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Laboratory Skills Essay

Cementum Regeneration: How Far Have We Come?

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Table of Contents:

Abstract:

Gingivitis is a prevalent disease which is characterized by swollen and bleeding gums [5]. If left untreated, it can lead to the destruction of the supporting framework of the tooth called the bone-periodontal ligament-cementum complex or periodontal complex [6]. Inflammation of this complex is called periodontitis which if not halted results in lose or lost teeth [6]. Regeneration of periodontium lost as a result of periodontitis is essential to prevent tooth loss. The complexity of the PDL apparatus makes its complete and functional regeneration challenging to achieve [3]. The transplantation of periodontal ligament stem cells into the periodontal defect has been shown to form periodontal ligament fibers similar to the natural periodontal ligament fibers without any adverse effects [100-102]. Alveolar bone regeneration has been successfully stimulated using different bone grafts and biomaterials [104,105]. Cementum is the least understood of all the structures in the periodontal complex [6]. Its regeneration, therefore, is a challenge. This review aims at analyzing how far have we been able to understand cementum or cementogenesis to be able to regenerate or engineer it. For successful regeneration of cementum, the important factors which are prerequisites to understanding its multifactorial nature should be taken into account. Cementoblastic progenitor selection and the possibility of involvement of specific integrins and signalling events in their recruitment has since created enigma about the mechanism of cementogenesis [16,18,19]. Here we are going to explore how far we have come in understanding these enigmatic factors in the light of recent research in the field. This review also explores the role of stem cells and scaffolds in cementum tissue engineering.

Introduction:

Gum disease or gingivitis is a prevalent disease which is characterized by swollen and bleeding gums [5]. If left untreated, it can cause the destruction of the supporting framework of the tooth [5]. This supporting framework holding the tooth in jaw is called the bone-periodontal ligament-cementum complex or periodontal complex [6]. Inflammation of this complex is called periodontitis, which is the sixth most prevalent disease worldwide [2]. If this inflammation is not halted, it destroys the complex and results in lose or lost teeth [6]. Regeneration of periodontium lost as a result of periodontitis is essential to prevent tooth loss.

Several procedures are available to regenerate the periodontium. These include non-surgical therapies such as scaling and root planing and surgical treatments such as guided tissue regeneration [1,97]. In severe cases, teeth are lost and are replaced with artificial teeth in the form of implants or bridges [1]. These artificial treatment options provide a temporary relief [1].

For the long term permanent relief, recently a lot of work has been dedicated to the regeneration and tissue engineering of the periodontal complex to be used as a natural way to re-functionalize the tooth after loss of its supporting structure due to periodontitis. The complexity of the PDL apparatus makes its complete and functional regeneration challenging to achieve [3]. To develop such a treatment, all components of the periodontal complex must be regenerated [102,103]. One of these is cementum which is the mineralized tissue covering the root of the tooth into which periodontal ligament (PDL) fibers insert on one end and the bone in which periodontal ligament fibers insert on the other end. The transplantation of periodontal ligament stem cells into the periodontal defect has been shown to form periodontal ligament fibers similar to the natural periodontal ligament fibers without any adverse effects [100-102]. Alveolar bone regeneration has been successfully stimulated using different bone grafts and biomaterials [104,105].

The formation of cementum, however, is essential for the attachment of periodontal ligament fibers to the root surface of the tooth. Cementum is the least understood of all the structures in the periodontal complex [6]. Its regeneration, therefore, is a challenge. This review aims at analyzing how far have we been able to understand cementum or cementogenesis to be able to regenerate or engineer it.

Cementum in periodontitis:

In early and moderate periodontal disease, the coronal/ upper half of the root is affected. This part is covered by acellular extrinsic fiber cementum (AEFC) [96]. In advanced lesions, the damage extends to apical/ lower part of root which is covered by cellular intrinsic fiber cementum (CIFC) [96]. There is a constant deposition of CFIC as a compensation of occlusal abrasion (Fig. 1.) [4]. However, in periodontitis the periodontal attachment is affected, which is provided by AEFC [4]. Though both types are produced by cementoblasts but a lack of cells in the acellular cementum limits its regenerative capacity [4].

