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Overexpressing the NH₂-terminal fragment of dentin sialophosphoprotein (DSPP) aggravates the periodontal defects in *Dspp* knockout mice

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Abstract

Objective—Previous studies have shown that dentin sialophosphoprotein (DSPP) is not only essential to the formation and mineralization of dentin but also plays an important role in forming and maintaining a healthy periodontium. Under physiological conditions, DSPP is proteolytically processed into the NH₂-terminal and COOH-terminal fragments, and these fragments are believed to perform different functions in the mineralized tissues. Previous studies in our group have demonstrated that the NH₂-terminal fragment of DSPP inhibits the formation and mineralization of dentin, while the role of this fragment in periodontium is unclear.

Methods—We analyzed the periodontal tissues of the transgenic mice overexpressing the NH₂-terminal fragment of DSPP in the *Dspp* knockout background (referred to as “*Dspp* KO/DSP Tg” mice), in comparison with wild type mice and *Dspp* knockout mice. The approaches used in this study included histology, micro-computed tomography, back scattered scanning electron microscopy and resin-casted scanning electron microscopy.

Results—*Dspp* KO/DSP Tg mice exhibited a greater reduction of the alveolar bone, more remarkably altered canalicular systems around the osteocytes, less cementum, more radical migration of the epithelial attachment towards the apical direction, and more severe inflammation in molar furcation region, than in the *Dspp* knockout mice.

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ETHICAL APPROVAL

The animal protocols used in this study were approved by the Animal Welfare Committee of Texas A&M University, Baylor College of Dentistry (Dallas, TX, USA).

CONFLICT OF INTEREST

No potential conflicts of interest exist.

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Conclusion—Overexpressing the NH₂-terminal fragment of DSPP worsened the periodontal defects in *Dspp* knockout mice, indicating that the NH₂-terminal fragment of DSPP may exert an inhibitory role in the formation and mineralization of hard tissues in the periodontium.

Keywords

Periodontitis; Gene regulation; Cementum; Bone biology; Cell biology

1. INTRODUCTION

Dentin sialophosphoprotein (DSPP) discovered by cDNA cloning using an odontoblast library was initially thought to be dentin-specific [1, 2]. Later on, its expression was also found in the long bone, alveolar bone and cementum [3, 4]. Genetic studies have shown an association of DSPP mutations with dentinogenesis imperfecta (DGI) in humans [5–8]. Gene ablation studies revealed that *Dspp* knockout (*Dspp* KO) mice have severe dental defects characterized by dentin hypomineralization, widened predentin zone and enlarged pulp chamber, resembling the tooth defects observed in human DGI [9]. These *in vivo* studies have confirmed the critical role of DSPP in dentinogenesis. A recent study by our group has shown that *Dspp* KO mice develop periodontal diseases manifesting a reduction of alveolar bone, decreased deposition of cementum and altered morphology of osteocytes in the alveolar bone [10]. These recent findings indicate that in addition to its critical role in dentin formation, DSPP is also essential for the formation and maintenance of a healthy periodontium.

Physiologically, DSPP is proteolytically cleaved by astacin proteases into the NH₂-terminal and COOH-terminal fragments [2, 11–13]. The NH₂-terminal fragments of DSPP exist in the extracellular matrix (ECM) of rat/mouse dentin as two forms, dentin sialoprotein (DSP) and the proteoglycan form of this fragment (DSP-PG); the former has no glycosaminoglycan (GAG) chains while the latter has two GAG chains made of chondroitin-4-sulfate [14, 15]. The COOH-terminal fragment of DSPP is referred to as dentin phosphoprotein (DPP) [14]. Previous studies in our group have established that the posttranslational processing (cleavage) of mouse DSPP at the NH₂-terminus of Asp⁴⁵² is an activation step essential to the formation and mineralization of dentin and alveolar bone [16, 17]. We have shown that the substitution of Asp⁴⁵² by Ala⁴⁵² prevents the cleavage of DSPP into fragments, which subsequently leads to defects in the mouse dentin and periodontium [16, 17].

