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Targeted Expression of csCSF-1 in *op/op* Mice Ameliorates Tooth Defects

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Abstract

Objective—The aim of this study was to characterize the tooth phenotype of CSF-1-deficient *op/op* mice and determine whether expression of csCSF-1 in these mice has a role in primary tooth matrix formation.

Design—Ameloblasts and odontoblasts, isolated from *wt/wt* frozen sections using laser capture microdissection, were analyzed for csCSF-1, sCSF-1 and CSF-1R mRNA by RT-PCR. Mandibles, excised from 8 day *op/op* and *wt/wt* littermates, were examined for tooth morphology as well as amelogenin and DMP1 expression using *in situ* hybridization. *Op/opCS* transgenic mice, expressing csCSF-1 in teeth and bone using the osteocalcin promoter, were generated. Skeletal x-rays and histomorphometry were performed; teeth were analyzed for morphology and matrix proteins.

Results—Normal dental cells *in vivo* express both CSF-1 isoforms and CSF-1R. Compared to *wt/wt*, *op/op* teeth prior to eruption showed altered dental cell morphology and dramatic reduction in DMP1 transcripts. *Op/opCS* mice showed marked resolution of osteopetrosis, tooth eruption and teeth that resembled amelogenesis imperfecta-like phenotype. At 3 weeks, *op/op* teeth showed severe enamel and dentin defects and barely detectable amelogenin and DMP1. In *op/opCS* mice, DMP1 in odontoblasts increased to near normal and dentin morphology was restored; amelogenin also increased. Enamel integrity improved in *op/opCS*, although it was thinner than *wt* enamel.

Conclusions—Results demonstrate that ameloblasts and odontoblasts are a source and potential target of CSF-1 isoforms *in vivo*. Expression of csCSF-1 within the tooth microenvironment is essential for normal tooth morphogenesis and may provide a mechanism for coordinating the process of tooth eruption with endogenous matrix formation.

Keywords

Macrophage colony-stimulating factor (CSF-1); osteopetrosis; transgenic mice; osteocalcin promoter; teeth

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Introduction

Macrophage colony stimulating factor (CSF-1), a key regulatory molecule for cells of the mononuclear phagocytic lineage, is highly expressed in mineralized tissues. During tooth development, we and others^{1,2} have shown that CSF-1 transcripts are expressed in murine odontoblasts, pulp cells, ameloblasts and in the surrounding dental follicle and alveolar bone osteoblasts. *In vitro*, osteoblasts and odontoblasts have been shown to synthesize the soluble (s) and cell surface (cs) forms of CSF-1 that mediate their biologic effect via the high-affinity CSF-1 (*c-fms*) tyrosine kinase receptor.^{3–5} The cell surface form is expressed as an integral transmembrane glycoprotein that is slowly shed from the membrane, whereas the soluble form is rapidly secreted as either a glycoprotein or proteoglycan.^{6–8} sCSF-1, released by osteoblasts and csCSF-1, through direct cell contact, stimulate the proliferation and differentiation of osteoclast progenitors and survival of osteoclasts.^{9–11}

The importance of CSF-1 in osteoclast-mediated bone remodeling and tooth eruption has been shown in osteopetrotic (*op/op*) mice. Absence of CSF-1 in osteoblasts/stromal cells of these mice decreases osteoclasts and leads to osteopetrosis and failure of tooth eruption.^{12,13} *Op/op* teeth gradually become invaded by alveolar bone and develop ankylosis with odontoma formation in incisors.^{14,15} In gene rescue studies, we have shown that sCSF-1 selectively expressed in osteoblasts of *op/op* mice using the osteocalcin promoter, reversed osteopetrosis and normalized tooth eruption.¹⁶ csCSF-1 has been reported to either completely or partially correct osteopetrosis and induce eruption; however, bone histomorphometry and tooth morphology were not examined.^{17,18} Whether dental cells *in vivo* also express csCSF-1 or CSF-1R has not been described. As previously stated, cell-cell contact mediates csCSF-1 action. Odontoblasts and ameloblasts are initially in contact and then separate as odontoblasts secrete dentin and ameloblasts secrete enamel. Dentin matrix contains the noncollagenous protein, dentin matrix protein 1 (DMP1) that is expressed by odontoblasts and required for dentin mineralization and maturation of predentin into dentin. Enamel is composed of amelogenins which account for 90% of the matrix.^{19,20} In humans and rodent models, altered production of matrix proteins can lead to dentin or enamel defects.^{20–24} The effect of CSF-1 deficiency or csCSF-1 on tooth matrix formation *in vivo* has not been explored. Thus, the objective of the present study was to determine if *wt/wt* dental cells express csCSF-1, sCSF-1 and CSF-1R *in vivo* and if CSF-1 deficiency in *op/op* teeth leads to altered amelogenin and DMP1 expression. To determine the potential role of csCSF-1 in modulating these matrix proteins, we utilized the osteocalcin promoter, which targets genes to odontoblasts and osteoblasts, to establish *op/op* transgenic mice selectively expressing csCSF-1 in teeth and bone.

