

Functional Role of Periostin Expression in Development and Wound Repair: Implications for Connective Tissue Disease.

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Abstract

Integrity of the extracellular matrix (ECM) is essential for maintaining the normal structure and function of connective tissues. ECM is secreted locally by cells and organized into a complex meshwork providing physical support to cells, tissues, and organs. Initially thought to act only as a scaffold, the ECM is now known to provide a myriad of signals to cells regulating all aspects of their phenotype from morphology to differentiation. Matricellular proteins are a class of ECM related molecules defined through their ability to modulate cell-matrix interactions. Matricellular proteins are expressed at high levels during development, but typically only appear in postnatal tissue in wound repair or disease, where their levels increase substantially. Members of the CCN family, tenascin-C, osteopontin, secreted protein acidic rich in cysteine (SPARC), bone sialoprotein, thrombospondins, and galectins have all been classed as matricellular proteins. Periostin, a 90kDa secreted homophilic cell adhesion protein, was recently added to matricellular class of proteins based on its expression pattern and function during development as well as in wound repair. Periostin is expressed in connective tissues including the periodontal ligament, tendons, skin and bone, and is also prominent in neoplastic tissues, cardiovascular disease, as well as in connective tissue wound repair. This review will focus on the functional role of periostin in tissue physiology. Fundamentally, it appears that periostin influences cell behaviour as well as collagen fibrillogenesis, and therefore exerts control over the structural and functional properties of connective tissues in both health and disease.

Keywords: Periostin, matricellular protein, connective tissue, Marfan's Syndrome,

Wound Repair.

Abbreviations:

ECM: Extracellular matrix

CTGF: Connective Tissue Growth Factor

FA: Focal adhesion

HGF: Human Gingival Fibroblast

OSF-2: Osteoblast Specific Factor-2

RCO: Rat Calvarial Osteoblast

SPARC: Secreted Protein Acidic Rich in Cysteine

TSP: Thrombospondin

Introduction

The extracellular matrix (ECM) is a key regulator of cell behaviour, providing molecular signals to resident cell populations that are essential for maintenance of normal connective tissue structure and function (Berrier and Yamada, 2007; Culav et al., 1999; Lukashev and Werb, 1998; Stamenkovic, 2003). ECM is composed of many different proteins, including the structural proteins fibronectin, collagens, laminins, vitronectin, as well as specialized proteins such as proteoglycans, glycoproteins, growth factors, and matrix metalloproteinases (Stamenkovic, 2003; Tayebjee et al., 2003). It is the amount, type, and composition of the ECM that give connective tissues their unique properties (Culav et al., 1999; Lukashev and Werb, 1998). The ECM is a dynamic structure, continually remodeling in response to mechanical stimuli, integrin signaling, and pathology (Berk et al., 2007; Gallagher et al., 2007; Hinz and Gabbiani, 2003; Larsen et al., 2006). As molecular techniques have advanced, it has been possible to learn more about ECM remodeling and the functions of each protein subclasses. In particular genetic knockout mice have proven an excellent model for investigating ECM molecules (Muller, 1999). Such models have highlighted that during development and wound repair, synthesis of matrix components can be highly transient, providing organizational cues to specific cell populations in a tightly controlled, time dependent manner. Adhesion of cells to ECM through integrin receptors regulates their shape, proliferation, intracellular signaling and differentiation, thus maintaining normal tissue function (Humphries et al., 2004; Lock et al., 2008; Zelenka, 2004).

During wound repair and certain pathologies, changes occur in the composition of the ECM, providing signals to the cells that mediate repair or, if misregulated, can result

in development of various pathologies (Berk et al., 2007; Darby and Hewitson, 2007; Grzesik and Narayanan, 2002; Midwood et al., 2004; Raines, 2000). In 2000, Paul Bornstein proposed that there was a family of secreted ECM proteins that could be linked through their common functionality. They termed these proteins “matricellular” proteins to highlight their influence on cell-matrix interactions. Matricellular proteins are important during development, but are typically restricted to tissue remodeling and wound repair in the normal adult. Matricellular proteins interact with cell surface receptors such as integrins and are able to bind growth factors as well as to the structural components of the matrix such as collagen (Baril et al., 2007; Butcher et al., 2007; Gillan et al., 2002; Shimazaki et al., 2008). Based on this definition, several proteins have now been identified as matricellular proteins (Bornstein, 2000), including the CCN family (including CCN2/connective tissue growth factor) (Leask and Abraham, 2006), thrombospondins (Bornstein et al., 2000; Chen et al., 2000), and galectins (Elola et al., 2007; He and Baum, 2006). A comprehensive list of known matricellular proteins is shown in Table 1 and functions of matricellular proteins in Table 2. However, in this review, the focus will be on periostin, a relatively recent addition to the matricellular protein family, despite being first identified 15 years ago as osteoblast specific factor-2 (OSF-2) (Takeshita et al., 1993).