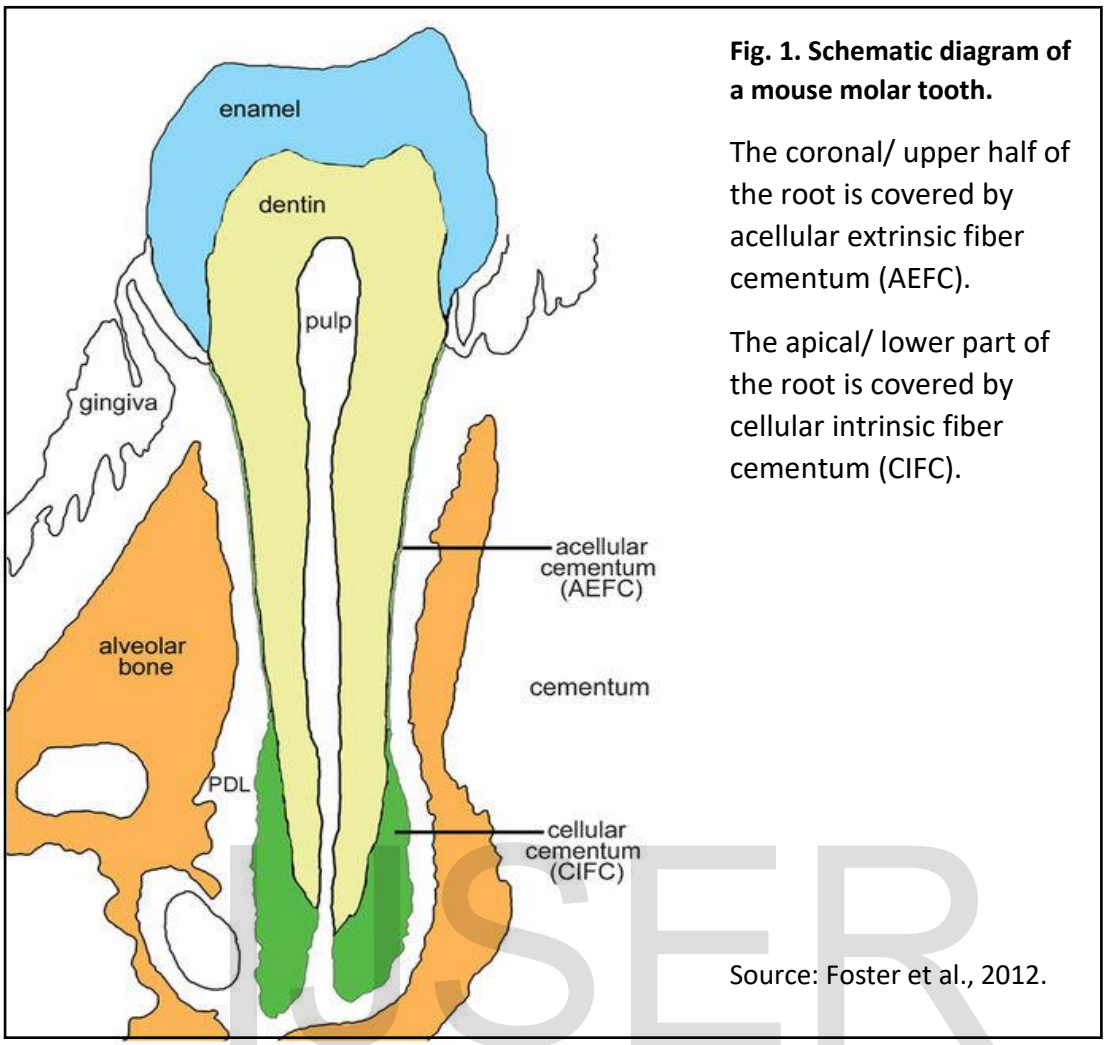

Fig. 1. Schematic diagram of a mouse molar tooth.

The coronal/ upper half of the root is covered by acellular extrinsic fiber cementum (AEFC).

The apical/ lower part of the root is covered by cellular intrinsic fiber cementum (CIFC).

Source: Foster et al., 2012.

Homeostasis of cementum and PDL:

The understanding of the factors contributing to maintaining the normal physiological environment of cementum and PDL can give us an insight in to achieving this homeostasis during regeneration.