Materials and Methods

Analysis of CSF-1 isoforms and receptor expression in *wt/wt* dental cells

Mandibles, isolated from three 1 day B6C3 *wt/wt* mice, were frozen and 8 μ thick sections were stained with HistoGene LCM stain. Early ameloblasts and odontoblasts were captured from first mandibular molars using PixiCell II LCM (Arcturus, Mountain View, CA), and total RNA was isolated using Pico-Pure RNA isolation kit according to manufacturer's instructions. Gene expression was analyzed by RT-PCR using previously published methods and primer pairs for csCSF-1, sCSF-1, CSF-1R and GAPDH (control gene) which yield a 163 bp, 240 bp, 552 bp and 407 bp amplicon, respectively.^{4,25,26} PCR products were separated by electrophoresis in a 1.5% agarose gel containing ethidium bromide and photographed under UV light.

Generation of transgenic *op/op* mice expressing *csCSF-1*

For the transgene construct, the 960 bp CSF-1 cDNA that encodes the human csCSF-1 cDNA was excised from pcCSF17 (American Type Culture Collection, Manassas, VA) with *SmaI-EcoRI*, subcloned into the *HincII* site of pUC7 and then inserted downstream of the 1.8 kb rat osteocalcin promoter in the *EcoRI*-digested pBSpKCR3 that we have previously described.^{16,27} The osteocalcin promoter has been shown to target exogenous genes to mature osteoblasts and odontoblasts.^{28,29} Preparation of DNA for microinjection, production of transgenic mice and genotyping of mice harboring the *op* allele, the transgene or both were carried as described for sCSF-1 *op/op* transgenic mice.^{16,27} All transgenic lines were established and bred using the B6C3 strain which is the same background as the *op/op* mutant. Three founders expressing csCSF-1 were identified with 2–4 copies of the transgene per haploid genome. CSF-1 transgenic *op/wt* mice were then bred with *op/wt* to establish *op/op* mice expressing the transgene (*op/opCS*). Age-matched *wt/wt* and *op/op* littermates served as normal and mutant controls, respectively. Six mice from each group were analyzed for eruption and tooth morphology and, at 6 and 14 weeks, hind limbs were x-rayed using a Faxitron x-ray cabinet. Animals used in this study were approved by the University of Texas Health Science Center, San Antonio Animal Care and Use Committee.

Analysis of transgene expression and bone histomorphometry

CSF-1 protein levels were measured in plasma, bone, teeth and tissue extracts as previously described.^{16,27} To analyze CSF-1 on the cell surface, primary bone marrow stromal cells were isolated from femurs, and the membrane fraction was isolated using the Mem-PER kit (Pierce). Whole tissue, membrane and plasma samples were then assayed using the human CSF-1 ELISA kit (R&D Systems, Minneapolis, MN). For bone histomorphometry, femurs from *wt/wt*, *op/op* and *op/opCS* mice (3–6/group) were fixed in 10% formalin and undecalcified bones were embedded in polymethylmethacrylate. Four-millimeter-thick sections were stained for tartrate-resistant acid phosphatase followed by counterstaining. Two-dimensional parameters of cancellous bone were measured in a 2-mm square, 0.5 mm distal to the lowest point of the growth plate. Cancellous bone volume (BV/TV), trabecular measurements, and osteoclast numbers were calculated using a digitized image analysis system and Osteomeasure software³⁰ (Osteometrics, Atlanta, GA) and values were expressed according to Parfitt et al.³¹

Scanning electron microscopy (SEM)

Molars, whole and after fracture, were analyzed by SEM. Mandibles from three *wt/wt*, *op/op* and *op/opCS* mice were fixed in 2% glutaraldehyde in 0.1M cacodylate buffer, post-fixed with osmium, dehydrated in a series of graded ethanols, with a final immersion in 100% hexaethyldisilazane. Samples were then mounted on carbon stubs, coated with gold/palladium with a Balzar Med 10 evaporator and examined with a Leo 435VP scanning electron microscope.