Periostin: A Novel Matricellular Protein

Periostin was originally identified as an 811-amino acid protein secreted by murine osteoblasts, which had structural homology to the insect axonal guidance protein fasciclin-1 (Takeshita et al., 1993). Originally termed osteoblast specific factor-2 (OSF-2),

it was renamed periostin due localized expression in the periosteum and the periodontal ligament (Kruzynska-Frejtag et al., 2004). In humans, the periostin gene is located on chromosome 13, at map position 13q13.3, and the protein is 835 amino acids in size. Periostin is a disulfide linked 90-kDa heparin-binding N terminus-glycosylated protein, containing 4 tandem fasciclin (Fas1) domains (Kudo et al., 2007; Litvin et al., 2004). Norris and colleagues, in 2008, were the first to propose that periostin should be classed as a matricellular protein, based on its apparent biological functions in the developing murine cardiac system (Norris et al., 2008a).

Due to an explosion of papers in the last two years, expression of periostin has now been confirmed in many other tissues and pathologies. Thus far, periostin has been found in bone (Horiuchi et al., 1999; Litvin et al., 2004; Nakazawa et al., 2004; Oshima et al., 2002), skin (Norris et al., 2007; Roy et al., 2007; Tilman et al., 2007), periodontal ligament (Horiuchi et al., 1999; Kii and Kudo, 2007; Kruzynska-Frejtag et al., 2004; Lallier and Spencer, 2007; Suzuki et al., 2004), muscle injury (Goetsch et al., 2003; Kudo et al., 2004), vascular injury (Lindner et al., 2005), myocardial infarction (Dorn, 2007; Iekushi et al., 2007; Oka et al., 2007; Shimazaki et al., 2008), epithelial ovarian cancer (Gillan et al., 2002), colorectal cancer (Tai et al., 2005), and pulmonary vascular remodeling (Li et al., 2004; Woodruff et al., 2007). Furthermore, periostin expression is known to be prominent in fibrotic conditions, including sub-epithelial fibrosis in bronchial asthma (Takayama et al., 2006), as well as in bone marrow fibrosis (Oku et al., 2008). Many of the initial research performed on periostin was descriptive in nature, confirming periostin expression in different tissues and pathologies. However, with the derivation of the periostin knockout mouse model (Kii et al., 2006; Rios et al., 2005), the

functions of periostin in development, wound repair, and disease, are beginning to be revealed.

Phenotype of the Periostin Knockout Mouse

The first description of the periostin knockout mouse was by Rios and colleagues (Rios et al., 2005). The phenotype of the periostin knockout mouse is of great interest due to the number of tissues affected. As with other matricellular proteins, periostin deficiency does not result in embryonic lethality, although approximately 14% of the pups die postnatally prior to weaning. Periostin expression is most common in collagen rich connective tissues. Deletion of periostin results in severe growth retardation, suggesting periostin is essential for postnatal development. Histological analysis of the periostin knockout mice demonstrated a lack of trabecular bone, severe incisor enamel defects, periodontal disease, cartilage and cardiac valve defects. However, when the mice are placed on a soft diet, growth retardation is attenuated suggesting that this maybe due to eating difficulties as a result of the lesions in the periodontium (Rios et al., 2005).

Aside from the CCN2 (connective tissue growth factor/CTGF) knockout (Kuiper et al., 2007), mice that lack matricellular proteins tend to have mild phenotypes that become more severe under injury or disease conditions (Bornstein and Sage, 2002). Deletion of matricellular proteins such as TSP-1 and -2, SPARC, galectins, or tenascin-C affect many tissue types, but more commonly these mice exhibit an altered response to tissue injury (Bornstein et al., 2004; Bornstein et al., 2000; Elola et al., 2007; Gruber et al., 2005; Park et al., 2004; Yan and Sage, 1999; Yang et al., 2000). However, the loss of periostin appears to be more severe, with significant damage occurring in connective

tissues during postnatal development. Interestingly, all matricellular proteins are known to play a major role in normal wound repair, but the role of periostin is not as clear.