The Wnt/ β-catenin signalling pathway also called Canonical Wnt signalling pathway [20,76] has been shown to maintain homeostasis of PDL [21]. This is an intercellular cell signalling pathway which has been shown to play crucial role in regulating differentiation, proliferation and death of many cell types [77-79]. It employs a receptor and a ligand [20]. The receptor gets activated by the ligand. This results in an accumulation of β-catenin in the cytoplasm which is then translocated into the nucleus where it takes part in the transcription of target genes by interacting with Tcf/Lef sites (Fig. 2.) [20]. A study concluded that PDL is a Wnt-dependent tissue and that cellular fibrillar cementum is depleted in the absence of Wnt-signalling [21]. Other studies have demonstrated that an increased canonical Wnt signalling expression causes an increase in the formation of cementum [111] and vice versa [112]. This finding is a huge leap forward as this pathway has not only been shown to take part in homeostasis of several organs but is now also being considered as a major target in repair and regeneration of

tissues [80]. Recently several studies have demonstrated its pronounced involvement in cementum regeneration during tissue engineering and has been covered in this review in detail in light of these studies [22,27,36,37,43,45].

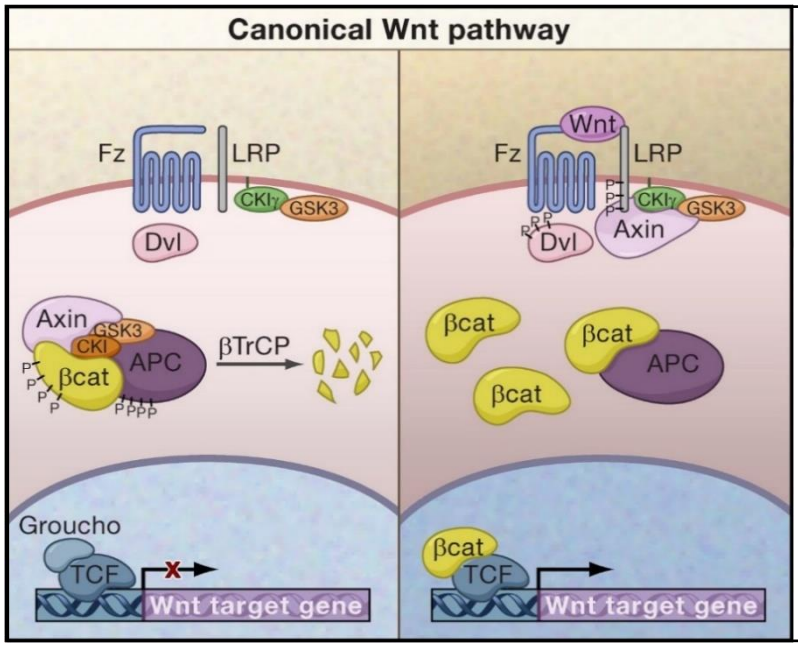

Fig. 2. Canonical Wnt Signalling Pathway. In the absence of Wnt ligand, there is phosphorylation of the complex containing β-Catenin, which leads to β-Catenin degradation (Left). In the presence of Wnt ligand, hypophosphorylation of the complex containing β-Catenin releases it from this degradation machinery. β-Catenin then enters the nucleus and takes part in the transcription of the target genes (Right) [20].

A study demonstrated that extracellular matrix (ECM) of cementum plays a vital role in maintaining physiological integrity of cementum and PDL [6]. It contains growth factors such as insulin-like growth factor (IGF), fibroblast growth factor (FGF) and bone morphogenetic proteins (BMPs) which form the basis of activities of different periodontal cell types [98,99]. The ECM proteins take part in crystal growth and regulation of mineralization [7]. The homeostasis of inorganic pyrophosphate (PP_i) in ECM (Fig. 3.) plays an important role in regulating mineralization of acellular cementum [7]. In mice deficient in PP_i the acellular cementum was found to have increased in thickness and its matrix composition was altered [7]. Pyrophosphate has been shown to regulate cementoblastic mineralization [7]. After initiation of cementogenesis, cementoblasts associated with AEFC mineralization were shown to express PP_i regulators [7]. One of these regulators is alkaline phosphatase (ALP) which plays an important role in regulating mineralization by transferring phosphate groups from the cells to the matrix [7,84-86].