In situ hybridization and tooth morphology

Mandibles and maxillae were fixed in 4% paraformaldehyde, demineralized, dehydrated and embedded in paraffin. 6-micron thick sections were cut onto glass slides and processed for *in situ* hybridization or H&E stain. For the amelogenin antisense probe, a pBluescriptIIKS+ vector containing a mouse amelogenin (AMGN) 885 bp cDNA fragment was linearized and transcribed with T₇ polymerase.³² DMP1 antisense and sense probes were prepared from a 623 bp mouse DMP1 *XhoI* and *NotI* linearized pCR-Script subclone and transcribed with T₃ and T₇ polymerase, respectively.³³ Single stranded probes were transcribed *in vitro* using the Riboprobe System kit (Promega, Madison, WI). *In situ* hybridization was performed according to the method of Sasson and Rosenthal³⁴ with minor modifications as we have described.³⁵

Sections were counterstained with hematoxylin and evaluated using bright and dark field microscopy

Statistical analysis

Bones from three to six mice were analyzed by histomorphometry. Statistical significance was assessed by Student's unpaired *t*-test. Significance was determined as $p < 0.05$ or 0.01 . Three to six animals were chosen per group which allows statistically significant detection (0.05) of mean differences between two groups of approximately 2 S.D. (1.98–2.29) with good power (0.8). For quantitation of amelogenin and DMP1 mRNA, darkfield *in situ* hybridization sections were analyzed from two mice in each group as previously described.³⁵ The intensity of signal in ameloblasts expressing amelogenin and odontoblasts expressing DMP1 was determined by densitometric analysis and measurements were performed by computer-assisted image analysis using ImageJ software (U.S. National Institutes of Health, Bethesda, MD). The optical density (OD) was analyzed on adjacent tissue sections (three per mouse) for a total of six specimens in each group. The OD of randomly selected areas was measured in comparable regions of molars and incisors. Intensity was measured as standard optical density ranging from 0 to 2 units. Analysis of variance and least significant difference multiple comparisons procedure (Statistical Analysis Software Version 8.2, SAS Institute, Cary, NC, USA) were used to separate the means and detect a difference in mRNA expression in experimental groups.

Results

To determine if dental cells express CSF-1 isoforms and receptor *in vivo*, early odontoblasts and ameloblasts were identified after HistoGene staining and isolated by LCM. Figure 1 shows that each cell type express two transcripts for CSF-1 at 240 bp and 163 bp that represent secreted and cell surface forms of CSF-1, respectively. This correlates with previous findings in cultured MO6-G3 odontoblast cells that expressed the same transcripts.⁴ Odontoblasts and ameloblasts from *wt/wt* mice also expressed the expected 552 bp CSF-1 receptor fragment.²⁶ Normal murine spleen that contains macrophages provided a positive control for CSF-1 isoforms and receptor.

The effect of CSF-1 deficiency on tooth morphology and matrix protein expression prior to eruption was analyzed in 8 day postnatal mice. Figure 2 shows *wt/wt* molars surrounded by a well-defined dental follicle. In contrast, the dental follicle in *op/op* molars was narrow and showed early invasion by bone trabeculae, with some trabeculae in contact with ameloblasts. Compared to *wt/wt*, *op/op* molars and incisors showed an abnormal contour with decreased enamel and dentin matrices. At this time point, cytodifferentiation of ameloblasts in mutants was altered, with mutant ameloblasts appearing shorter with diminished cytoplasm and rounder, less elongated nuclei compared to *wt*. By *in situ* hybridization, amelogenin was highly expressed in *op/op* molars and not significantly different from that detected in *wt/wt* molars. Similar findings were observed in incisors (data not shown). Mutant odontoblasts in molars and incisors (Figures 2 and 3) were shorter and less polarized along the predentin than those in *wt/wt* teeth. The relative amount of predentin to mature mineralized dentin was also increased in mutant teeth. As shown in Figure 3, abundant DMP1 transcripts were detected in *wt/wt* odontoblasts whereas *op/op* odontoblasts showed little DMP1 expression.

