RESEARCH REPORTS

Biological

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ABSTRACT

Bone sialoprotein (BSP) is an extracellular matrix protein found in mineralized tissues of the skeleton and dentition. BSP is multifunctional, affecting cell attachment and signaling through an RGD integrin-binding region, and acting as a positive regulator for mineral precipitation by nucleating hydroxyapatite crystals. BSP is present in cementum, the hard tissue covering the tooth root that anchors periodontal ligament (PDL) attachment. To test our hypothesis that BSP plays an important role in cementogenesis, we analyzed tooth development in a *Bsp* null (^{-/-}) mouse model. Developmental analysis by histology, histochemistry, and SEM revealed a significant reduction in acellular cementum formation on *Bsp*^{-/-} mouse molar and incisor roots, and the cementum deposited appeared hypomineralized. Structural defects in cementum-PDL interfaces in *Bsp*^{-/-} mice caused PDL detachment, likely contributing to the high incidence of incisor malocclusion. Loss of BSP caused progressively disorganized PDL and significantly increased epithelial down-growth with aging. *Bsp*^{-/-} mice displayed extensive root and alveolar bone resorption, mediated by increased RANKL and the presence of osteoclasts. Results collected here suggest that BSP plays a non-redundant role in acellular cementum formation, likely involved in initiating mineralization on the root surface. Through its importance to cementum integrity, BSP is essential for periodontal function.

KEY WORDS: bone sialoprotein, cementum, periodontal ligament, bone, alkaline phosphatase, extracellular matrix.

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Deficiency in Acellular Cementum and Periodontal Attachment in *Bsp* Null Mice

INTRODUCTION

Bone sialoprotein (BSP, or integrin binding sialoprotein) is an extracellular matrix protein in the Small Integrin Binding Ligand N-linked Glycoprotein (SIBLING) family, associated with mineralized tissues of the skeleton and dentition (Ganss *et al.*, 1999; Fisher and Fedarko, 2003). Like other SIBLING proteins, BSP is multifunctional, with roles in cell attachment and migration (through the integrin-binding RGD motif), cell signaling, collagen binding, and hydroxyapatite nucleation. The ability of BSP to act as a positive regulator of hydroxyapatite precipitation has been demonstrated *in vitro* (Hunter and Goldberg, 1993) and is of particular interest in terms of regulating mineralized tissue development. Mice null for the *Bsp* gene (*Bsp*^{-/-}) feature delayed bone growth and mineralization (Malaval *et al.*, 2008).

In addition to its inclusion in bone extracellular matrix, BSP is present in both acellular and cellular cementum (MacNeil *et al.*, 1994; McKee *et al.*, 1996). Acellular cementum is the thin, mineralized tissue covering the cervical portion of the tooth root, important for attachment of the periodontal ligament (PDL) to the root surface (Bosshardt, 2005; Foster *et al.*, 2007). Cellular cementum is a more bone-like tissue covering apical portions of roots. Developmental factors directing cementogenesis remain poorly defined, hampering progress toward more effective therapies for cementum regeneration. The finding that BSP was present in cementum and strongly expressed by cementoblasts led to speculation that this protein might contribute to cementum formation and mineralization (Somerman *et al.*, 1990; MacNeil *et al.*, 1995); however, this hypothesis has remained untested in the 2 decades since these findings. Here, we analyzed the function of BSP in cementogenesis and periodontal stability by determining the effects of the lack of BSP on tooth formation.

MATERIALS & METHODS

Animals

Animal procedures were performed in accordance with guidelines of the Canadian Council on Animal Care and Animal Care and Veterinary Services, University of Western Ontario. Preparation and genotyping of *Bsp*^{-/-} and wild-type (WT) mice have been described previously (Malaval *et al.*, 2008). Mice were maintained on a mixed 129/CD1 background and fed a standard mouse diet (2018 Tekland Global 18% protein diet, Harlan Laboratories, Indianapolis, IN, USA). From 3 to 6 animals were analyzed *per* genotype at ages 14, 26, 30, and 60 post-natal days (dpn). Serum biochemistry was analyzed at the NIH Veterinary Services Clinical Chemistry (Bethesda, MD, USA).