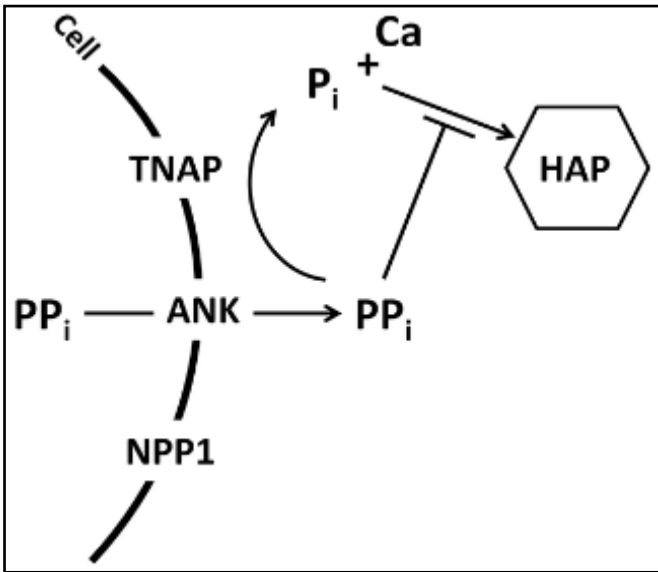

Fig. 3. Pyrophosphate homeostasis in the extracellular space of mineralized tissues. Inorganic phosphate (P_i) is a component of mineral hydroxy apatite (HAP). Pyrophosphate (PP_i) is an inhibitor of HAP. The enzymes involved in regulation of mineralization are tissue nonspecific alkaline phosphatase (TNAP), ankylosis protein (ANK) and ectonucleotide pyrophosphate phosphodiesterase 1 (NPP1). TNAP hydrolizes PP_i to release P_i and enhances mineralization. ANK and NPP1 increase PP_i and hence control mineralization [7].

PDL has been shown to supply progenitor cells to maintain homeostasis of the periodontal tissues [61]. Osteonectin is a collagen binding protein which was found to play an important role in maintaining homeostasis of collagenous ECM of PDL where clear alterations in collagen fiber content and morphology were seen in osteonectin-null mice [56]. Collagen homeostasis is crucial to maintaining healthy PDL [56].

Challenges and triumphs in cementum regeneration:

For successful regeneration of cementum, the important factors which are prerequisites to understanding its multifactorial nature should be taken into account. These factors include the unique composition of extracellular matrix of cementum, its poorly understood developmental regulation and our poor understanding of differentiation of the cementum forming cells called cementoblasts. Cementoblastic progenitor selection and the possibility of involvement of specific integrins and signalling events in their recruitment has since created enigma about the mechanism of cementogenesis [16,18,19]. Here we are going to explore how far we have come in understanding these enigmatic factors in the light of recent research in the field.

1. Extracellular matrix of cementum:

In periodontal disease, the structural integrity of the extracellular matrix of cementum and its biochemical composition is severely compromised [6]. More over the provisional matrix that is generated during healing has a different composition [6]. Hence, in order to generate new cementum for PDL fibers attachment, local environment must be favorable for the recruitment and functioning of cementum-forming cells. Therefore, the wound matrix should be rendered conducive for cementum repair. Though cementum is a mineralized tissue, its physiology, however, is one of its kind and it contains unique molecules that have not been detected in other tissues [6].

Inorganic pyrophosphate is the naturally occurring inhibitor of mineralization in extracellular matrix of mineralized tissues [7]. The acellular cementum is the mineralized type of cementum. This acellular cementum has been shown to be exceptionally sensitive to regulation of pyrophosphate [7]. The Foster and Nagatomo et al., 2012 group demonstrated in their experiment that in mice deficient in extracellular inorganic pyrophosphate, an increase in acellular cementum deposition was seen however there was no change in the composition of cellular cementum, nor in the PDL tissue and dentine, which shows that inorganic pyrophosphate is unique to acellular cementum in the periodontal complex [7].

For a long time, efforts have been made to prepare root surfaces that would allow cell attachment and hence periodontal regeneration. Identifying important attachment proteins expressed in cementum matrix can help us in developing effective clinical treatments. A study showed that an adhesion molecule called bone sialoprotein (BSP) was expressed by cells lining the root surface of murine molar tooth germs [13,14,46]. It was suggested that this protein might be involved in differentiation of cementoblasts and early mineralization of cementum matrix [13,14,46].