To determine the effect of csCSF-1 on teeth and bone, transgenic *op/opCS* mice were analyzed. High levels of CSF-1 were detected in bone (705 pg/mg protein) and, to a lesser extent, in teeth (160 pg/mg protein). Marrow stromal cells isolated from *op/opCS* mice showed detectable csCSF-1, with approximately 4.8 pg CSF-1/mg protein. In contrast, circulating csCSF-1 or expression of the transgene in tissues other than bone and teeth was not detected. *Op/opCS* mice showed marked resolution of osteopetrosis. In Figure 4, x-rays of *op/op* mice at 6 and 14 weeks show marked skeletal sclerosis, with dense radiopaque bone in the tibial and femoral

metaphysis with obliteration of marrow spaces. In contrast, 6 week *op/opCS* show radiolucent marrow spaces similar to *wt/wt* mice. There is mild residual metaphyseal sclerosis in the femur and tibia that continued to resolve until at least 14 weeks. By histomorphometry (Table 1), *op/opCS* mice at 14 weeks showed increased %BV/TV that correlated with increased numbers of bone trabeculae associated with increased bone remodeling. Compared to *op/op* mice, the growth plate width in *op/opCS* declined to near normal values and the number of osteoclasts per millimeter of bone surface in *op/opCS* was comparable to that in *wt/wt* mice.

Eruption of incisors and molars in *wt/wt* mice was observed 10 days after birth whereas teeth in *op/op* mice failed to erupt. As shown in Figure 5, csCSF-1 restored tooth eruption in *op/opCS*, although the onset of eruption was slightly delayed at 15 days. At 3 weeks, *op/opCS* mice showed chalky white incisors and at 10 weeks, incisors were malaligned with twisted tips that were prone to breakage. By SEM (Figure 6), first mandibular molars from 3 week *op/op* mice showed underdeveloped cusp tips compared to *wt/wt* with enamel chipping. On fracture analysis, *op/op* enamel was thin, lacked the normal parallel crystals seen in *wt/wt* enamel and it fractured on a different plane than the dentin, indicating a faulty dentin-enamel junction. The dentin showed decreased dentin tubules per dentin unit area and increased amount of intertubular dentin. Marked regression of these defects was observed in *op/opCS*, with molar contours on SEM similar to *wt/wt*. *Op/opCS* showed normal dentin morphology and thicker, more organized enamel and the enamel fractured on the same plane as dentin similar to that observed in *wt/wt* teeth. Although *op/opCS* enamel was improved, it was slightly thinner and less organized than *wt/wt* enamel and this likely contributed to the abnormal gross phenotype.

To determine if the tooth defects in *op/opCS* were associated with altered morphology and enamel/dentin matrix proteins, histologic preparations were examined and the expression pattern of amelogenin and DMP1 was assessed. By 3 weeks (Figure 7), *wt/wt* incisors showed well-organized periodontal ligaments (PDL), whereas those in *op/op* teeth were poorly formed with focal detachment from roots. The ameloblasts in mutant incisors and molars were flattened, atrophic and barely expressed amelogenin. Only ameloblasts in daughter tooth germs at the basal end of incisor roots showed abundant amelogenin transcripts. In contrast, in *op/opCS* mice, the PDL and root morphology resembled that in *wt/wt* mice, and odontomas were not identified. In *op/opCS*, amelogenesis was partially corrected. *Op/opCS* ameloblasts appeared more columnar with abundant cytoplasm than those in *op/op* mice and expressed amelogenin, although to a lesser extent than *wt/wt* ameloblasts. On H&E stained preparations shown in Figure 8, *op/op* molars showed disorganized odontoblasts and dentin with mineralization defects characterized by foci of globular mineralized dentin adjacent to unmineralized dentin. The predentin layer was widened and the mineralization front was irregular compared to that in *wt/wt* teeth. In *op/opCS* molars, an odontoblast layer, albeit flattened, was identified and the dentin morphology was restored to normal, with predentin and dentin being similar to *wt/wt*. At 3 weeks, DMP1 transcripts (Figure 8) remained persistently decreased in *op/op* odontoblasts, whereas expression of csCSF-1 in *op/opCS* mice increased DMP1 expression to near normal levels.

