Inherent physical characteristics and gene expression differences between alveolar and basal bones

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Objectives. The objective of this study was to evaluate the baseline differences between alveolar and basal areas of the rat mandible.

Study Design. Rat mandibular alveolar and basal bones were evaluated using histology and micro-computed tomography to compare osteocyte number as well as bone density and architecture and polymerase chain reaction to measure gene expression levels.

Results. Micro-computed tomography data indicated that basal bone is denser and less porous than alveolar bone. Histologic analysis showed that alveolar bone has more osteocytes per unit area compared with basal bone. Real-time polymerase chain reaction results showed higher levels of expression of the following genes in basal bone than in alveolar bone: *SOST*, *E-11*, *DMP-1*, and *MEPE*.

Conclusions. Three of these gene products are associated with mature osteocytes, and this suggests that basal bone has more mature osteocyte phenotypes compared with alveolar bone. These findings are suggestive of fewer bone mineralization units and therefore a slower remodeling rate. (Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122:35-42)

Several studies in the 1970s and 1980s showed that as a result of various factors, alveolar bone shows signs of bone resorption and deposition earlier compared with other bone types.[1,2] The mandible is constantly remodeling because of several factors, including mechanical stress, tooth extraction, orthodontic compression, tooth loss, and periodontitis.[3] The mandible is made up of two bone types—alveolar and basal—and it is not clear if these bone types significantly differ. For example, in Klemetti's report of his 1993 research, he stated that bone resorption begins at the alveolar part of the mandible, whereas the basal region of the mandible remains unchanged. He further explained that factors such as osteoporosis do not change the lower part of the mandible.[4]

Part of these data was presented at the following meetings: American Association of Orthodontist Annual Meeting, Hawaii, in May 2012 ("Alveolar and basal bone microstructure and osteogenic gene profile"); and The American Association for Dental Research Abstract, Tampa, FL, in March 2012 ("Microstructure and osteogenic gene profile variation between alveolar/basal bone").

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Much of the research on bone and bone cells has concentrated on cells from long bones, but less is known about mandibular basal and alveolar bones.[5] The majority of studies on dental bone have concentrated on the alveolar type. Alveolar and basal bones have significantly different resorption rates.[6,7] Because of the knowledge gap in this area, dental professionals have fewer treatment options for patients.

To better understand alveolar bone resorption and the limited resorption in basal bone, it is important to know the specifics about the mandible and how it differs from other bone types. Assumptions cannot be made about mandibular bone resorption based on other bone types or their corresponding bone cells. The main function of the osteocyte is to signal both osteoblasts and osteoclasts to maintain the structure and mass of bone.[8] As mechanosensors, osteocytes are important for studying bone resorption among bone types. However, differences between osteocytes within the types of bone must be understood.

In this study, the physical and gene expression profiles of the alveolar and basal bones of a rat mandible were investigated. There is a lack of significant knowledge regarding ways to prevent or reverse overall

Statement of Clinical Relevance

If fundamental differences in the gene expression of dental alveolar and basal bone could be identified, therapeutic targets may be identified to help reduce or totally eliminate the loss of alveolar dental bone caused by resorption.

bone loss in patients. Without bone grafting, many dental procedures are difficult to perform. Understanding the differences in physical and molecular properties between alveolar and basal bones is essential for better dental treatment outcomes. Therefore, our overall hypothesis is that there will be significant differences in the physical properties and gene expression of bone regulatory proteins between alveolar and basal bones.

MATERIALS AND METHODS

Animals

The Institutional Animal Care and Use Committee approved the use of six 6-month-old Sprague-Dawley male rats, which had been used as untreated controls in another study. The committee approved use of these animals after euthanization as cadaver tissue sources.

Tissue preparation and processing for micro-computed tomography

Bone preparation for micro-computed tomography (micro-CT) began by cutting portions of the incisor and leaving only the molar area of the rat mandibles. The collected bone samples were fixed in formalin for 48 hours, transferred to 70% ethanol, and stored at −20°C. The mandible samples were then placed in phosphate-buffered saline solution and scanned in 70% alcohol.

Micro-CT

Following harvest, the mandibles were frozen until the time of scanning and then were placed in a small saline-filled tube. For bone mineral density (BMD) measurement and three-dimensional (3-D) morphometric analysis, 4% paraformaldehyde-fixed mandibles were scanned by using Skyscan 1172 (Skyscan, Aartlesaar, Belgium). The mandibles were placed in a container filled with phosphate-buffered saline and scanned, using a 0.25-mm aluminum filter, at an image pixel size of 36.65 μm, 0.5° rotation step, and frame averaging of 3. Reconstruction of the scanned images was done with a Skyscan Nrecon program (Skyscan, Aartlesaar, Belgium). The reconstructed data sets were loaded into Skyscan CT-analyzer software (Skyscan, Aartlesaar, Belgium) for measurement of BMD and 3-D morphometric parameters. Four regions of interest were selected in alveolar and basal bones. BMD was measured in each region of interest after calibration with 0.25 and 0.75 density hydroxyl apatite phantoms. The average density of the alveolar and basal regions of interest was calculated.

The Skyscan 1174 Micro CT analyzer (Microphotonics, Allentown, PA) has the ability to study up to 29 parameters. Micro-CT analysis calculated the following 14 parameters: tissue volume (TV), bone volume (BV), percent bone volume (%BV), tissue surface (TS), bone surface (BS), bone surface/volume ratio (BS/V), mean total cross-sectional bone area (A), mean total cross-sectional bone perimeter (P), trabecular thickness (plate model, TbTh), trabecular diameter (rod model, TD), trabecular number (rod model, TN), closed porosity (percent) (Po), mean fractal dimension (MFD), and total intersection surface (S). To help clarify the results, we will discuss three parameters: bone surface/bone volume (BS/BV), trabecular thickness (TbTh), and porosity (Po).

BV is the sum of voxels above threshold, with an additional dilation−erosion step to fill in occasional small voids in the cortical wall. BS is measured in square millimeters. A lower bone surface is connected with increased bone strength and solidity. The micro-CT analyzer measures the surface based on the faceted surface of the "marching cubes volume model."[9] From these calculations, the BS/BV can be derived—a measurement of bone surface per given BV. This parameter was used as an indicator of bone strength.

TbTh has been standardized and is considered one of the descriptors of trabecular bone architecture.[10] Through a combination of several formulas, the TbTh of the object is calculated.

Po is a key parameter that can determine the performance of bone. The micro-CT analyzer measures the Po of bone as a percentage of the total area of binarized objects contained in fully enclosed spaces.[9]

Statistical analysis included analysis of variance (ANOVA), pairwise Student's t tests, with a level of significance chosen at $\alpha < 0.05$.

Tissue preparation and processing for histologic analysis

Mandibles were fixed, decalcified, and embedded in paraffin for histologic analysis. Five-micron sections were stained with hematoxylin and eosin, and the number of osteocytes per field area of each bone was counted. Other samples were stained with Podoplanin (E-11; Abbiotec, San Diego, CA) avidin-biotin complex by the Augusta University Histology Core facility in Augusta, Georgia. The blood vessel area and bone marrow space were eliminated to provide a final osteocyte number per unit area.

Tissue preparation and processing for RNA isolation and real-time polymerase chain reaction (PCR)

Bone samples were flash-frozen in liquid nitrogen, wrapped in foil, and crushed by using a steel ball mill. The crushed bone powder from each alveolar or basal bone sample underwent RNA isolation by Trizol