Osteopontin (OPN) is another adhesion molecule that was found to be localized to non-mineralized tissues which might suggest that it has a role in mineralization inhibition and that BSP and OPN are somehow collectively involved in regulating cementogenesis [13]. It was also localized intracellularly in cementoblasts [53]. Osteocalcin (OCN) is a non-collagenous protein hormone which was found to play significant role in cementogenesis [51]. It is a prominent component of cellular cementum [53,54]. SPARC/ Osteonectin (ONN) is a protein which is concerned with regulating the cell adhesion to the extracellular matrix [55]. Cellular cementum of rat showed intense expression of mRNA for ONN [54]. ONN is also found in PDL and alveolar bone, hence it is an important protein to be taken into consideration for regenerating periodontal attachment lost due to periodontitis [56].

2. Developmental regulation of cementum:

The developmental regulation of cementum is poorly understood and hence has hampered efforts of its regeneration [7].

As cementum is essential for appropriate maturation of the periodontium, a lack of cementum markers is another major challenge in understanding the molecular mechanisms that regulate periodontal regeneration [10]. A study, however, identified and characterized cementum protein 23 (CP-23) that appeared to be PDL and cementum-specific [10]. The study found that CP-23 was localized to the cementoid matrix of cementum throughout the entire root surface and that 98% of the cementoblasts and 15% of the PDL cells expressed this marker [10]. This novel human expressed gene might be helpful to better understand the cellular and molecular events that regulate the process of cementogenesis [10].

Cementum attachment protein (*CAP*) is another putative cementoblast marker alongwith CP23 [11]. This protein has been shown to promote migration and attachment of human alveolar bone cells and human periodontal cells onto root surface. Hence, cementum attachment protein seems to play a crucial role in cementogenesis [12].

Cementum matrix protein 1 (*CEMP1*) is a cementum molecule expressed by cementoblasts, some PDL cell subpopulations and mesenchymal stem cells located in the paravascular region of the PDL [24]. This protein is a human cementoblastoma derived marker of cementogenic differentiation and it has been shown to increase the expression of *CAP* when transfected to human gingival fibroblasts [24].

3. Differentiation of cementoblasts:

The cells that form the cementum are called cementoblasts. Their function is to deposit and mineralize the matrix of cementum [50]. Their origin and the molecular factors responsible for their recruitment and differentiation are not fully understood [6]. Dental follicle cells have been shown to differentiate into into cementoblasts in response to stimuli from epithelial components [8]. It has been shown that BMP-2 induces these cells towards cementoblastic phenotype [107]. The Liu et al., 1997 suggested that PDL may be the source of cementoblast progenitors [16]. Bar-kana et al., 1998 demonstrated that cells cultured from human PDL formed cementum-like mineralized nodules and showed cementum-specific markers [17]. It was suggested in a study that cementoblasts may be derived from stem cells in the PDL, gingiva and alveolar bone [6].

The role of *CEMP1* in cementoblastic differentiation of PDL stem cells was explored by a study done in 2012 [47]. It was demonstrated that its over-expression increases differentiation of PDL stem cells to cementoblastic phenotype and reduces their differentiation towards periodontal and osteoblastic lineages [47].

Cementum and bone are similar mineralized tissues and hence cementoblasts and osteoblasts have number of similar characteristics [91,92]. Both of these tissues express BSP, OCN and OPN [91,92]. Cementum, however, lacks vasculature and innervation [91,92]. Prostaglandin E_2 (PGE_2) receptor and its signalling pathway has been used as a target for therapeutic utility in treating various bone diseases [93]. A study demonstrated its role in cementoblastic differentiation in OCCM 30 mouse cell line [94]. The results showed that PGE_2 decreased proliferation of cementoblasts and increased activity of ALP and BSP [94]. Hence it might imply that it has a regulatory role in cementogenesis. Sclerostin is a small protein marked by *SOST* gene which is an inhibitor of osteoblastic activity and hence bone formation [87]. Its effect on proliferation and differentiation of cementoblasts was demonstrated by Bao et al., 2013. The results indicated that it not only inhibits the cementoblastic proliferation and differentiation but also increases their apoptosis [88]. Sclerostin inhibitors have been shown to increase bone formation and decrease its resorption [87]. Strontium, in this regard, has been used to treat bone resorption diseases [89]. The effect of strontium on cementoblasts was investigated in 2014 as an inhibitor of sclerostin [90]. Strontium was found to inhibit sclerostin in cementoblasts in vitro and facilitated cementogenesis by promoting

differentiation of cementoblasts [90]. Strontium had been previously considered as a potential agent for cementum regeneration. It was incorporated into a mesoporous glass scaffold seeded with PDL stem cells where it induced cementogenic differentiation of these cells with enhanced expression of cementogenesis-related genes including *CEMP1* [35].