Discussion

The present study demonstrates, for the first time, that csCSF-1, sCSF-1 and CSF-1R are expressed in early ameloblasts and odontoblasts *in vivo*. Absence of CSF-1 isoforms in *op/op* teeth at 8 days prior to eruption was associated with shortened ameloblasts, loss of odontoblastic polarization and dramatically reduced DMP1 expression compared with *wt/wt* teeth. These findings indicate that CSF-1 is important for optimal cytodifferentiation and dentin matrix protein expression. Lack of DMP1 expression in *op/op* teeth may have contributed to altered tooth morphology since *op/op* mice share a number of defects reported in DMP1 null pups including reduced enamel, widened predentin zone and decreased mineralized dentin.

³⁶ The observation that DMP1 is markedly diminished in *op/op* odontoblasts is novel and suggests that CSF-1 may directly or indirectly regulate DMP1.

Expression of csCSF-1 in *op/opCS* mice resulted in tooth eruption and substantial resolution of osteopetrosis by x-ray and histomorphometric analysis, indicating that the csCSF-1 form alone is sufficient to support the formation of mature osteoclasts and induce osteoclast resorption. In previous studies, human csCSF-1 targeted to bone in *op/op* mice using the rat 2.4 kb collagen type I alpha promoter, completely rescued osteopetrosis by 4 weeks, whereas murine csCSF-1 targeted to endogenous sites using the murine CSF-1 promoter resulted in partial remission of osteopetrosis at 12 weeks.^{17,18} Tooth eruption was restored in these studies; however, the effect of csCSF-1 on bone histomorphometric parameters or tooth morphology was not examined. Our findings indicate that csCSF-1 restored osteoclast numbers in *op/opCS* mice at 14 weeks, although mild metaphyseal sclerosis persisted. This suggests that csCSF-1 may not be adequate to sustain osteoclastic bone resorption (ie., osteoclast activity cannot keep pace with the new trabecular bone formation.). Conflicting results between studies regarding the ability of csCSF-1 to rescue osteopetrosis may be due, in part, to the use of different promoters. For example, the rat 2.3 kb type I collagen promoter has been shown to drive GFP reporter gene expression in a larger population of osteoblasts and odontoblasts in transgenic mice compared to the rat 1.7 kb osteocalcin promoter.²⁹

In *op/opCS* mice, decreased bone resorption likely contributed to the minor delay in tooth eruption. In previous studies using *op/op* mice and CSF-1 deficient toothless (*tl*) rats, failure of eruption due to a lack of osteoclast-mediated bone resorption was implicated as the cause of altered tooth morphogenesis.^{14,37} Indeed, *in vitro* explants of *op/op* tooth germs, cultured in the presence of surrounding bone, showed poor development compared to those cultured in the absence of bone.³⁸ A report by D'Errico et al.,³⁹ however, indicated that *op/op* teeth, analyzed prior to eruption, have defective dentinogenesis. This is supported by the observation that dental pulp cells in one week *op/op* tooth germs have disordered extracellular matrix protein expression that may affect odontoblast differentiation and matrix production.⁴⁰ Our studies also showed altered odontogenesis and amelogenesis in *op/op* teeth as early as day 8. Thus, we propose that the severe dentin and enamel defects observed in *op/op* teeth at 3 weeks characterized by disorganized odontoblasts, decreased DMP1, abnormal dentin mineralization, atrophic ameloblasts, thin enamel and decreased amelogenin may be due, in part, to failure of eruption as well as to persistent absence of CSF-1 in dental cells during development.

csCSF-1, expressed in *op/opCS* teeth, improved odontoblast cell morphology compared to that in *op/op* mice and restored dentin on SEM and histologic analysis. The ability of csCSF-1 to correct dentin defects seen in *op/op* mice suggests that csCSF-1 may act in an autocrine fashion in odontoblasts to regulate DMP1 since odontoblasts express the CSF-1 receptor. Alternatively, csCSF-1 may indirectly regulate DMP1 via the release of other cytokines. Regulation of amelogenin may also occur through interaction of csCSF-1 on the surface of odontoblasts with its cognant receptor on ameloblasts. It is tempting to speculate that partial rather than complete rescue of the enamel defect in *op/opCS* mice is due, in part, to loss of cell contact between odontoblasts and ameloblasts during tooth development. Whether the mild delay in eruption contributed to the incomplete rescue of the enamel defect in *op/opCS* mice remains to be determined.