Wound Repair

Wound repair is a series of overlapping events that begin immediately after wounding (platelet aggregation) until matrix contraction results in tissue closure (Midwood et al., 2004). While fibrin, collagen, and fibronectin provide structural support to the matrix during wound repair, matricellular proteins act by providing specific signals to the constituent cell populations, modulating their phenotype (Alford and Hankenson, 2006; Kyriakides and Bornstein, 2003). Each protein is expressed at different stages of wound repair and some patterns are more transient than others. However, to date, the expression profile of periostin in the wound repair process has yet to be elucidated. Thus far, expression of periostin in tissue repair has been investigated predominantly within the vascular and cardiac systems (Dorn, 2007; Kuhn et al., 2007; Lindner et al., 2005; Litvin et al., 2007; Norris et al., 2008a; Shimazaki et al., 2008), and to a lesser extent in chronic dermal wounds (Roy et al., 2007), muscle (Goetsch et al., 2003), and bone fracture (Nakazawa et al., 2004). Interestingly, the regulatory processes, including matricellular protein expression, responsible for normal development of bone, cartilage and cardiac tissue also play a major role in their pathogenesis in adults.

Periostin was initially identified in periosteum and bone (Horiuchi et al., 1999; Takeshita et al., 1993), and in bone fracture, periostin mRNA is upregulated 2-fold and localizes to preosteoblastic cells within the periosteum, as well as in undifferentiated mesenchymal cells close to the fracture site (Nakazawa et al., 2004). The periostin

mRNA signal peaks at day 7, and is significantly reduced by day 14, where the undifferentiated mesenchymal cells no longer express periostin mRNA. It seems likely that periostin plays a role in recruitment of pre-osteoblast cells into the provisional callus during fracture healing (Kojima et al., 2007; Nakazawa et al., 2004). However, the importance of periostin for mesenchymal cell physiology is not limited to only bone and periosteum.

In periostin knockout mice, large numbers of undifferentiated mesenchymal cells remain in the heart tissue after development (Butcher et al., 2007; Norris et al., 2008b), suggesting that periostin maybe required in the differentiation of mesenchymal progenitors to cardiomyocytes. Periostin has been observed to increase the number of cardiomyocytes actively replicating DNA in rats after myocardial infarction (Kuhn et al., 2007). In particular, periostin is expressed by cardiac fibroblasts where it interacts with integrins on cells likely modulating their behaviour during the remodeling process following infarct (Shimazaki et al., 2008). However, it is still not clear if periostin acts on cardiomyocytes directly, or support cells only such as the cardiac fibroblasts (Dorn, 2007).

In vascular remodeling, which can be induced through balloon injury, periostin mRNA levels strongly increase (Lindner et al., 2005). Periostin expression after injury was localized to smooth muscle cells of the neointima and the adventitia. This expression pattern is similar to the expression pattern of other matricellular proteins including tenascin-C after balloon injury (Majesky, 1994; Wallner et al., 2002), suggesting that periostin may perform similar functions in such situations. Significantly, over expression of periostin enhances smooth muscle cell migration *in vitro* (Li et al., 2006), and may

have a de-adhesive activity similar to tenascin-C and other matricellular proteins (Murphy-Ullrich, 2001). While matricellular proteins appear important in arterial remodeling, in vein grafts molecules such as tenascin-C appear to contribute to intimal hyperplasia and ultimate graft failure (Wallner et al., 1999). It will be of great interest to assess if periostin is also expressed in vein grafts during remodeling in the arterial system.

From the information highlighted above, it appears that periostin, like other matricellular proteins, can be considered to play a fundamental role in tissue remodeling. Periostin is known to interact with integrins, influencing cell-matrix interactions, adhesion, proliferation and differentiation processes (Kudo et al., 2007). The focus in our laboratory is on wound healing in the periodontium, particularly in the presence of biomaterials (Hamilton and Brunette, 2007; Hamilton et al., 2007; Hamilton et al., 2006; Schuler et al., 2006). We have recently shown secretion of periostin in to the ECM by human gingival fibroblasts cultured on titanium *in vitro*, but only in the presence of ascorbic acid (Figure 1). This is suggestive that periostin may closely associate with collagen synthesis on titanium. Indeed, it appears that periostin is essential for certain parts of the collagen assembly process.