The exact mechanisms of how the cleaved fragments of DSPP function in the dentin and periodontal tissues remain largely unknown. *In vitro* analyses have shown that DPP is a strong initiator and regulator for the formation and growth of hydroxyapatite crystals [18, 19], while DSP (the NH₂-terminal fragment of DSPP without the GAG chains) has no significant effects on the formation and growth of hydroxyapatite crystals [20]. Recent *in vivo* studies have demonstrated that overexpressing the NH₂-terminal fragment of DSPP (including both DSP and DSP-PG) worsened the dentin defects in *Dspp* KO mice [21], indicating that DSP and/or DSP-PG may inhibit the formation and mineralization of dentin. This investigation focuses on the specific roles of the NH₂-terminal fragments of DSPP in periodontium, by analyzing the alveolar bone, cementum and periodontal ligaments in the mutant mice overexpressing the NH₂-terminal fragment of DSPP but lacking the

endogenous *Dspp* gene. Our results showed that overexpressing the NH₂-terminal fragment of DSPP worsened the periodontal defects in *Dspp* KO mice.

2. MATERIALS AND METHODS

2.1 Generation of transgenic mice overexpressing the NH₂-terminal fragment of DSPP in the *Dspp*-knockout background (*Dspp* KO/DSP Tg mice)

Detailed procedures for the generation of transgenic mice overexpressing the NH₂-terminal fragment of DSPP driven by a type I collagen promoter have been described in our previous publication [21]; these transgenic mice are named “DSP Tg mice”. We crossbred DSP Tg mice with *Dspp* KO mice obtained from the Mutant Mouse Regional Resource Center (MMRRC, UNC, Chapel Hill, NC), to generate a line of mutant mice that express the transgene encoding the NH₂-terminal fragment of DSPP but lack the endogenous *Dspp*; this line of mutant mice is referred to as “*Dspp* KO/DSP Tg” mice. Detailed information regarding the genotyping primers and the expression level of the transgene can be found in our previous report [21]. We employed histology, micro-computed tomography and scanning electron microscopy to assess the periodontal tissues of *Dspp* KO/DSP Tg mice, in comparison with those of the wild type (WT) and *Dspp* KO mice.

2.2 Histology (H&E staining)

For histology analyses, 3-month-old *Dspp* KO/DSP Tg, WT and *Dspp* KO mice were perfused from the ascending aorta with 4% paraformaldehyde (Sigma Aldrich Corporation, St. Louis, MO) in 0.1 M phosphate buffer. The heads from the sacrificed animals were then fixed in 4% paraformaldehyde for 48 hours and then decalcified in 8% EDTA (pH 7.4) (Sigma Aldrich Corporation, St. Louis, MO) at 4°C for approximately 2 weeks. The dissected mandibles were embedded in paraffin, and serial sections of 5 µm were prepared from these paraffin blocks. The sections were stained with hematoxylin & eosin (H&E) and observed under an Olympus microscope (Olympus BX51, Olympus Corporation, Center Valley, PA USA).

2.3 Micro-computed tomography (µ-CT)

We dissected the mandibles from the 3-month-old and 6-month-old WT, *Dspp* KO, and *Dspp* KO/DSP Tg mice (3 mice for each age group; i.e., totally, 6 animals for each type of mice). The mandibles were analyzed using the µ-CT (µ-CT 35 imaging system, Scanco Medical, Basserdorf, Switzerland), as we previously described [17, 22]. We performed high-resolution scans (3.5 µm/slice) and three-dimensional reconstructions to assess the morphological characteristics of the mouse mandibles and to calculate the ratios of alveolar bone volume to total volume (BV/TV), among the three groups of mice at both ages. The BV/TV values were expressed as mean ±SD. Student's t-test was adopted in our statistical analyses to determine the significance levels of differences between two individual groups, using SPSS (SPSS v.17.0, IBM, Somers, NY).