Decreased production of amelogenin in 3 week *op/opCS* teeth was associated with gross abnormalities that mimicked an amelogenesis imperfecta (AI)-like phenotype similar to that described in amelogenin-deficient mice.²⁴ In the latter model, incisors were chalky white with broken tips and, by SEM, enamel was disorganized and hypoplastic. In humans, AI is a diverse hereditary disorder selectively affecting enamel with individuals showing a hypoplastic, hypomaturation or hypocalcified phenotype.⁴¹ The hypoplastic form shows thin pitted enamel,

while hypomaturation results in chalky white enamel due to failure of matrix degradation, and hypocalcification leads to severe enamel chipping. The molecular basis of AI is not fully understood. Some cases are associated with decreased enamel matrix proteins. In X-linked or autosomal dominant forms, a haploinsufficiency of amelogenin or enamelin leads to AI.^{42–44} Delayed and/or failed eruption occurs more frequently in patients with AI (27%) than in non-AI subjects (8%) and has been identified in autosomal dominant and recessive, as well as X-linked AI.^{45–47} Cosegregation of hypoplastic enamel and delayed eruption in cases of AI suggests that the mutation causing this type of AI involves a gene expressed in ameloblasts as well as in surrounding odontogenic tissues.⁴⁷ The combination of defective enamel, dentin and eruption as seen in *op/op* mice has also been rarely reported in humans.⁴⁸ Thus, results in *op/op* models suggest that csCSF-1 may be a common link involved in mediating tooth matrix formation and eruption.

Our findings indicate that CSF-1 is a multifunctional molecule that plays a central role in regulating endogenous matrix formation and eruption *in vivo*. Genetic analysis would be relevant for determining whether a specific mutation in the human CSF-1 gene on 1p21 is a candidate gene for AI and/or dentin dysplasia, especially with eruption defects. Targeting csCSF-1 to the tooth microenvironment may provide novel therapeutic strategies for regulating enamel/dentin matrix proteins and eruption in dental disorders.

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Figure 1.

RT-PCR analysis of CSF-1R, sCSF-1 and csCSF-1 in ameloblasts and odontoblasts isolated by LCM. Ethidium bromide staining of amplified PCR products separated on 1.5% agarose gels is shown. GAPDH was included as a control for mRNA/cDNA integrity. A positive control, RNA from mouse spleen, was run in parallel in a separate gel. The 552 bp CSF-1R transcript and two transcripts for CSF-1 at 240 bp and 163 bp that represent secreted and cell surface forms of CSF-1 are shown.

Figure 2.

Morphologic analysis and *in situ* hybridization of amelogenin gene expression in *wt/wt* and *op/op* first molars at day 8. Hematoxylin stained *in situ* hybridization (A,D) and adjacent histologic sections (B,E) show flattened odontoblasts and ameloblasts, widened predentin zone and reduced enamel in *op/op* teeth compared to *wt/wt*. Bright field (A,D) and dark field (C,F) *in situ* hybridization images showed abundant amelogenin transcripts in *op/op* and *wt/wt* ameloblasts (black grains in A,D; white grains in C,F). Quantitative analysis (G) showed that amelogenin expression in *op/op* ameloblasts was similar to that detected in *wt/wt* ameloblasts. ^a*wt/wt* vs *op/op*, $p=0.22$ (NS) Od, odontoblasts; AM, ameloblasts; E, enamel

Figure 3.

Morphologic analysis and *in situ* hybridization of DMP1 gene expression in *wt/wt* and *op/op* incisors at day 8. Hematoxylin stained *in situ* hybridization preparations (A,B,D,E) show lack of polarized Od, widened predentin zone and decreased dentin in *op/op* incisors compared to *wt/wt*. Bright (B,E) and dark field images (C, F) and quantitative analysis (G) show a significant reduction in DMP1 transcripts in *op/op* Od compared to *wt/wt* Od. ^a *wt/wt* vs *op/op*, $p < 0.0001$ PD, predentin; D, dentin

Figure 4. Skeletal x-rays of *wt/wt*, *op/op* and *op/opCS* at 6 and 14 weeks. Compared to *wt/wt*, *op/op* mice showed diffuse osteosclerotic bone. At 6 weeks, *op/opCS* mice show radiolucent marrow spaces with mild residual metaphyseal sclerosis (arrows) that continued to resolve until at least 14 weeks.

Figure 5.

