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ORIGINAL

Differential bone histomorphometric characters of the mandible in senescence-accelerated mice (SAMP6 and SAMP8): murine models for senile osteoporosis and temporomandibular joint osteoarthritis

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Abstract: Histomorphometric analyses of the mandible and femur were performed in senescence-accelerated mice (SAM) using SAMP6 as a senile osteoporosis (OP) model, SAMP8 as a temporomandibular joint osteoarthritis (TMJOA) model, and SAMR1 as control. Thirty-six male mice at 2 or 4 months of age (6 for each strain and age) were used. The cortical thickness index (CTI), bone area (B.Ar/T.Ar), trabecular width (Tb.Wi), trabecular number (Tb.N), trabecular separation (Tb.Sp), osteoblast perimeter (N.Ob/B.Pm), and osteoclast perimeter (N.Oc/B.Pm) were assessed in the distal femoral metaphysis and in the mandibular ramus including the alveolar bone. Compared with SAMR1, SAMP6 showed lower B.Ar/T.Ar and Tb.Wi in the femur and mandible at 2 and 4 months of age. This strain showed lower CTI in both bones and higher Tb.N and Tb.Sp in the femur at 4 months of age. SAMP8 showed higher CTI in both bones at 2 months of age and maintained a high index in the mandible but not in the femur at 4 months of age; however trabecular bone mass was not reduced. SAMP6 exhibited lower N.Ob/B.Pm in the femur but not in the mandible at 4 months of age, while SAMP8 showed lower N.Oc/B.Pm in the mandible but not in the femur at 2 and 4 months of age. The differential histomorphometric features of the mandible in different SAM strains may imply a difference in mandibular bone property between SAM mice with senile OP and TMJOA genetic background.

Keywords: bone, mandible, osteoarthritis, osteoporosis, mouse

Introduction

Osteoporosis (OP) and osteoarthritis (OA) are common skeletal disorders in the elderly, the incidence of which appears to be inversely related to each other[1]. OP and OA in oral bone are also crucial diseases for dental treatment. Systemic OP likely has an effect on human oral bone status, showing a positive relationship with oral bone loss[2-6] and tooth loss[7-11]. Temporomandibular (TMJ) OA occurs in isolation and not simultaneously with other joints including hip, knee, and hand. Temporomandibular OA shows initial fibrillation and eventually loss of the articular cartilage, resulting in cavitation and erosion of the exposed bone with gradual changes of condylar shape[12].

Primary OP is classified into postmenopausal OP seen in postmenopausal women and senile OP occurring in older men and women. The former shows a rapid loss of trabecular bone after menopause as a result of deficiency of endogenous estrogen, while the latter shows a slow loss of cortical and trabecular bone as an end result of age-related bone loss[13]. Previous studies of postmenopausal OP using ovariectomized rat models suggest that estrogen deficiency causes bone loss and trabecular fragmentation in the mandibular alveolar bone[14,15]. However, the histological changes in the oral bone following senile OP have not been defined. With regard to OA, the etiology remains to be elucidated but one possible explanation is that increased stiffness of the subchondral bone caused by the healing of microfractures may result in overloaded cartilage[16,17]. This hypothesis suggests that OA may be a disease of the bone rather than of the cartilage. In the oral bone, TMJOA may also represent stiffness of the subchondral bone, possibly showing similar characteristics in alveolar bone. Stiffness of the oral bone associated with current or potential TMJOA would affect, in part, alveolar bone loss and tooth loss. Histological analysis of the mandibular bone with TMJOA is thus necessary.

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The senescence-accelerated mouse (SAM) is a murine model of accelerated senescence consisting of two series, the aging-accelerated SAMP series and the control SAMR series[18]. All strains show a peak of bone mass at 4 to 5 months of age. Within the SAMP series, SAMP6 shows the lowest bone mass and is regarded as a model for senile OP[19]. All SAMP strains except SAMP6 exhibit OA in the TMJ but not in other joints[20]. SAMP8 strain exhibits degenerative changes in the TMJ after 10 months of age[20,21]. Thus, SAMP6 and SAMP8 strains are good models for histological analysis of the oral bone in senile OP and TMJOA. Elucidation of the oral bone properties in senile OP and TMJOA will provide crucial information for dental diagnosis and treatment. The purpose of this study was to analyze the histomorphometric characteristics of the mandibular and femoral bones in SAMP6 and SAMP8 compared with those of SAMR1.