2.4 Back scattered and resin-casted scanning electron microscopy (SEM)

For these experiments, the mandibles from 3-month-old mice (n = 3 for each type of mice) were dissected and fixed in 2% paraformaldehyde with 2.5% glutaraldehyde (Sigma Aldrich

Corporation, St. Louis, MO) in 0.1 M cacodylate buffer solution (pH 7.4) at room temperature. The detailed protocols used for processing, polishing and coating samples for back scattered and resin-casted SEM (Philips XL30 FEI scanning electron microscope, Philips, Hillsboro, OR, USA) analyses have been described in previous studies [10, 17].

3. RESULTS

3.1 Inflammatory infiltration and migration of epithelial attachment in the periodontium of *Dspp* KO/DSP Tg mice

Results from H&E staining demonstrated that when compared to the WT controls (Fig. 1A), the furcation region of the mandibular molar in *Dspp* KO mice (Fig. 1B) and *Dspp* KO/DSP Tg mice (Fig. 1C) had obvious inflammatory infiltration. The inflammatory reaction appeared more prevalent and severe in the *Dspp* KO/DSP Tg (Fig. 1C) than in the *Dspp* KO mice (Fig. 1B).

In comparison with the WT mice (Fig. 2A), both types of the mutant mice (Fig. 2B, 2C) showed the detachment and migration of the junctional epithelium, along with the disruption of periodontal ligaments in the interdental region between the mandibular first and second molars. The above defects in the *Dspp* KO/DSP Tg mice (Fig. 2C) were much more severe than in the *Dspp* KO mice (Fig. 2B).

3.2 Reduction of alveolar bone in *Dspp* KO/DSP Tg mice

Results from both H&E staining (Figs. 1, 2) and μ -CT analyses (Fig. 3) showed that the reduction of alveolar bone in the *Dspp* KO/DSP Tg mice was greater than in *Dspp* KO mice. In the H&E-stained sections, *Dspp* KO mice (Fig. 1B) showed a moderate loss of alveolar bone in the furcation region of the first mandibular molar when compared to the WT control (Fig. 1A), while the same region in the *Dspp* KO/DSP Tg mice (Fig. 1C) was completely engulfed with the inflammatory cells and had only a few islands of bone left with the majority of the alveolar bone structures being destroyed.

When the alveolar bone volume fractions (BV/TV) were compared among the three types of mice (Fig. 3A and B), the *Dspp* KO/DSP Tg mice had the least values of BV/TV in either the 3- or 6-month-old age groups. The differences in BV/TV values were statistically significant between the *Dspp* KO and *Dspp* KO/DSP Tg mice at both ages.

3.3 Loss of cellular cementum in *Dspp* KO/DSP Tg mice

Using backscattered and resin-casted SEM approaches (Fig. 4), we observed an overall reduction of cellular cementum in the mandibular first molars of the *Dspp* KO (Figs. 4B and 4E) and *Dspp* KO/DSP Tg mice (Figs. 4C and 4F). The periapical region of the mandibular first molars in the *Dspp* KO/DSP Tg mice (Figs. 4C and 4F) had the least amounts of cementum when compared to the WT control (Figs. 4A and 4D) or the *Dspp* KO mice (Figs. 4B and 4E).

3.4 Alteration of the osteocyte lacunae and canalicular system in the alveolar bone of *Dspp* KO/DSP Tg mice

The osteocyte lacunae in the alveolar bone of WT mice were well organized and evenly spaced with most of the canaliculi radiating in directions perpendicular to the long axis of the osteocyte bodies (Fig. 5A). In contrast, the osteocyte lacunae in the alveolar bone of the *Dspp* KO mice appeared irregular with disorganized and fewer canaliculi (Fig. 5B). The osteocyte lacunae in the alveolar bone of *Dspp* KO/DSP Tg mice were extremely disorganized and had very few osteocyte canaliculi (Fig. 5C). The osteocyte canaliculi in the alveolar bone of the *Dspp* KO/DSP Tg mice (Fig. 5C) also had a reduced extension into the surrounding bone compared to *Dspp* KO mice (Fig. 5B). It was apparent that the defects of the osteocyte lacunae and the structures surrounding the lacunae in the alveolar bone of *Dspp* KO/DSP Tg mice were worse than those of the *Dspp* KO mice.