Tooth analysis in *op/op*, *wt/wt*, *op/opCS* at 3 wks and *wt/wt* and *op/opCS* at 10 wks. Gross appearance at 3 wks (upper panel) showed absence of teeth in *op/op* and normal incisors in *wt/wt* mice. *op/opCS* showed slightly delayed eruption and chalky white teeth. By 10 wks, *op/opCS* incisors were malaligned and chalky white with twisted tips prone to breakage (inset).

Figure 6.

SEM of whole and fractured first mandibular molars in *op/op*, *wt/wt* and *op/opCS* at 3 wks. Compared to *wt/wt* (A–C), *op/op* molars, had underdeveloped cusp tips with enamel chipping (D, arrow); on fracture, *op/op* molars showed thin, poorly organized enamel (E, arrow E) that fractured on a different plane than dentin (dotted line, dentin-enamel junction) and decreased amount of dentin tubules per unit area (F, arrow). *Op/opCS* molars showed regression of these defects with normal contour (G) and dentin morphology (I) and thicker more organized enamel (H).

Figure 7.

Comparative morphology and *in situ* hybridization of amelogenin gene expression in *wt/wt*, *op/op* and *op/opCS* incisors at 3 wks. Hematoxylin stained *in situ* hybridization sections of *wt/wt* incisors showed a well-formed periodontal ligament (A,C) and mature ameloblasts with abundant amelogenin (B), whereas *op/op* incisors showed a poorly developed periodontal ligament focally detached from bone (E,G, arrow) and flattened ameloblasts with little amelogenin (F). In *op/opCS*, the periodontal ligament was normal (I,K) and ameloblasts expressed amelogenin (J). Dark field images (D,H,L) and quantitative analysis (M) showed significantly increased amelogenin in *op/opCS* compared to *op/op*, with expression partially

restored to *wt/wt* levels. ^a*wt/wt* vs *op/op*, $p<0.0001$; ^b*wt/wt* vs *op/opCS*, $p<0.05$; ^c*op/op* vs *op/opCS*, $p<0.001$

Figure 8.

Comparative morphology and *in situ* hybridization of DMP1 gene expression in *wt/wt*, *op/op* and *op/opCS* molars at 3 wks. On H&E stained sections, *wt/wt* molars (A,B) show polarized Od, normal predentin and a smooth transition to dentin which is uniformly mineralized. *Op/op* molars (D,E) show disorganized Od, widened predentin and altered dentin mineralization with globular mineralized dentin (dark pink round areas) adjacent to unmineralized dentin (pale pink). In *op/opCS* molars (G,H), an Od layer is identified; predentin and dentin are similar to that in *wt/wt*. Dark field images (C,F,I) and quantitative analysis (J) showed significantly increased DMP1 in *op/opCS* compared to *op/op*, with expression restored to near normal levels. ^a*wt/wt* vs *op/op*, $p < 0.0001$; ^b*wt/wt* vs *op/opCS*, $p < 0.02$; ^c*op/op* vs *op/opCS*, $p = 0.0002$

Table 1

Histomorphometric Analysis

	wt/wt	op/op	op/opCS
BV/TV(%)	20.8+/-1.9	0	32.9+/-1.2 ($p=0.004$) **
Trabecular Thickness (um)	43.4+/-3.2	NT	40.8+/-0.52 ($p=0.597$) NS
Trabecular No./mm	4.5+/-0.3	NT	6.5+/-0.7 ($p=0.027$) *
Trabecular Separation (um)	188+/-20.6	NT	116.7+/-14.9 ($p=0.059$) NS
OC No./mmBS	2.9+/-0.4	NT	1.9+/-0.1 ($p=0.133$) NS
Growth Plate Width (mm)	115+/-4.3	364+/-35.2	143+/-10.3 ($p=0.022$) *

Histomorphometric analysis of the distal femoral metaphysis from *wt/wt*, *op/op* and *op/opCS* at 14 wks. The abnormal BV/TV and growth plate width in *op/op* mice was partially corrected in *op/opCS*. Osteoclast numbers per millimeter of bone surface in *op/opCS* were comparable to *wt/wt*. (%BV/TV in *op/op* mice is nil due to the absence of normal cancellous bone. Measurements not tested (NT) in *op/op* mice cannot be calculated due to the absence of cancellous bone (n = 3–6 mice/group). Data are expressed as mean+/-SE.

*
 $p<0.05$ or

**
 $p<0.01$ *op/opCS* vs *wt/wt*. NS, not significant, OC osteoclast; No., number.