Micro-computed Tomography (microCT) and Scanning Electron Microscopy (SEM)

MicroCT of formalin-fixed hemi-mandibles was performed with eXplore Locus SP (GE Healthcare, Mississauga, ON, Canada). 2D images were acquired at 80 kVp and 80 μ A, with an integration time of 1,600 msec/frame, 4 frames/view, and a total of 900 views at increments of 0.4 degrees. Images were constructed at a spatial resolution of 13 μ m and calibrated with a cortical bone phantom (SB3; Gammex RMI, Milwaukee, WI, USA) having a hydroxyapatite equivalent of 1,100 mg/cc (White, 1978). Data were analyzed with MicroView ABA version 2.2 (GE Healthcare).

For SEM analysis, extracted and cleansed molars were osmium-coated. For fracture analysis, an ultra-fine diamond burr (Brasseler DET6UF-31, Brasseler USA, Savannah, GA, USA) was used to drill through crowns, and teeth were fractured longitudinally. SEM images were analyzed in secondary electron mode at 1-10 kV on a Leo 1540 XB FIB/SEM (Carl Zeiss, Western Nanofabrication Facility, University of Western Ontario, London, ON, Canada).

Histological Procedures

Procedures for histology, *in situ* hybridization (ISH), and immunohistochemistry (IHC) were previously described on Bouin's fixed, decalcified, paraffin-embedded samples (Foster, 2012; Foster *et al.*, 2012). Histomorphometry was used to quantify cementum, root-lining cells, and epithelial attachment, and statistical analysis was performed by independent-samples *t* test. ISH for *Bsp* mRNA used digoxigenin (DIG)-labeled cRNA probe (Marian Young, NIDCR, Bethesda, MD, USA) with NBT/BCIP substrate. IHC used biotinylated secondary antibodies and peroxidase substrate. Primary antibodies included rabbit anti-BSP (1:200; Renny Franceschi, University of Michigan, Ann Arbor, USA), rat anti-alkaline phosphatase (TNAP/ALPL, 1:200; R&D Systems, Minneapolis, MN, USA), LF-175 rabbit anti-osteopontin (OPN; 1:200) (Larry Fisher, NIDCR), rabbit anti-cytokeratin (Abcam, Cambridge, MA, USA), goat anti-RANKL (1:50; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and rat anti-neutrophil antibody (1:200; NIMP; Abcam). Undecalcified, glutaraldehyde/formalin-fixed tissues were LR white-embedded for ultramicrotome sectioning (1 μ m) for

von Kossa staining (Everts *et al.*, 2012), or processed and methylmethacrylate-embedded for microtome sectioning (6 μ m) for Goldner's trichrome staining (Kacena *et al.*, 2004).

RESULTS

Bsp^{-/-} Mice Exhibit Defective Acellular Cementum Formation

Bsp mRNA is highly expressed by cementoblasts during tooth root formation, as well as by osteoblasts of the alveolar bone (Fig. 1A). BSP protein is localized to alveolar bone and to acellular and cellular cementum covering the cervical and apical portions of the molar, respectively (Figs. 1B, 1C). The absence of BSP immunostaining was confirmed in *Bsp*^{-/-} mice (Figs. 1D, 1E).

Over the course of root development, *Bsp*^{-/-} mouse molar teeth displayed deficiency in acellular cementum formation. At 14 post-natal days (dpn), a basophilic layer of acellular cementum covered the root dentin in WT mice (Figs. 1F, 1G), while in *Bsp*^{-/-} molars, this layer was thinner (Figs. 1H, 1I). At 26 dpn, WT acellular cementum had grown thicker, and inserted PDL fibers radiated from the surface of the cervical root (Figs. 1J, 1K). Conversely, *Bsp*^{-/-} acellular cementum showed no additional growth (Figs. 1L, 1M). It was common to find PDL disorganization in *Bsp*^{-/-} molars, and a structural defect in the cementum-PDL interface was indicated by detachment in all *Bsp*^{-/-} samples (possibly partly due to tissue processing, but not present in WT samples). By 60 dpn, acellular cementum remained stunted in *Bsp*^{-/-} mice, and PDL detachment was present in all *Bsp*^{-/-}, but not WT, samples (Figs. 1N vs. 1P). Histomorphometry confirmed that the apparent acellular cementum thickness was significantly reduced in *Bsp*^{-/-} compared with WT molars (Fig. 1R). Alcian blue staining supported diminished acellular cementum, and Goldner's trichrome staining indicated a lack of mineralized cementum matrix on *Bsp*^{-/-} molar surfaces (Appendix Fig. 1). The continuously erupting mouse incisor features acellular cementum on the lingual root analog, and *Bsp*^{-/-} incisors featured reduced cementum thickness and PDL disorganization (Appendix Fig. 2), contributing to a high rate of incisor malocclusion in these mice. The quantity of cellular cementum covering the apical molar root was unaffected in *Bsp*^{-/-} compared with WT molars at 60 dpn (Figs. 1O vs. 1Q), though at 26 dpn mineralization was delayed in this layer (Appendix Fig. 3).