Materials and Methods

Animals and assessment of bone size

A total of 36 male SAM comprising SAMP6, SAMP8, and their control SAMR1 (12 mice each) were purchased from Japan SLC, Inc. (Shizuoka, Japan). The mice were housed up to five per cage, with 12:12-h light:dark cycles and access to standard mouse chow and water ad libitum, until 2 or 4 months of age. Mice aged 2 or 4 months were sacrificed under diethyl ether anesthesia. Six left femurs and mandibles were used for each strain and each age group in this study. Before tissue processing, bone size was assessed. The femurs were measured using a distal caliper; the length was recorded as the distance between the superior side of the proximal condyle and the inferior side of the distal condyle, and the width as the diameter of the bone at mid-diaphysis. The mandibles were measured using a digital microscope (VHX-100, Keyence, Osaka, Japan); and the length was measured as the perpendicular distance from the alveolar crest of the incisor to the line joining the posterior side of the mandibular condyle and the posterior side of the mandibular angle, and the height as the perpendicular distance from the superior side of the mandibular condyle to the mandibular plane. The mean and standard deviation (SD) of the six values were calculated for each group.

Tissue preparation

After the bone measurement, the left femoral and mandibular bones were fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) at pH 7.4 for 24 h at 4^{o}C. They were rinsed with PBS, followed by decalcification with 10% EDTA in PBS for 2 weeks at 4^{o}C. From each whole femur, the distal portion was cut off as sample (distal femur) (Fig. 1). From each whole mandible, two portions were sampled. The first portion comprised the first and second molars (the mid mandible), and the second portion contained the third molar and the mandibular angle, ramus, and condyle (the posterior mandible) (Fig. 2). After dehydration with a graded series of ethanol, the samples were passed through xylene and embedded in paraffin. The embedded samples of the distal femur, the mid mandible, and the posterior mandible were serially sectioned in frontal, sagittal, and horizontal planes (3 µm in thickness), and the sections were deparaffinized. Some sections were stained with picrosirius red that selectively stains the collagenous matrix[22] equivalent to the bone matrix. Other sections were stained with toluidine blue to stain the osteoid being produced or newly produced by osteoblasts, or with tartrate resistant acid phosphatase (TRAP) to stain the osteoclasts.

Bone histomorphometry

Bone histomorphometric analysis of the femoral and mandibular bones was basically performed according to the principles recommended by the American Society for Bone and Mineral Research[23]. Measurements were based on observations of the sections under a microscope and calculated using an image and analysis program (Scion Image, Scion Corporation, Frederick, MD, USA). Cortical structure was evaluated by the cortical thickness index [CTI = (bone diameter – trabecular or marrow diameter) / bone diameter]; and trabecular bone volume and structure were evaluated by the bone area (B.Ar/T.Ar), trabecular width (Tb.Wi), trabecular number (Tb.N), and trabecular separation (Tb.Sp). Osteoblast perimeter (N.Ob/B.Pm) and osteoclast perimeter (N.Oc/B.Pm) were measured in the trabecular bone. For the femur, the measurements were made using the frontal sections of the distal metaphysis in an area beginning 0.5 mm and ending 2.0 mm proximal to the growth plate/metaphyseal junction to exclude the primary spongiosa junction (Fig. 1). The CTI was expressed as the mean of three measurements of the cortical area at 0.5, 1.0 and 1.5 mm proximal to the junction. The B.Ar/T.Ar, Tb.Wi, Tb.N, and Tb.Sp were calculated in the trabecular area upon removal of the cortical area. The N.Ob/B.Pm and N.Oc/B.Pm were calculated in the same trabecular area, based on the assumption that the cells overlying the toluidine blue-stained osteoid are osteoblasts, and the TRAP-positive cells are osteoclasts. For the mandible, the CTI was measured buccolingually using a horizontal section of the mandibular ramus at the occlusal plane (Fig. 2). The other histomorphometric indices were calculated in a trabecular area defined by the interradicular septum between the distal root of the first molar and the mesial root of the second molar (Fig. 3). The mean and SD of six measurements are presented for each group. The experimental protocol described above was approved by the Ethical Committee for Animal Experiments in our institute.

Statistical analysis

Comparison among the three strains was performed using one-factor ANOVA with Bonferroni/Dunn test. A minimum P value of 0.005 for ANOVA and of 0.0167 for Bonferroni/Dunn test was the necessary condition for statistical significance. Data were processed on a Macintosh computer using the StatView 5.0 software (SAS Institute, Cary, NC, USA).

