R, right chew; L, left chew; Con-Chn 1–3, control side strain gage channels; Ext-Chn 1–3, extraction side strain gage channels. Strains were lower on the extraction side. **(D) Summary of masticatory strain (mean, S.D.).** The extraction side of the IE group tended to have lower strain than the non-extraction side; sides did not differ in the SE group. See Table 2 for full data.

Fig. 3. Alveolar bone morphology and mineralization

(A) Control sites (SE non-extraction). The buccal surface contour is illustrated by a broken line, and the deflection angle of the buccal surface is indicated by an arrow. Green, calcein; red, alizarin complexone, which was injected 1 week after calcein. The boxes show, at higher magnification, (a) buccal gingival crest; (b) buccal ridge middle level; (c) lingual gingival crest; (d) lingual ridge middle level. S_B, buccal surface; S_L, lingual surface; B_B, buccal bundle bone; B_L, lingual bundle bone. +, bone formation surface; -, resorptive lacunae. Note bone formation at the crests, the buccal surface and the lingual bundle bone, and resorptive lacunae at the lingual surface and the buccal bundle bone. These features were also seen on both sides of the IE group. **(B) Experimental sites (SE extraction).** Abbreviations and symbols are the same as in (A). The buccal deflection angle is nearly flat, the socket opening to the gingiva is closed, and bundle bone is no longer distinguishable. Bone formation is still prominent at the crests and buccal surface, and the lingual surface still features resorptive lacunae.

Fig. 4. Tissue and cell features of alveolar bone growth and resorption

(A) Control sites (SE non-extraction). The buccal surface contour is illustrated by a broken line and the deflection angle is indicated by an arrow. The boxes show, at higher magnification, (a) buccal surface (S_B); (b) buccal bundle bone (B_B); (c) lingual surface (S_L); (d) lingual bundle bone (B_L). +, bone formation surfaces, lined with osteoblasts (brackets); -, resorptive surface, surrounded by multinucleated osteoclasts (arrowheads). The distribution of these cellular features is consistent with the pattern of mineralization (Fig. 3A). These features were also seen on both sides of the IE group. **(B) Experimental sites (SE extraction).** Abbreviations and symbols are the same as in (A). Except for the nearly flat buccal deflection angle, the buccal (a) and lingual (c) surface features are similar to those of control sites, but the interior is not. Instead of bundle bone, this region has large unfilled spaces around a developing tooth bud. Osteoclasts are present not only at the lingual surface, but also inside where bundle bone originally resided.

A. Vertical growth

B. Transverse growth: buccal surface

C. Intracortical remodeling: lingual ridge

Fig. 5. Quantitative findings for mineral apposition

Vertical columns indicate mean values and error bars indicate standard deviations. There was one degree of freedom for factorial and interaction tests. **(A)** Vertical mineral apposition rate (MAR_V) at the alveolar crests tested by 2-way repeated measures ANOVA. **(B)** Transverse mineral apposition rate (MAR_T) and transverse mineral apposition zone (MAZ_T) at the buccal surface tested by repeated measures MANOVA. **(C)** Osteonal mineral apposition rate (MAR_O) and bone formation rate (BFR) of the lingual alveolar ridge tested by repeated measures MANOVA.

Table 1

Live animal procedures

Pig Age (weeks)	12	13-14	15	18-19	20
IE Group (n=7)	Arrival Baseline EMG	Fluorescent bone labels	Tooth extraction, final functional recording (alveolar bone strain measurement, EMG), then euthanasia	--	--
SE Group (n=9)	Arrival Baseline EMG	Tooth extraction	--	Fluorescent bone labels	Final functional recording (alveolar bone strain measurement, EMG), then euthanasia

Table 2

Masticatory shear strain analysis mean $\mu\epsilon \pm$ S.D. (sample size; coefficient of variation)

Group	Extraction site	Non-extraction site	t-value; degrees of freedom; p-value for extraction vs. non-extraction site (2-sample t-tests)
IE	44.8 $\pm$ 47.6 (5; 1.062)	110.6 $\pm$ 58.0 (5; 0.524)	1.962; 8; 0.086
IE minus outlier	24.5 $\pm$ 17.1 (4; 0.698)	121.0 $\pm$ 61.3 (4; 0.507)	3.033; 6; 0.029
SE	55.8 $\pm$ 27.7 (4; 0.496)	52.7 $\pm$ 21.5 (6; 0.407)	0.199; 8; 0.846