Failure of Acellular Cementum Mineralization in *Bsp*^{-/-} Mice

SEM analysis supported histochemical observations that the thin layer of acellular cementum on *Bsp*^{-/-} molar roots was not mineralized, because no mineralized cementum ultrastructure could be identified on the cervical root surfaces of *Bsp*^{-/-} molars at 60 dpn, in contrast to the mineralized cementum layer on age-matched WT molars (Fig. 2).

We considered developmental possibilities predisposing to the inhibition of cementum formation in *Bsp*^{-/-} mice. By histology and von Kossa staining, dentin, required as a foundation for cementum deposition, appeared sound and well-mineralized in *Bsp*^{-/-} mice (Fig. 1; Appendix Figs. 4A, 4B). Acellular cementum

Figure 1. Bone sialoprotein is required for proper acellular cementum formation. (A) *Bsp* mRNA is expressed by cementoblasts (cb) and osteoblasts (ob) during molar tooth root development at 14 dpn. (B, C) BSP protein is localized to alveolar bone (b), acellular cementum (ac), and cellular cementum (cc) at 26 dpn. White box in B is shown at higher magnification in C to highlight acellular cementum. BSP immunolabeling in (D) WT alveolar bone is absent in (E) *Bsp*^{-/-} mice. (F, G) Newly formed acellular cementum anchors periodontal ligament (pdl) attachment in WT molars. (H, I) Acellular cementum in *Bsp*^{-/-} molars is thin compared with that in WT mice. In contrast to the thickened acellular cementum in (J, K) WT mice at 26 dpn, (L, M) *Bsp*^{-/-} molars feature stunted acellular cementum, and regions of PDL detachment from the tooth (*) were common, indicating defects in the cementum-PDL interface. The well-organized attachment of the acellular cementum, PDL, and bone in (N) WT molars at 60 dpn is severely degraded in (P) *Bsp*^{-/-} molars, where stunted acellular cementum is associated with PDL disorganization. In contrast, cellular cementum in (O) WT and (Q) *Bsp*^{-/-} mice at 60 dpn is not different in size. (R) Both buccal and lingual acellular cementum samples are significantly reduced in thickness in *Bsp*^{-/-} mice compared with their WT counterparts (*indicates significant difference, $p < 0.05$). Scale bar is 200 μ m in panels A, B, F, H, O, and Q (original, 100X), 50 μ m in panels C, D, E, G, I, K, M, N, and P (original, 400X), and 400 μ m in panels J and L (original, 50X).

formation requires migration of dental follicle/cementoblast precursors to the nascent root surface. Root-lining cells were present in similar numbers in *Bsp*^{-/-} vs. WT first molar roots at 14 dpn (Appendix Fig. 4C). PDL fringe fibers at the root surface suggested that the proper acellular cementum matrix was present for cementogenesis (Appendix Figs. 4D, 4E). Immunostaining revealed the deposition of osteopontin (OPN) on the root surface, confirming functional cementoblasts in *Bsp*^{-/-} mice (Appendix Figs. 4F-4I). Markers of local and systemic mineral metabolism were assayed. TNAP immunolabeling in *Bsp*^{-/-} vs. WT molars (Appendix Figs. 4J-4M), and serum alkaline phosphatase, calcium, and phosphorus, were undiminished in *Bsp*^{-/-} mice (Appendix Table), ruling out systemic mineral ion disturbance as an underlying cause of the cementum defect.