Results

Bone size

The sizes of the femur and mandible differed significantly among the three SAM strains (Table 1). SAMP8 but not SAMP6 had shorter and narrower femora compared with SAMR1 at 2 months of age. At 4 months of age, SAMP8 had shorter but wider femora, while SAMP6 had the same length but wider femora compared with SAMR1. The mandibles of SAMP6 and SAMP8 were equal in size as that of SAMR1 at 2 months of age, except for the height of SAMP6. At 4 months of age, however, the mandible of SAMP6 was shorter in length and height, while that of SAMP8 was longer in length and height compared with SAMRI.

Cortical thickness

The CTI values of the femur and mandible of SAMP6 were not different from those of SAMR1 at 2 months of age but became smaller at 4 months of age (Table 2 and Fig. 3). In contrast, SAMP8 had larger CTI in the femur and mandible compared with

Table 1. Bone sizes

Age	2 months				4 months			
	Femur		Mandible		Femur		Mandible	
	Length*	Width*	Length	Height*	Length*	Width*	Length*	Height*
SAMR1	15.67 ± 0.22	1.79 ± 0.02	10.83 ± 0.19	5.36 ± 0.11	16.41 ± 0.29	1.77 ± 0.02	11.82 ± 0.12	5.86 ± 0.06
SAMP6	15.74 ± 0.20	1.83 ± 0.04	10.55 ± 0.65	5.02 ± 0.18†	16.12 ± 0.13	1.86 ± 0.04†	11.57 ± 0.08†	5.61 ± 0.07†
SAMP8	15.30 ± 0.18†	1.60 ± 0.04†	10.62 ± 0.06	5.27 ± 0.11	16.06 ± 0.06†	1.84 ± 0.06†	12.36 ± 0.14†	6.32 ± 0.05†

Data are presented as mean ± SD ($n = 6$) in mm.
*: significant difference among three SAM strains by one-factor ANOVA ($p < 0.05$), †: significant difference between SAMR1 and other strain by Bonferroni/Dunn test ($p < 0.0167$).

Table 2. Structural index of cortical bone (Cortical thickness index; CTI)

Age	2 months		4 months	
	Femur*	Mandible*	Femur*	Mandible*
SAMR1	16.8 ± 3.3	21.8 ± 0.9	16.4 ± 0.7	22.9 ± 1.4
SAMP6	17.4 ± 3.0	22.0 ± 1.3	13.9 ± 1.2†	19.5 ± 1.9†
SAMP8	24.4 ± 2.1†	24.7 ± 1.1†	14.6 ± 1.6	25.9 ± 1.0†

Data are presented as mean ± SD ($n = 6$) in %. Cortical thickness index (CTI) = bone diameter – trabecular or marrow diameter) / bone diameter.
*: significant difference among three SAM strains by one-factor ANOVA ($p < 0.05$), †: significant difference between SAMR1 and other strain by Bonferroni/Dunn test ($p < 0.0167$).

SAMR1 at 2 months of age, and the mandible remained larger but not the femur at 4 months of age.

Trabecular bone characteristics in structure

Compared with SAMR1, SAMP6 showed smaller bone mass in the trabecular area of the femur and mandible (Table 3 and Fig. 5). Smaller B.Ar/T.Ar and Tb.Wi of both the femur and mandible were observed at 2 and 4 months of age; while smaller Tb.N and larger Tb.Sp of the femur but no differences in the mandible were observed at 4 months of age compared with SAMP1. Conversely, SAMP8 did not show smaller bone mass in the trabecular area (Table 3 and Fig. 5). All four indices in the femur of this strain were the same as those of SAMR1 at 2 and 4 months of age, except Tb.Wi at 4 months of age. The mandible of this strain showed larger B.Ar/T.Ar and smaller Tb.Sp at 2 months of age but only larger Tb.N at 4 months of age.

Number of osteoblasts and osteoclasts in the trabecular bone

The numbers of osteoblasts and osteoclasts differed between SAMP6 and SAMP8 (Table 4). Compared with SAMR1, SAMP6 showed no differences in N.Ob/B.Pm in both the femur and mandible at 2 months of age. However, N.Ob/B.Pm in the femur became smaller than while that in the mandible was similar to that of SAMR1 at 4 months of age. No difference in N.Oc/B.Pm was observed between SAMP6 and SAMR1. In SAMP8, N.Ob/B.Pm in the femur was the same as those of SAMR1 at 2 and 4 months of age. The mandible of this strain exhibited smaller N.Ob/B.Pm at 2 months of age but increased to the same level as SAMR1 at 4 months of age. The N.Oc/B.Pm in the femur of SAMP8 were similar to those of SAMR1 at 2 and 4 months of age but the values in the mandible were smaller than those of SAMPR1 both at 2 and 4 months of age.