Osterix (*Osx*) is a transcriptional factor which is essential for osteogenesis [83]. Investigations regarding its role in cementogenesis revealed that cementoblasts have a mesenchymal origin and hence this factor has a primary role in the formation of cellular cementum [42]. In a later study, *Osx* over-expression in cementoblasts was found to not only upregulate expression of cementogenesis markers such as OPN, OCN and BSP but also *DKK1* which is a canonical Wnt antagonist [43]. It was hence concluded that Osx enhances cementoblastic proliferation and differentiation by downregulation of canonical Wnt signalling expression [43]. Another study demonstrated that transforming growth factor beta (TGF-β) signalling pathway [48,82] is an upstream regulator of *Osx* in maintaining differentiation of cementoblasts and formation of cellular cementum through a Smad-dependent pathway [49]. It is a canonical signalling pathway which employs a receptor and a ligand (Fig. 4.). *Osx* expression was found to be decreased in a TGF-β receptor II (TβRII) lacking mouse cementoblast cell line OCCM-30 [49]. In these cells, activity of ALP and expression of cementogenesis related genes was decreased [49]. ALP is considered as an early marker of cementoblastic differentiation [84]. The cells also had an abnormal phenotype and were partially rescued by transduction of *Osx* [49]. In-vivo, in *osteocalcin-Cre* mediated *Tgfbr2* conditional knockout mice, which lack functional TβRII, a sharp reduction was seen in cellular cementum, matrix secretion and mineral apposition rates [49]. Hence, TGF-β signalling pathway appears to play a major role in cementoblastic differentiation and cementum formation [49].

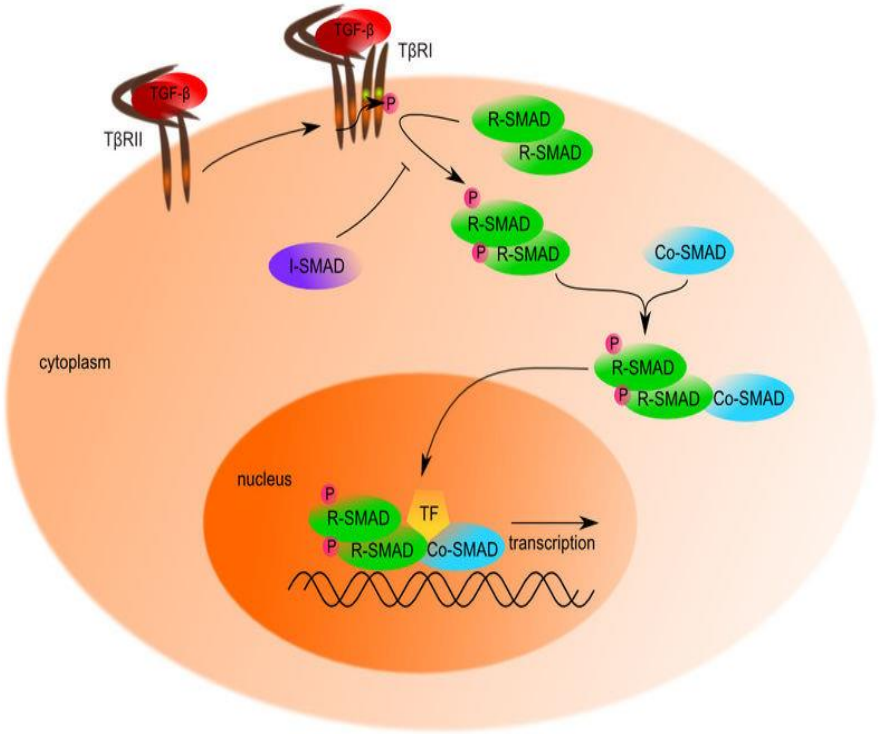

Fig. 4. TGF-β canonical signalling pathway. TGF-β ligands bind TGF-β receptor II which causes phosphorylation of receptor I. Type I receptor then phosphorylates SMADs which bind with co-SMADs. These complexes enter the nucleus and take part in the transcription of the target genes.