Periodontal Breakdown in *Bsp*^{-/-} Mice

Defective acellular cementum in *Bsp*^{-/-} mice preceded a progressive breakdown of periodontal organization at later ages. WT molars at 26 dpn displayed dense and highly organized collagen fiber insertions in the cervical root, visualized by picrosirius red staining under polarized light (Figs. 3A, 3B). Conversely, *Bsp*^{-/-} molars lost PDL organization in the cervical root by this age (Figs. 3C, 3D), a condition that degenerated further over time (Appendix Fig. 5). Defective cementum-PDL attachment in *Bsp*^{-/-} molars allowed for significant down-growth of gingival epithelial tissues into the periodontal region, initiating a long junctional epithelium (Figs. 3E, 3F; Appendix Fig. 6). Periodontal disarray in *Bsp*^{-/-} mice was accompanied by increased incidence of root resorption by 60 dpn (Figs. 3G-3J) and increased numbers of osteoclast-like cells, RANKL immunostaining, and bone resorption in alveolar bone (Figs. 3K-3N). There was no indication that inflammation played a significant role in the periodontal breakdown, based on histology and the lack of neutrophil infiltration into the periodontia (Appendix Fig. 7).

Tooth and bone resorption was documented further by SEM and microCT. SEM revealed extensive pitting in *Bsp*^{-/-} vs. WT molars, concentrated in the cervical roots (Figs. 4A-4F). Lack of acellular cementum was indicated by clearly visible dentinal tubules on the external surfaces of *Bsp*^{-/-} molar roots. MicroCT analysis revealed extensive reduction in alveolar bone height in *Bsp*^{-/-} vs. WT mandibles, particularly along the lingual aspect (Figs. 4G-4J).

DISCUSSION

BSP is an extracellular matrix protein present in bone and cementum. Here we demonstrate for the first time that lack of BSP inhibited the formation and mineralization of acellular cementum. Further, reduction and loss-of-function of the acellular cementum in *Bsp*^{-/-} mice led to structural defects in the cementum-PDL interface, PDL disorganization, down-growth of epithelium, and increased tooth and bone resorption. Results collected here demonstrated that BSP plays a non-redundant role in acellular cementum formation, likely involved in initiating mineralization and/or promoting appositional cementum growth. Because of its importance to cementum formation, BSP is essential for the formation of a functional periodontium.

Figure 2. Lack of mineralized acellular cementum ultrastructure on *Bsp*^{-/-} mouse molar teeth. Secondary electron SEM analysis on fractured first molars at 60 dpn indicated that, compared with the mineralized acellular cementum layer visible in WT molars at various magnifications (A, C, E), no mineralized cementum ultrastructure could be identified on the roots of *Bsp*^{-/-} molars (B, D, F). Some cracking is evident as an artifact of sample preparation. Abbreviations: enam = enamel; ac = acellular cementum; pdl = periodontal ligament. Scale bar is 50 μ m in panels A and B (original, 500X), 10 μ m in panels C and D (original, 2,000X), and 2 μ m in panels E and F (original, 10,000X).

Acellular Cementum as a Mineralization-sensitive Tissue

Lack of BSP led to a severe developmental inhibition of acellular cementum formation, shown here by histology, histochemistry, immunohistochemistry, and SEM. As a late stage in odontogenesis, cementum formation depends on a number of prior developmental stages. Our results suggested that the defect did not result from defective dentin, lack of migration/position of precursor cells, or root-surface collagen fringe fibers [considered the extracellular matrix proper for acellular cementum (Bosshardt and Schroeder, 1996)]. Local TNAP deficiency (by IHC), hypophosphatemia, and hypocalcemia were ruled out as causes of cementum inhibition in *Bsp*^{-/-} mice. It is notable that acellular cementum formation is similarly inhibited in the *Alpl*^{-/-} mouse model featuring loss of TNAP function (Beertsen *et al.*, 1999; McKee *et al.*, 2011; Foster *et al.*, 2012). *Alpl*^{-/-} mice harbor increased levels of the mineral inhibitor pyrophosphate (PP_i) and serve as a model of hypophosphatasia (HPP), where lack of acellular cementum predisposes to tooth loss (van den Bos *et al.*, 2005). We have demonstrated that reduction of PP_i, in turn,