Discussion

This study demonstrates for the first time the differential bone histomorphometric characteristics of the mandible in senile OP model and TMJOA model mice. In SAMP6, reduced cortical and trabecular volumes and fragmented trabeculae were observed in the mandible as well as in the femur. The femoral findings in the present study are supported by a number of previous studies that

Table 3. Structural indices of trabecular bone

	Femur				Mandible			
	B.Ar/T.Ar	Tb.Wi	Tb.N	Tb.Sp	B.Ar/T.Ar	Tb.Wi	Tb.N	Tb.Sp
Age 2 months	*	*	*		*	*		*
SAMR1	14.7 ± 2.5	27.8 ± 4.0	5.3 ± 0.6	165.4 ± 21.9	75.6 ± 6.0	95.3 ± 12.6	8.2 ± 0.9	30.3 ± 6.9
SAMP6	10.0 ± 0.8†	22.4 ± 3.3†	4.5 ± 0.4	203.2 ± 16.3	62.2 ± 4.5†	67.3 ± 19.8†	10.3 ± 3.0	40.2 ± 10.1
SAMP8	12.9 ± 3.7	27.3 ± 3.1	4.7 ± 1.0	192.5 ± 38.8	83.8 ± 3.5†	84.0 ± 14.6	10.4 ± 1.7	15.8 ± 2.5†
Age 4 months	*	*	*	*	*	*	*	*
SAMR1	8.9 ± 2.1	24.6 ± 1.7	3.6 ± 0.7	269.0 ± 66.8	74.1 ± 6.0	84.3 ± 16.9	9.1 ± 1.4	28.8 ± 6.7
SAMP6	5.1 ± 1.3†	18.6 ± 2.3†	2.7 ± 0.4†	357.5 ± 64.2†	63.6 ± 6.2†	58.7 ± 8.3†	11.0 ± 1.4	33.5 ± 7.8
SAMP8	10.1 ± 1.5	30.2 ± 4.7†	3.3 ± 0.2	273.5 ± 17.3	77.4 ± 4.6	68.9 ± 10.6	11.6 ± 1.5†	19.9 ± 4.8

B.Ar/T.Ar, bone area; Tb.Wi, trabecular width; Tb.N, trabecular number; Tb.Sp, trabecular separation.
Data are presented as mean ± SD ($n = 6$) in % for B.Ar/T.Ar, µm for Tb.Wi, /mm for Tb.N, and µm for Tb.Sp).
*: significant difference among three SAM strains by one-factor ANOVA ($p < 0.05$), †: significant difference between SAMR1 and other strain by Bonferroni/Dunn test ($p < 0.0167$).

Table 4. Number of osteoblasts and osteoclasts in trabecular bone

Age	2 months				4 months			
	Femur		Mandible		Femur		Mandible	
	N.Ob/B.Pm	N.Oc/B.Pm	N.Ob/B.Pm*	N.Oc/B.Pm*	N.Ob/B.Pm*	N.Oc/B.Pm	N.Ob/B.Pm	N.Oc/B.Pm*
SAMR1	34.6 ± 4.4	4.29 ± 0.29	43.1 ± 1.8	8.69 ± 0.94	41.5 ± 6.9	3.52 ± 0.54	36.6 ± 3.3	5.72 ± 0.62
SAMP6	31.9 ± 4.8	4.25 ± 0.68	42.4 ± 5.0	9.25 ± 0.61	30.8 ± 4.0†	3.06 ± 0.76	39.4 ± 4.7	6.27 ± 1.42
SAMP8	37.4 ± 4.6	4.00 ± 0.42	34.3 ± 7.6†	5.34 ± 0.79†	37.9 ± 1.5	3.59 ± 0.52	41.5 ± 2.7	4.06 ± 0.35†

N.Ob/B.Pm, osteoblast perimeter; N.Oc/B.Pm, osteoclast perimeter. The average values (*n* = 6) and SDs are shown as number/mm in unit. The asterisk (*) and symbol (†) indicates statistic significance among three SAM strain groups by one-factor ANOVA ($p < 0.05$) and between SAMR1 group and other strain group by Bonferroni/Dunn test ($p < 0.0167$).