Periostin-ECM Interactions: Influence on Collagen Fibrillogenesis

Collagen fibrils are the ECM component allowing connective tissues to withstand tensile forces, and tissues such as ligaments, tendons, bone, cartilage, and skin contain large numbers of collagen fibrils thus allowing dispersal of such forces (Canty and Kadler, 2005; Culav et al., 1999). Collagen fibrillogenesis is a complex multi-step process that is

still poorly understood (Canty and Kadler, 2005). Although it was initially thought that secreted collagen might self assemble, evidence is now quickly accumulating that other ECM proteins are required. Matricellular proteins in particular appear to be of importance in collagen assembly (Bornstein et al., 2004; Bornstein et al., 2000; Yang et al., 2000). For example, deletion of the thrombospondin-2 gene has shown to disrupt collagen fibrillogenesis (Bornstein et al., 2000), and in SPARC null mice, collagen fibrils are significantly smaller (Martinek et al., 2007).

Expression of periostin is common in collagen rich tissues, suggesting that it may influence collagen fibrillogenesis (Borg and Markwald, 2007). Interestingly, periostin protein is routinely present in adult animals in tissues such as the periodontal ligament (Horiuchi et al., 1999; Tomokiyo et al., 2008; Wilde et al., 2003), unlike many of the other matricellular proteins, further suggesting that it plays a key role in adult tissues. Norris et al, 2007, investigated the role of periostin in collagen I fibrillogenesis in murine connective tissues including periodontal ligament, tendon, skin and atrioventricular valves. Using co-immunoprecipitation techniques, they identified that periostin directly binds to collagen type I, and in periostin knockout mice, collagen fiber diameter and cross-linking are significantly reduced. Furthermore, biomechanical testing of skin samples from knockout and wild type mice highlighted a reduced modulus of elasticity and lower ultimate stress in samples from periostin knockouts. They concluded that periostin appeared to influence maturation and assembly of collagen I fibrils. This hypothesis is backed further from the observations that periostin null mice also appear to be unable to support normal valvular remodeling or maturation of the cardiac skeleton (Butcher et al., 2007; Norris et al., 2008b; Snider et al., 2008). The hypothesis that

periostin plays an important role in collagen fibrillogenesis has significant implications for connective tissue disease, where defects in collagen and elastin production result in chronic fibrotic conditions (Abraham et al., 1982; Leask et al., 2004; Uitto, 1979; Uitto and Lichtenstein, 1976).

Connective Tissue Disease and Periostin Expression: Insights from the Periostin^{-/-} Mouse Phenotype

Connective tissue diseases are disorders featuring abnormalities commonly involving the ECM proteins collagen and elastin. Several chronic connective tissue diseases have been identified, including Marfan's syndrome (fibrillin defect), scleroderma (collagen over-production), Ehlers-Danlos (defect in collagen synthesis), and pseudoxanthoma elasticum (elastin defect). Matricellular proteins have already been implicated in such disease conditions; CCN2 in scleroderma (Leask et al., 2004), and thrombospondin-2 in rheumatoid arthritis (Park et al., 2004).

As more is learned about connective tissue diseases, insights are gained into the possible involvement of periostin (Erkan et al., 2007; Oku et al., 2008; Takayama et al., 2006). In this review, we will specifically deal with Marfan's syndrome (Callewaert et al., 2008). Although the condition has been genetically linked to defects in fibrillin (Nasuti et al., 2004), the exact mechanisms underlying the condition are still not well understood (Boileau et al., 2005; Collod-Beroud and Boileau, 2002; Robinson and Booms, 2001; Whiteman et al., 2006). *In vitro* studies have shown that type I collagen secreted by cells isolated from Marfan's patients is more soluble (Francis et al., 1976; Priest et al., 1973). This is indicative of lower levels of collagen crosslinking, which has