4. DISCUSSION

DSPP is expressed in dentin, cementum, alveolar bone, long bone and several non-mineralized tissues [14, 23]. Previously, our group showed that the expression level of DSPP in the alveolar bone and cellular cementum is significantly higher than that in the long bone [3, 14]. In the periodontal structures, DSPP was found in the osteocytes of the alveolar bone, cementocytes of cellular cementum in the apical region as well as in the matrices surrounding these mineralized tissue-forming cells [3]. There are a number of reports regarding the biological roles of DSPP and the mechanistic aspects of this protein in dentinogenesis [5–9, 17, 21], whereas little information is available regarding the biological functions of this molecule in periodontium. Recent work from our group has shown that deletion of DSPP leads to periodontal disease in mice and has established the essential role of this molecule in maintaining the integrity of the periodontium [14]. The current study provides new knowledge about the molecular mechanisms by which DSPP functions in the periodontal tissues.

DSPP is present in the ECM of dentin as three forms found at significant levels [14]: 1) DSP (the NH₂-terminal fragment without GAG chains, containing little or no phosphorylation), 2) DSP-PG (the proteoglycan form of the NH₂-terminal fragment, possessing two GAG chains), and 3) DPP (the COOH-terminal fragment, very highly phosphorylated). Based on their differences in chemical property and tissue distribution profiles, these three molecular variants derived from the amino acid sequence of DSPP are believed to play different functions [14, 24]. In this study, we analyzed the specific role of the NH₂-terminal fragment of DSPP in periodontium, using transgenic mice overexpressing this fragment.

We found that overexpressing the NH₂-terminal fragment of DSPP aggravated the periodontal defects in *Dspp* KO mice; the periodontal deterioration in the *Dspp* KO/DSP Tg mice evident in the multipronged analyses included more severe inflammatory infiltration around the alveolar bone, more significantly reduced propagation of the osteocyte processes into surrounding alveolar bone, greater reduction of interradicular and interdental bone, greater loss of cellular cementum, and more radical migration of junctional epithelium towards the apex, in comparison with the *Dspp* KO mice. The periodontal deteriorations in *Dspp* KO/DSP Tg mice were likely due to more severe defects in the alveolar bone and

cementum, which lead to secondary effects (including inflammation) on the periodontal ligament and to the formation of deeper periodontal pockets. The exacerbated abnormality in the morphology of osteocytes and canaliculi in the alveolar bone of *Dspp* KO/DSP Tg mice indicate that the defect of the alveolar bone must be due to intrinsic factors of bone cells associated with the overexpression of the NH₂-terminal fragment of DSPP.

In a previous study, Suzuki et al [25] generated transgenic mice expressing the NH₂-terminal fragment of DSPP driven by the mouse *Dspp* promoter and showed that transgenic expression of this fragment partially rescued the tooth defects in *Dspp*-null mice. We think that the variance between our studies and the previous one may be attributed to the use of different promoters.

The NH₂-terminal fragments of DSPP exist as two forms: DSP and DSP-PG [14]. While the exact ratio of DSP to DSP-PG is unknown, it appears that DSP-PG is a major proteoglycan in the dentin matrix [14, 26, 27]. *In vitro* mineralization analyses showed that DSP has no significant effects on the formation and growth of hydroxyapatite crystals [20] while information regarding the biological roles of DSP-PG is lacking. In this study, overexpressing the NH₂-terminal fragment of DSPP in the type I collagen-expressing tissues (dentin, bone, cementum and periodontal ligaments) aggravated the periodontal defects of *Dspp* KO mice, indicating that the NH₂-terminal fragment of this protein may play an inhibitory role in the formation and mineralization of hard tissues in the periodontium. However, we do not know which of the two molecular species (i.e., DSP or DSP-PG) derived from the NH₂-terminal fragment of DSPP is responsible for the exacerbated phenotypic changes in the *Dspp* KO/DSP Tg mice. Future studies are warranted to define which of the two NH₂-terminal forms is exerting the inhibitory functions in the formation and mineralization of hard tissues in the periodontium.