Figure 3. Periodontal breakdown in *Bsp*^{-/-} mice. (A, B) Picosirius-red-stained sections viewed under polarized light reveal a high degree of organization and directionality (indicated by high-intensity yellowish fibers) of periodontal ligament (pdl) collagen fibrils between acellular cementum (ac) and bone (b) in WT molars at 26 dpn. (C, D) *Bsp*^{-/-} molars at 26 dpn feature loss of organization of collagen fibers in the cervical PDL region (#), where detachment (*) is also present. (E) Pan-keratin immunohistochemistry (red-brown color) indicates a well-maintained junctional epithelium (je) in WT molars at 60 dpn, whereas (F) *Bsp*^{-/-} molars feature down-growth and establishment of a long junctional epithelium in association with loss of PDL attachment. Compared with (G, H) WT molars at 60 dpn, (I, J) *Bsp*^{-/-} molar teeth feature extensive root resorption (chevrons). Compared with (K, L) WT molars, *Bsp*^{-/-} molars at 60 dpn feature (M) dramatically increased localization of receptor activator of nuclear factor kappa-B ligand (RANKL) near tooth and bone surfaces, and (N) numerous osteoclast-like cells [arrowheads; large, multinucleated, tartrate-resistant acid phosphatase (TRAP)-positive] on alveolar bone surfaces. Scale bar is 200 μ m in panels A and C (original, 100X), 50 μ m in panels B, D, H, J, L, and N (original, 400X), 100 μ m in panels G, I, K, and M (original, 200X), and 100 μ m in panels E and F (original, 100X).

promotes increased acellular cementum formation (Foster *et al.*, 2011, 2012), and hypothesized that acellular cementum represents a tissue strongly regulated at the physicochemical level of mineralization, *i.e.*, hydroxyapatite nucleation and growth. Cellular cementum size was undiminished by the loss of BSP, though mineralization was delayed at earlier ages; functional consequences of this are under study. Cellular cementum was unaffected in *Alpl*^{-/-} mice (Beertsen *et al.*, 1999) and in mice where acellular cementum increased *via* decreased PP_i (Foster *et al.*, 2012), supporting the view that these tissues are subject to different developmental influences.

Acellular cementum forms as a progressive mineralization of the PDL collagen fiber bundles inserting into the root surface. We have previously shown BSP binds collagen with high affinity (Tye *et al.*, 2005) and mediates hydroxyapatite formation (Hunter and Goldberg, 1993; Harris *et al.*, 2000) and that hydroxyapatite nucleation potency is increased 10-fold when BSP is incubated with collagen (Baht *et al.*, 2010). Considering that BSP is localized across the width of the acellular cementum (McKee *et al.*, 1996; Bosshardt *et al.*, 1998), and based on our past and present findings, it is likely that BSP is critical for proper cementum formation and function.

Another SIBLING protein, OPN, was present at *Bsp*^{-/-} root surfaces, as in WT controls. OPN functions as an inhibitor of hydroxyapatite crystal growth *in vitro* and *in vivo* (Hunter *et al.*, 1994; Boskey *et al.*, 2002). The presence of OPN in the absence of BSP may additionally tip the balance toward inhibition of cementum mineralization. It is also possible that another function of BSP (*e.g.*, cell adhesion, signaling) may contribute to the cementum phenotype of the *Bsp*^{-/-} mouse, and this is currently under study.

Loss of BSP Leads to Periodontal Breakdown

A functional periodontal complex depends on stable insertion of PDL collagen fibers into the acellular cementum, as well as ability for remodeling on the bone surface

of the PDL space. We propose that loss of acellular cementum in *Bsp*^{-/-} mice led to a progressive degradation of periodontal integrity over time. Lack of PDL-tooth anchorage was followed by PDL disorganization, epithelial down-growth, and tooth and alveolar bone resorption. These are hallmarks of periodontal breakdown associated with periodontal disease; however, this phenotype developed without an apparent inflammatory response in the periodontal compartment, suggesting failure of function/adaptation of the periodontium. Similar examples of periodontal degradation have resulted in transgenic mouse models with compromised periodontal function, e.g., *Dmp1* null (Ye *et al.*, 2008) and *periostin* null (Rios *et al.*, 2008) mice. Substitution of a soft diet for pelletized chow is currently being studied to determine the contribution of occlusal stress to the incidence of periodontal breakdown and incisor malocclusion.