already been identified in periostin knockout mice (Norris et al., 2007). This is backed by other research that suggest that the Marfan's phenotype may be due to the expression of a variety of primary structure alterations in the chains of type I collagen that interfere with normal crosslink formation (Byers et al., 1981). In our laboratory, we have recently performed analysis of periostin knockout mice skulls, confirming the observations by Norris et al, of severe periodontal disease, significant reduction in bone density, and incisor defects (Figures 2 and 3). Our analysis has also described for the first time that bones are missing in the orbit of the mice, or fail to properly fuse (Figure 4). In Marfan's patients, craniofacial abnormalities are common (De Coster et al., 2004; Pirinen, 1998; Westling et al., 1998), and we hypothesize that the periostin knockout mouse has a Marfan's like phenotype. Whether periostin impacts on collagen synthesis and assembly directly or through fibrillin is not yet known, but the prospect is intriguing. Furthermore, as Marfan's syndrome, periostin deficient mice have severe defects in their heart's valves, especially the mitral valve (Rios et al., 2005). The valve leaflets become extremely floppy and do not close tightly, allowing blood to leak backwards across the valve, back into the ventricles. This provides further evidence that the periostin knockout mouse suffers from a Marfan's-like phenotype. Further analysis of the periostin knockouts could provide important information for human connective tissue diseases, particularly those where collagen synthesis and assembly is defective.

Conclusions

Periostin is a novel secreted protein with very diverse functions that appear necessary for postnatal development. The expression of periostin is most common in collagen rich

connective tissues, where it appears essential for proper ECM synthesis, particularly with respect to collagen I fibrillogenesis. Mice deficient in periostin show a phenotype similar to Marfan's syndrome, suggesting that periostin may be involved in this pathology, in addition to fibrillin-1.

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Table 1

Matricellular Proteins	
<i>Bone Sialoprotein</i>	<i>Periostin</i>
<i>CTGF</i>	<i>SPARC (Osteonectin)</i>
<i>Cyr61</i>	<i>Tenascin-C</i>
<i>Galectin 1, 2, 3, 4, 8, and 9</i>	<i>Thrombospondin-1 and 2</i>
<i>Nov</i>	<i>WISP-1, 2 and 3</i>
<i>Osteopontin</i>	

Table 2

Known Effects of Matricellular Proteins Cell Physiology	
<i>Cell Adhesion</i>	<i>Cell de-adhesion</i>
<i>ECM synthesis</i>	<i>Collagen Fibrillogenesis</i>
<i>Proliferation</i>	<i>Apoptosis</i>
<i>Differentiation</i>	<i>De-differentiation</i>
<i>Migration</i>	<i>Growth factor production</i>
<i>Morphology</i>	<i>Bio-mineralization</i>