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References

1. Butler WT. Dentin matrix proteins. *Eur J Oral Sci.* 1998; 106 (Suppl 1):204–10. [PubMed: 9541227]
2. MacDougall M, Simmons D, Luan X, Nydegger J, Feng J, Gu TT. Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a single transcript coded by a gene on human chromosome 4. Dentin phosphoprotein DNA sequence determination. *J Biol Chem.* 1997; 272:835–42. [PubMed: 8995371]
3. Baba O, Qin C, Brunn JC, Jones JE, Wygant JN, McIntyre BW, Butler WT. Detection of dentin sialoprotein in rat periodontium. *Eur J Oral Sci.* 2004; 112:163–70. [PubMed: 15056114]
4. Qin C, Brunn JC, Cadena E, Ridall A, Tsujigiwa H, Nagatsuka H, Nagai N, Butler WT. The expression of dentin sialophosphoprotein gene in bone. *J Dent Res.* 2002; 81:392–4. [PubMed: 12097430]
5. Kim JW, Nam SH, Jang KT, Lee SH, Kim CC, Hahn SH, Hu JCC, Simmer JP. A novel splice acceptor mutation in the DSPP gene causing dentinogenesis imperfecta type II. *Hum Genet.* 2004; 115:248–54. [PubMed: 15241678]
6. Rajpar MH, Koch MJ, Davies RM, Mellody KT, Kielty CM, Dixon MJ. Mutation of the signal peptide region of the bicistronic gene DSPP affects translocation to the endoplasmic reticulum and