An unexpected finding was the increased incidence of tooth and alveolar bone resorption in *Bsp*^{-/-} mice. Previous studies in *Bsp*^{-/-} mice showed that BSP deficiency impairs osteoclastogenesis and bone resorption *in vivo* and *in vitro* (Malaval *et al.*, 2008; Boudiffa *et al.*, 2010). Conversely, BSP over-expression increased osteoclastic activity (Valverde *et al.*, 2008). We propose that increased resorption in the periodontal compartment of *Bsp*^{-/-} mice is the result of local loss of integrity and function in a tissue that functions under high mechanical stress and depends on rapid remodeling for proper maintenance. While increased presence of RANKL and TRAP-positive osteoclast-like cells was noted in periodontia of *Bsp*^{-/-} mice, the cause for increased tooth and bone resorption is being explored further in this model.

In summary, results from these studies show, for the first time, that BSP is critical for acellular cementum formation and periodontal attachment. The role of BSP in acellular cementogenesis is likely to be in promoting mineralization at the root surface to anchor PDL fibers and provide strong attachment of the tooth to the alveolar bone. Loss-of-function of BSP may predispose to periodontal disease through defective cementum-PDL attachment and inability to prevent establishment of biofilm in the periodontal space. Ongoing studies will further elucidate potential roles of BSP in cementum formation and regeneration, and in wider periodontal biology.

Figure 4. Increased tooth root and alveolar bone resorption in *Bsp*^{-/-} mice. Compared with the smooth surfaces of (A, C) 10-week-old WT molars viewed by backscattered SEM, (B, E) *Bsp*^{-/-} teeth exhibit abundant pitting (white arrows in B and E) of the root surface, consistent with external root resorption, concentrated on the cervical portion of the root surface. White boxes in A and B indicate regions shown at higher magnification in C and E, respectively. Compared with the (D) WT molar root surface at high magnification, (F) *Bsp*^{-/-} molar roots display exposed dentinal tubules, indicating the absence of cementum in regions of root resorption. MicroCT revealed that, compared with (G, I) WT mandibles, (H, J) *Bsp*^{-/-} mandibles display extensive reduction in alveolar bone height, especially on the lingual aspect (black arrows in H and J). Abbreviations: M1 = first molar; M2 = second molar; M3 = third molar.