Figure legends

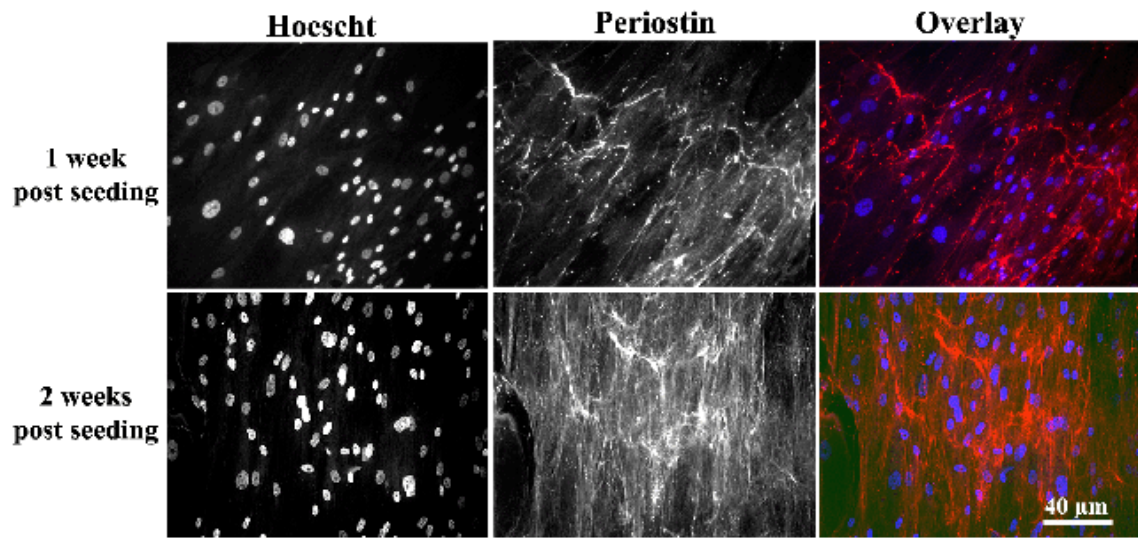


Figure 1: Matricellular protein effects on cell behaviour

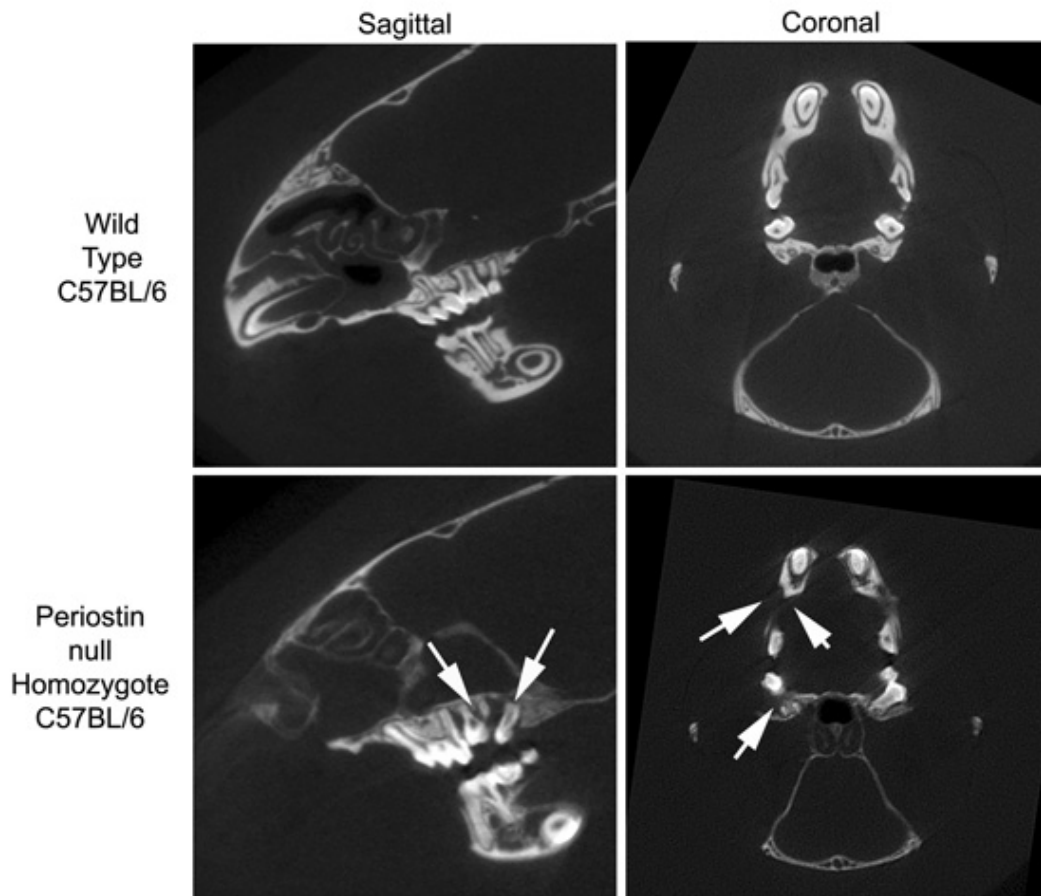


Figure 2: MicroCT analysis of wild type C57BL/6 and periostin knockout C57BL/6 mice at a scanning distance of 40 μ m. Overall skull shape differed between the mice types, and the orbital bones appear to be missing entirely in the periostin knockout (see arrows) which is a characteristic of Marfan's syndrome.