Source: Kubiczkova et al., 2012.

BMP-2 is a member of TGF-β superfamily [106]. It has been shown to enhance cementoblastic differentiation [107, 108]. BMP-signalling pathways in cementoblasts have been shown to downregulate canonical Wnt signalling expression by activation of Wnt/ β-Catenin inhibitors: Dkk1 and sclerostin [109,110]. However, as already mentioned by Bao et al., 2013 that sclerostin causes inhibition of cementoblastic differentiation [88], which might suggest that there might be an optimum expression of canonical Wnt signalling which might be required to achieve appropriate cementoblastic differentiation. The discussion under 'homeostasis of cementum and PDL' shows that cementogenesis might not be dependent solely on Wnt/β-Catenin signalling expression of cementoblasts. The balance may be achieved by an interplay of its expression in cementoblasts and some other component/s during different stages of cementogenesis. As during physiological repair of cementum after resorption during orthodontic tooth movement, reparative cementum was shown to be produced by epithelial-mesenchymal transition of epithelial rest cells of Malassez by upregulation of Wnt/β-Catenin signalling expression [42,109]. This resulted in cementoblast-like mesenchymal cells which then formed reparative cementum [42,109].

Box. 1. Cementoblasts and Osteoclasts. Cementoblasts have been shown to form and recruit osteoclasts (bone resorbing cells) [95,115,116]. Osteoclasts are always found in close association with cementum/ tooth root. Does that mean that during orthodontic tooth movement, the remodelling of alveolar bone proper is mediated mainly by cementoblasts and do these cells have osteoclastogenic memory which causes post-treatment relapse of teeth?

This cementum remodelling that occurs during orthodontic tooth movement can give us an insight into the factors necessary for cementum repair and subsequently regeneration. It was demonstrated that the cells laying down reparative cementum within the resorption lacunae expressed a transcription factor *Runx 2* which then upregulated ALP, collagen type 1, OCN and OPN [113,114]. It was shown that during this cementum repair, components of ECM (OPN, OCN) from resorbed cementum caused cementoblastic progenitor recruitment to the root surface and then their adhesion, proliferation and differentiation. These processes were facilitated by local cytokines and growth factors (IGF, FGF, BMP, TGF-β) [113].

4. Cementum Tissue Engineering:

Tissue engineering is defined as an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain and improve tissue function [31]. The goal of tissue engineering is to design bioactive, bioresorbable 3D scaffolds that can mimic the structure and integrity of the natural tissues even under load-bearing conditions [28].

Tissue engineering is based on the concept of combining a scaffold [29] with living cells/ bioactive molecules that in the presence of sufficient bloody supply will be able to promote repair/ regeneration of the tissues [59]. Bioactivity, is referred to as the capability of a material to affect its surroundings [29]. Upon implantation, such materials form a bond with the surrounding tissues [30]. In PDL regeneration, it is important to

generate hard and soft tissues of the complex and create attachment conducive environment for both of these tissues [67].

- Cementum regeneration using PDL stem cells:

Periodontal ligament stem cells were isolated from extracted human third molars in 2004 [41]. These cells expressed mesenchymal stem cell markers, had the ability of self-renewal and were able to differentiate into cementoblasts, osteoblasts and adipocytes [41]. On ectopic transplantation into immunocompromised mice, these cells formed cementum-like and PDL-like structures [41].

When seeded onto the scaffold, cementogenic differentiation of these cells is essential to be able to use them for cementum regeneration. Several different approaches have been used to induce cementogenic differentiation of PDL stem cells in the recent times. Here we are going to briefly discuss some recent work regarding successful induction of cementogenic differentiation of PDL stem cells.