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REFERENCES

- Baht GS, O'Young J, Borovina A, Chen H, Tye CE, Karttunen M, et al. (2010). Phosphorylation of Ser136 is critical for potent bone sialoprotein-mediated nucleation of hydroxyapatite crystals. *Biochem J* 428:385-395.
- Beertsen W, VandenBos T, Everts V (1999). Root development in mice lacking functional tissue non-specific alkaline phosphatase gene: inhibition of acellular cementum formation. *J Dent Res* 78:1221-1229.
- Boskey AL, Spevak L, Paschalis E, Doty SB, McKee MD (2002). Osteopontin deficiency increases mineral content and mineral crystallinity in mouse bone. *Calcif Tissue Int* 71:145-154.
- Bosshardt DD (2005). Are cementoblasts a subpopulation of osteoblasts or a unique phenotype? *J Dent Res* 84:390-406.
- Bosshardt D, Schroeder H (1996). Cementogenesis reviewed: a comparison between human premolars and rodent molars. *Anat Rec* 245:267-292.
- Bosshardt D, Zalzal S, McKee M, Nanci A (1998). Developmental appearance and distribution of bone sialoprotein and osteopontin in human and rat cementum. *Anat Rec* 250:13-33.
- Boudiffa M, Wade-Guëye NM, Guignandon A, Vanden-Bossche A, Sabido O, Aubin JE, et al. (2010). Bone sialoprotein deficiency impairs osteoclastogenesis and mineral resorption in vitro. *J Bone Miner Res* 25:2669-2679.
- Everts V, Niehof A, Tigchelaar-Gutter W, Beertsen W (2012). Transmission electron microscopy of bone. *Methods Mol Biol* 816:351-363.
- Fisher L, Fedarko N (2003). Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connect Tissue Res* 44(Suppl 1):33-40.
- Foster BL (2012). Methods for studying tooth root cementum by light microscopy. *Int J Oral Sci* 4:119-128.
- Foster BL, Popowics TE, Fong HK, Somerman MJ (2007). Advances in defining regulators of cementum development and periodontal regeneration. *Curr Top Dev Biol* 78:47-126.
- Foster BL, Nagatomo KJ, Bamashmous SO, Tompkins KA, Fong H, Dunn D, et al. (2011). The progressive ankylosis protein regulates cementum apposition and extracellular matrix composition. *Cells Tissues Organs* 194:382-405.
- Foster BL, Nagatomo KJ, Nociti FH, Fong H, Dunn D, Tran AB, et al. (2012). Central role of pyrophosphate in acellular cementum formation. *PLoS One* 7:e38393.
- Ganss B, Kim R, Sodek J (1999). Bone sialoprotein. *Crit Rev Oral Biol Med* 10:79-98.
- Harris NL, Rattray KR, Tye CE, Underhill TM, Somerman MJ, D'Errico JA, et al. (2000). Functional analysis of bone sialoprotein: identification of the hydroxyapatite-nucleating and cell-binding domains by recombinant peptide expression and site-directed mutagenesis. *Bone* 27:795-802.
- Hunter GK, Goldberg HA (1993). Nucleation of hydroxyapatite by bone sialoprotein. *Proc Natl Acad Sci USA* 90:8562-8565.
- Hunter GK, Kyle CL, Goldberg HA (1994). Modulation of crystal formation by bone phosphoproteins: structural specificity of the osteopontin-mediated inhibition of hydroxyapatite formation. *Biochem J* 300(Pt 3):723-728.
- Kacena MA, Troiano NW, Wilson KM, Coady CE, Horowitz MC (2004). Evaluation of two different methylmethacrylate processing, infiltration, and embedding techniques on the histological, histochemical, and immunohistochemical analysis of murine bone samples. *J Histotechnol* 27:119-130.
- MacNeil R, Sheng N, Strayhorn C, Fisher L, Somerman M (1994). Bone sialoprotein is localized to the root surface during cementogenesis. *J Bone Miner Res* 9:1597-1606.
- MacNeil R, Berry J, D'Errico J, Strayhorn C, Piotrowski B, Somerman M (1995). Role of two mineral-associated adhesion molecules, osteopontin and bone sialoprotein, during cementogenesis. *Connect Tissue Res* 33:1-7.
- Malaval L, Wade-Guëye NM, Boudiffa M, Fei J, Zirngibl R, Chen F, et al. (2008). Bone sialoprotein plays a functional role in bone formation and osteoclastogenesis. *J Exp Med* 205:1145-1153.
- McKee MD, Zalzal S, Nanci A (1996). Extracellular matrix in tooth cementum and mantle dentin: localization of osteopontin and other noncollagenous proteins, plasma proteins, and glycoconjugates by electron microscopy. *Anat Rec* 245:293-312.
- McKee MD, Nakano Y, Masica DL, Gray JJ, Lemire I, Heft R, et al. (2011). Enzyme replacement therapy prevents dental defects in a model of hypophosphatasia. *J Dent Res* 90:470-476.
- Rios H, Ma D, Xie Y, Giannobile W, Bonewald L, Conway S, et al. (2008). Periostin is essential for the integrity and function of the periodontal ligament during occlusal loading in mice. *J Periodontol* 79:1480-1490.
- Somerman M, Shroff B, Agraves W, Morrison G, Craig A, Denhardt D, et al. (1990). Expression of attachment proteins during cementogenesis. *J Biol Buccale* 18:207-214.
- Tye CE, Hunter GK, Goldberg HA (2005). Identification of the type I collagen-binding domain of bone sialoprotein and characterization of the mechanism of interaction. *J Biol Chem* 280:13487-13492.
- Valverde P, Zhang J, Fix A, Zhu J, Ma W, Tu Q, et al. (2008). Overexpression of bone sialoprotein leads to an uncoupling of bone formation and bone resorption in mice. *J Bone Miner Res* 23:1775-1788.
- van den Bos T, Handoko G, Niehof A, Ryan L, Coburn S, Whyte M, et al. (2005). Cementum and dentin in hypophosphatasia. *J Dent Res* 84:1021-1025.
- White DR (1978). Tissue substitutes in experimental radiation physics. *Med Phys* 5:467-479.
- Ye L, Zhang S, Ke H, Bonewald LF, Feng JQ (2008). Periodontal breakdown in the Dmp1 null mouse model of hypophosphatemic rickets. *J Dent Res* 87:624-629.