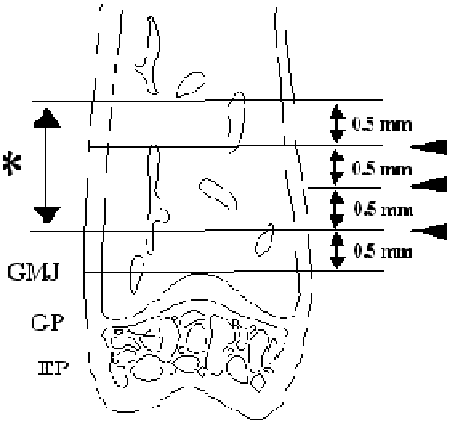

Figure 1. A schema of the distal metaphysis of the femur in a frontal section. The measurements for bone histomorphometric indices in the trabecular bone were performed in an area beginning 0.5 mm and ending 2.0 mm proximal to the growth plate/metaphyseal spongiosa junction (asterisk). The measurement for cortical thickness index was performed at three points (arrowhead) in the area. GMJ, growth plate/metaphyseal spongiosa junction; GP, growth plate; EP, Epiphysis.

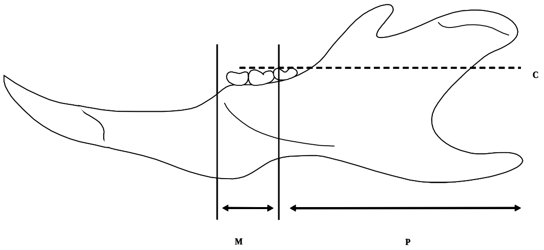

Figure 2. A schema of the mandible. The mid region (M) consisting of the first and second molars and the posterior region (P) consisting of the third molar and the mandibular angle, ramus, and condyle were used for bone histomorphometric analyses. The measurement for cortical thickness index was performed in a horizontal section in the mandibular ramus at the occlusal plane (C).

indicated low bone formation and bone mineral density[19, 24-27] accompanied by increased brittleness of the bone in this strain[28]. In the mandible, previous studies using ovariectomized rats have shown similar findings of bone loss and trabecular fragmentation in the alveolar bone[14,15]. The histological characteristics and structure of the mandible of SAMP6 observed in this study are similar to those of the femur of this strain and presumably also similar to those of the mandible of the ovariectomized rats. The number of osteoblasts in the femur of SAMP6 was low compared with that of SAMR1, consistent with previous findings concerning this bone of SAMP6 in which osteoblastogenesis was disturbed[29] but adipogenesis and myelopoiesis were accelerated[30,31]. In the mandible of this strain, however, the number of osteoblasts was not reduced as was observed in the femur. The preservation of osteoblast number in the OP mandible but not in the OP femur may be attributed to the occlusal stress through the teeth. Nevertheless, the senile OP mandible showed bone loss in the alveolar area without any change in osteoclast number, possibly implying that the osteoblasts secrete less or brittle bone matrix.

This study has shown that, in SAMP8, the cortex thickens and the number of osteoclasts in the trabeculae decreases in the mandible but not in the femur. The thickening of subchondral bone appears to precede destruction of articular cartilage in OA[32]. Cortical thickening of the mandibular ramus in SAMP8 at 4 months of age, 6 months before the degradation of the mandibular condylar cartilage[20,21], may imply a potential thickening of the cortex of the whole mandible even before the onset of TMJOA attack. The rate of remodeling in cortical bone is lower throughout life and may normally be 5 to 10 times lower than that in trabecular bone in the adult[33]. Indeed, cortical bone is thought to be more stable than trabecular bone from the findings of collagen cross-linking[34] and cross-linking alteration[35-37] as well as findings of bone loss by skeletal unloading[38,39]. Thus, cortical thickening implies an increase in old bone matrix. Furthermore, a decreased number of osteoclasts in the alveolar trabecular bone without bone loss in SAMP8 may suggest older bone matrix in the alveolar bone. Taken together, SAMP8 may have older bone matrix in the cortex and trabeculae area of the mandible before the onset of TMJOA attack.