- results in defective dentine biomineralization. *Hum Mol Genet.* 2002; 11:2559–65. [PubMed: 12354781]
7. Xiao S, Yu C, Chou X, Yuan W, Wang Y, Bu L, Fu G, Qian M, Yang J, Shi Y, Hu L, Han B, Wang Z, Huang W, Liu J, Chen Z, Zhao G, Kong X. Dentinogenesis imperfecta 1 with or without progressive hearing loss is associated with distinct mutations in DSPP. *Nat Genet.* 2001; 27:201–4. [PubMed: 11175790]
 8. Zhang X, Zhao J, Li C, Gao S, Qiu C, Liu P, Wu G, Qiang B, Lo WH, Shen Y. DSPP mutation in dentinogenesis imperfecta Shields type II. *Nat Genet.* 2001; 27:151–2. [PubMed: 11175779]
 9. Sreenath T, Thyagarajan T, Hall B, Longenecker G, D'Souza R, Hong S, Wright JT, MacDougall M, Sauk J, Kulkarni AB. Dentin sialophosphoprotein knockout mouse teeth display widened predentin zone and develop defective dentin mineralization similar to human dentinogenesis imperfecta type III. *J Biol Chem.* 2003; 278:24874–80. [PubMed: 12721295]
 10. Gibson MP, Zhu Q, Liu Q, D'Souza RN, Feng JQ, Qin C. Loss of dentin sialophosphoprotein leads to periodontal diseases in mice. *J Periodontal Res.* 2013; 48:221–7. [PubMed: 22934831]
 11. Gu K, Chang S, Ritchie HH, Clarkson BH, Rutherford RB. Molecular cloning of a human dentin sialophosphoprotein gene. *Eur J Oral Sci.* 2000; 108:35–42. [PubMed: 10706475]
 12. Ritchie HH, Wang L-H, Knudtson K. A novel rat 523 amino acid phosphophoryn: nucleotide sequence and genomic organization. *Biochim Biophys Acta.* 2001; 1520:212–22. [PubMed: 11566357]
 13. Tsuchiya S, Simmer JP, Hu JC, Richardson AS, Yamakoshi F, Yamakoshi Y. Astacin proteases cleave dentin sialophosphoprotein (Dspp) to generate dentin phosphoprotein (Dpp). *J Bone Mineral Res.* 2011; 26:220–8.
 14. Zhu Q, Sun Y, Prasad M, Wang X, Yamoah AK, Li Y, Feng J, Qin C. Glycosaminoglycan chain of dentin sialoprotein proteoglycan. *J Dent Res.* 2010; 89:808–12. [PubMed: 20400719]
 15. Prasad M, Butler WT, Qin C. Dentin sialophosphoprotein in biomineralization. *Connect Tissue Res.* 2010; 51:404–17. [PubMed: 20367116]
 16. Gibson MP, Jani P, Liu Y, Wang X, Lu Y, Feng JQ, Qin C. Failure to process dentin sialophosphoprotein into fragments leads to periodontal defects in mice. *Eur J Oral Sci.* 2013; 121:545–50. [PubMed: 24112131]
 17. Zhu Q, Gibson MP, Liu Q, Liu Y, Lu Y, Wang X, Feng JQ, Qin C. Proteolytic processing of dentin sialophosphoprotein (DSPP) is essential to dentinogenesis. *J Biol Chem.* 2012; 287:30426–35. [PubMed: 22798071]
 18. Boskey AL, Maresca M, Doty S, Sabsay B, Veis A. Concentration-dependent effects of dentin phosphophoryn in the regulation of in vitro hydroxyapatite formation and growth. *Bone Miner.* 1990; 11:55–65. [PubMed: 2176557]
 19. Saito T, Arsenault AL, Yamauchi M, Kuboki Y, Crenshaw MA. Mineral induction by immobilized phosphoproteins. *Bone.* 1997; 21:305–11. [PubMed: 9315333]
 20. Boskey A, Spevak L, Tan M, Doty SB, Butler WT. Dentin Sialoprotein (DSP) Has Limited Effects on In Vitro Apatite Formation and Growth. *Calcif Tissue Int.* 2000; 67:472–8. [PubMed: 11289697]
 21. Gibson MP, Liu Q, Zhu Q, Lu Y, Jani P, Wang X, Liu Y, Paine ML, Snead ML, Feng JQ, Qin C. Role of the NH2-terminal fragment of dentin sialophosphoprotein in dentinogenesis. *Eur J Oral Sci.* 2013; 121:76–85. [PubMed: 23489896]
 22. Sun Y, Lu Y, Chen L, Gao T, D'Souza R, Feng JQ, Qin C. DMP1 Processing is Essential to Dentin and Jaw Formation. *J Dent Res.* 2011; 90:619–24. [PubMed: 21297011]
 23. Prasad M, Zhu Q, Sun Y, Wang X, Kulkarni A, Boskey A, Feng JQ, Qin C. Expression of dentin sialophosphoprotein in non-mineralized tissues. *J Histochem Cytochem.* 2011; 59:1009–21. [PubMed: 22043023]
 24. Huang B, Sun Y, Maciejewska I, Qin D, Peng T, McIntyre B, Wygant J, Butler WT, Qin C. Distribution of SIBLING proteins in the organic and inorganic phases of rat dentin and bone. *Eur J Oral Sci.* 2008; 116:104–12. [PubMed: 18353003]
 25. Suzuki S, Sreenath T, Haruyama N, Honeycutt C, Terse A, Cho A, Kohler T, Muller R, Goldberg M, Kulkarni AB. Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. *Matrix Biol.* 2009; 28:221–9. [PubMed: 19348940]

26. Qin C, Brunn JC, Baba O, Wygant JN, McIntyre BW, Butler WT. Dentin sialoprotein isoforms: detection and characterization of a high molecular weight dentin sialoprotein. *Eur J Oral Sci.* 2003; 111:235–42. [PubMed: 12786955]
27. Yamakoshi Y, Hu JC, Fukae M, Iwata T, Kim JW, Zhang H, Simmer JP. Porcine dentin sialoprotein is a proteoglycan with glycosaminoglycan chains containing chondroitin 6-sulfate. *J Biol Chem.* 2005; 280:1552–60. [PubMed: 15537641]

Fig. 1.