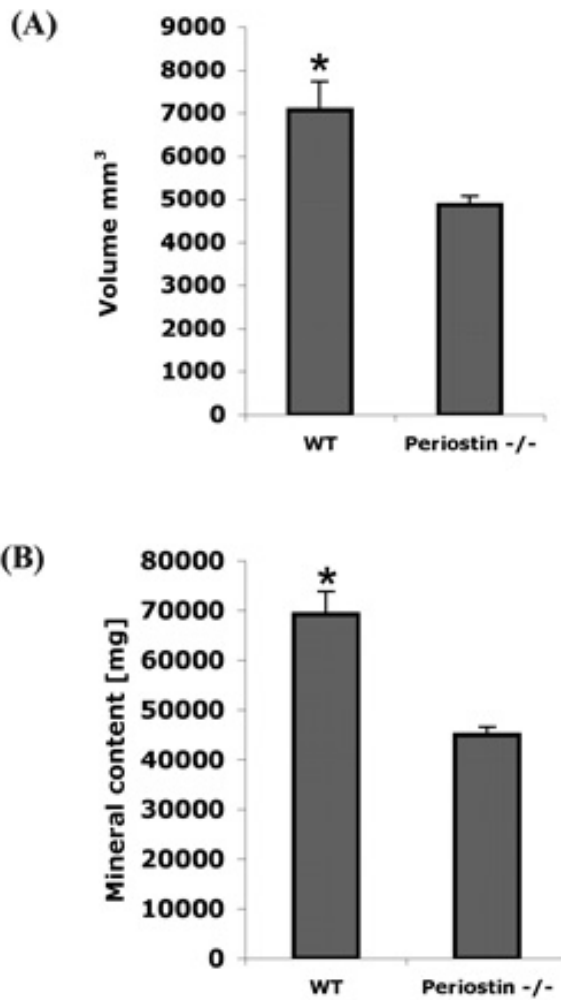
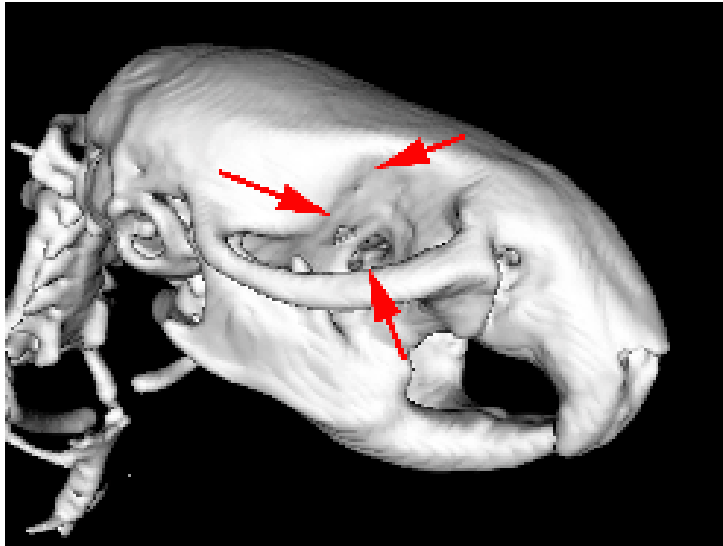


Figure 3: 6 week old periostin knockout C57BL/6 and litter matched wild types were analyzed using microCT imaging. In wild type mice, the molars are well arranged, with healthy periodontal ligament evident in the sagittal view. Periostin knockout mice have significant defects around the bone and periodontal ligaments, particularly in the back molars (Arrow, sagittal view). In the coronal view, bone loss is evident in the jaw (arrows) when periostin knockout mice are compared with wild type litter matched controls

Wild type C57BL/6



Periostin Knockout C57BL/6

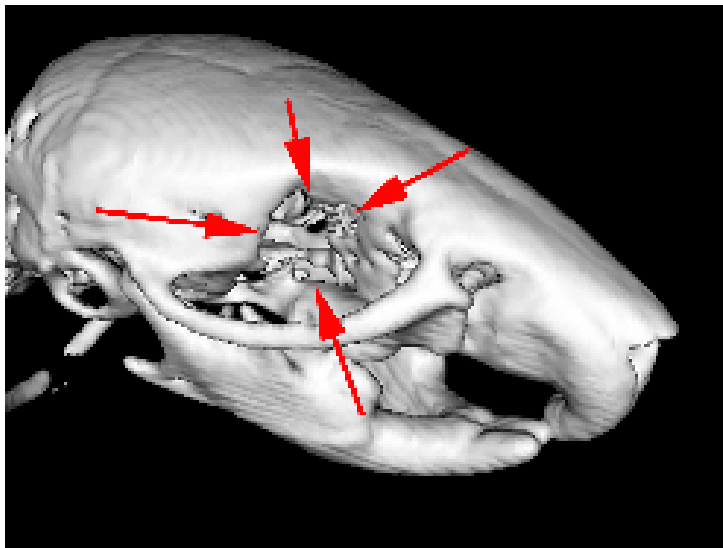


Figure 4: Analysis of the bone volume and mineral content reveals that the loss of periostin influences formation of bone, which is consistent with the findings of Rios et al, 2005.