Biochemical analysis concerning the major bone matrix protein, collagen, has suggested that changes observed in OP and OA are more likely to be disease- rather than age-related[40]. No biochemical change of collagen in human bone has been found to be caused by aging, although the content declines throughout life[41]. The collagen in the human OP bone exhibits a high level of lysyl hydroxylation, a small amount of immature cross-links, and a small amount of mature cross-link (pyrrole), and the collagen fibrils are correspondingly narrower[40,42,43]. The subchondral bone in human femoral OA has an increased content of collagen, a high ratio of $\alpha 1$ and $\alpha 2$ chains (homotrimer of $\alpha 1$ chains) and a high level of lysyl hydroxylation, together with narrower collagen fibrils and reduced mineralization and mechanical strength[40,44,45]. These differential biochemical and structural conditions of collagen in OP and OA patients probably determine the bone properties in these diseases. It is possible that similar characteristics reflect the OP and TMJOA mandibular bones. The differential histomorphometric findings of the mandible between SAMP6 and SAMP8 together with some differences between the

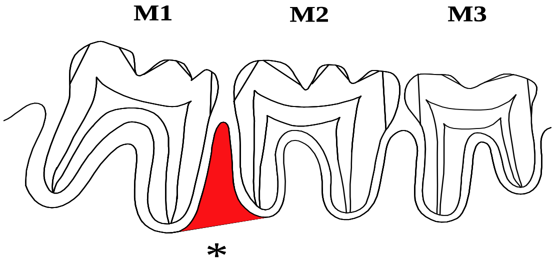

Figure 3. A schema of the alveolar bone in a sagittal section. The measurements for bone histomorphometric indices in the trabecular bone were performed in a trabecular area defined by the interradicular septum between the distal root of the first molar and the mesial root of the second molar (asterisk). M1, the first molar; M2, the second molar; M3, the third molar.

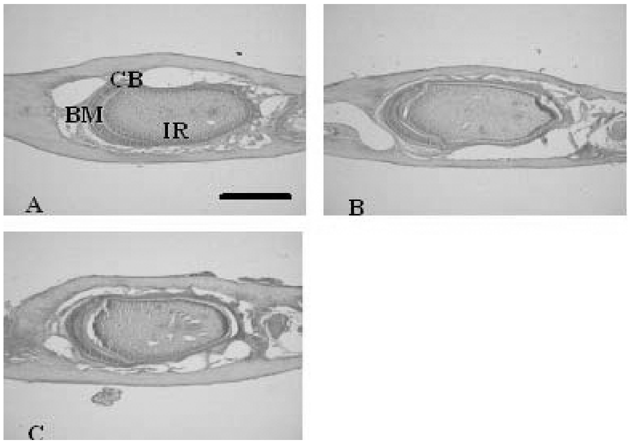

Figure 4. Cortical bone at the mandibular ramus of three SAM strains at 4 months of age. A, SAMR1; B, SAMP6; C, SAMP8. Cortical thickness was different. CB, cortical bone; BM, bone marrow; IR, incisor root. Bar indicates 0.5 mm.

mandible and femur may imply different and specific characteristics of the mandibular bone matrix in senile OP and TMJOA. Although it remains to be elucidated by biochemical analysis, the bone matrix status of the oral bone in potential patients with senile OP and TMJOA appears to predict future conditions of the bone and teeth, affecting the proper choice of dental treatment.

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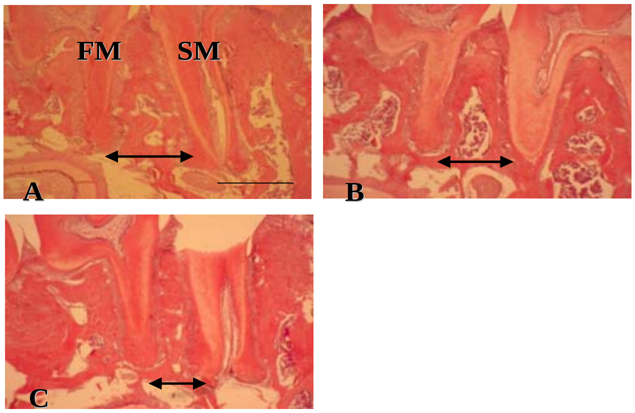

Figure 5. Mandibular alveolar bone of three SAM strains at 4 months of age. A, SAMR1; B, SAMP6; C, SAMP8. Compared with SAMR1 and SAMP8, SAMP6 shows narrower bone area and larger bone marrow area at the interradicular septum between the distal root of the first molar and the mesial root of the second molar (double-headed arrow). CB, cortical bone; BM, bone marrow; IR, incisor root. Bar indicates 0.5 mm.

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