H&E staining for the furcation regions of the first mandibular molars from 3-month-old WT (A), *Dspp* KO (B) and *Dspp* KO/DSP Tg (C) mice. While the inflammatory reaction (black arrows in B and C) was present in the molar furcation region of both *Dspp* KO and *Dspp* KO/DSP Tg mice, the inflammation in the latter mice was more remarkable than in the former. Note that there was very little alveolar bone left in the molar furcation region of *Dspp* KO/DSP Tg mice. Bar: 100 μm.

Fig. 2.

H&E staining for the interdental regions between the first and second molars of mandibles in the 3-month-old WT (A), *Dspp* KO (B) and *Dspp* KO/DSP Tg (C) mice. Black arrows indicate the cemento-enamel junctions. Blue arrows indicate the site of the epithelial attachment (bottom of the gingival sulcus). The reduction of the interdental alveolar bone in the *Dspp* KO/DSP Tg (C) was greater than in the *Dspp* KO mice. The epithelial attachment receded more apically in the *Dspp* KO/DSP Tg mice (C, indicated by blue arrow) than in the *Dspp* KO mice. Bar: 100 μ m.

Fig. 3.

The quantitative μ -CT analyses of the interradicular alveolar bone in the 3-month-old (A) and 6-month-old (B) mice. Student's *t-test* was used to statistically compare the mean differences between two groups in each of the paired analyses. The differences in the bone volume fractions (BV/TV) between the *Dspp* KO and *Dspp* KO/DSP Tg mice at both ages were statistically significant. The data represent mean $\pm$ SD ($n = 3$). BV, bone volume; TV, total volume; *: $P = 0.05$.

Fig. 4.

Backscattered SEM (A–C) and resin-casted SEM analyses for the periapical regions of first molars in the mandibles of 3-month-old WT (A, D), *Dspp* KO (B, E) and *Dspp* KO/DSP Tg (C, F) mice. In these SEM images, the dentin is denoted as ‘D’, pulp as ‘P’, and cementum as ‘C’. In the backscattered SEM images, the black areas represent unmineralized or hypomineralized areas, and thus, the dental pulp (P) appears black. Cementum (C) is less mineralized (appears darker) than dentin (D). In comparison with the WT mice (A), the periapical regions of *Dspp* KO (B) and *Dspp* KO/DSP Tg (C) mice had broader areas of blackness in the backscattered SEM images and contained smaller areas of cementum. Among the three types of mice, *Dspp* KO/DSP Tg mice had the least amounts of cellular cementum. The resin-casted SEM images (D–F) also revealed that among the three groups, the *Dspp* KO/DSP Tg mice (F) had the least amounts of cellular cementum in the periapical region. Bar: 200 μ m.

Fig. 5.

Resin-casted SEM analyses of the osteocyte lacunae and canaliculi in the alveolar bone of 3-month-old WT (A), *Dspp* KO (B) and *Dspp* KO/DSP Tg (C) mice. In the WT mice the canaliculi radiated out in directions perpendicular to the long axis of the osteocyte bodies. In contrast, the osteocyte lacunae in the alveolar bone of the *Dspp* KO and *Dspp* KO/DSP Tg mice appeared irregular with disorganized and fewer canaliculi. The defects of the osteocyte lacunae and the structures surrounding the lacunae in the alveolar bone of *Dspp* KO/DSP Tg mice were apparently worse than those of the *Dspp* KO mice. Bar: 5 μm.