It was found in 2012 that Lithium ions possess the cementogenic potential [36]. Studies showed that incorporation of Lithium ions into mesoporous bioactive glass scaffolds or β-tricalcium phosphate bioactive ceramics seeded with PDL cells enhanced the proliferation, differentiation and cementogenic gene expression of these cells by upregulating the expression of Wnt/β-Catenin signalling [36,37]. In 2013, inorganic stimuli derived from bioactive bredigite bioceramic extract upregulated *CEMP1* and CAP expression in PDL cells along with Wnt/β-Catenin activation indicating that this can be an interesting tissue engineering technique to stimulate cementogenic differentiation of PDL cells [27]. In 2014, a study revealed that $Ca_3ZrSi_2O_9$ ceramic disks can enhance expression of *CEMP1* and CAP in these cells along with the expression of *AXIN2* and *Ctnnb* which are the Wnt/β-Catenin pathway related genes [38]. In 2015, a study demonstrated that hydroxyapatite bioceramics with micro-nano-hybrid surface when seeded with PDL cells enhanced the expression of *CEMP1*, *CAP*, *LRP5* and β-*Catenin* in these cells. Moreover, they found that the expression of the cementogenic genes was repressed when Wnt-signalling was inhibited [45].

However, in another study it was found that activation of Wnt signalling inhibited the cementogenic differentiation of PDL stem cells and promoted their differentiation towards osteoblastic lineage [22]. The study also explored the role of hypoxia in cementogenic differentiation of these cells, as hypoxia is known to maintain stemness of PDL stem cells by enhanced expression of pluripotency markers [23]. Hypoxia was found to inhibit β-Catenin expression which caused down regulation of Wnt signalling and an increased expression of cementum protein 1 (*CEMP1*). It was concluded that hypoxia enhances cementogenic differentiation of PDL cells by down regulating Wnt signalling [22].

It was suggested in 2012 that *CEMP1* has the potential to be used for PDL bioengineering since it was found to stimulate PDL cells into different phenotypes in three-dimensional

culture conditions [25]. *CEMP1* has since been used for cementum bioengineering as a cementum-specific protein that induces cementogenic differentiation of PDL stem cells [47]. When loaded in an electrospun multiphasic scaffold [63] in the form of recombinant *CEMP1*, its controlled release was found to suppress PDL stem cell proliferation and upregulation of cementoblastic markers which included *CEMP1* and *CAP*. Hence this construct can be used for cementum tissue engineering [26].

Nagelschmidtite is another bioactive bioceramic which has been used to stimulate cementogenic differentiation of PDL cells [32]. The NAGEL scaffold made with this material has been shown to have supported the attachment as well as proliferation of PDL cells. It has been shown to enhance the cementogenesis-related gene expression of PDL cells in-vivo [32]. Another study produced similar results using DIOP ceramics to prepare bioactive scaffolds for cementum regeneration [33]. Ionic products from a ceramic powder $Ca_7Si_2P_2O_{16}$ were found to possess excellent mineralization ability in stimulated body fluids and it also induced cementogenic differentiation of PDL cells with enhanced expression of cementogenesis-related genes including CEMP1 [34]. Similar results were produced with strontium-containing mesoporous bioactive glass scaffolds [35].

Vitamin C was regarded as a useful stimulus for cementogenic differentiation of PDL stem cells by a study done in 2016 [39]. This group treated PDLSCs in-vitro with Vitamin C and qPCR showed an upregulation of *CAP* and *CEMP1* cementogenic genes in these cells. Moreover, when these cells were transplanted into immunocompromised mice, it resulted in the formation ectopic cementum and bone-like tissues [39].

A study done in 2012 demonstrated the effect of pre-existing root cementum on cementogenic differentiation of PDL cells [11]. When PDL cells were inoculated onto root slices in culture in absence of pre-existing root cementum, fibrous tissue formed along the root surface and splits were seen between new fibrous tissue and dentine surface [11]. New cementum was either not formed or poorly formed. However, in presence of pre-existing cementum, PDL cells inoculation onto root surface produced cementum-like matrix on old cementum. Furthermore, there were no splits seen between new and old layers of cementum [11]. This might explain why cementum mediated attachment is successful if the avulsed tooth is immediately replanted. There might be a survival threshold for the cementum

Box. 2. Scurvy and Vitamin C. Scurvy was a menace for 18th century sailors. Its first symptoms were swollen and bleeding gums which soon led to lose or lost teeth and other systemic signs. This disease was finally ascribed to Vitamin C deficiency. Vitamin C was then discovered to play an essential role in the formation of collagen [118]. Collagen is an essential component of ECM of cementum and PDL fibres. Hence, the lose teeth were a result of loss of these important components of the tooth attachment apparatus!

Fig. 2-5 Periodontal disease seen in scurvy.

when its exposed to unfavorable conditions above which cementum fails to survive and hence late replantation results in ankylosis [15].

- Cementum regeneration using other stem cells:

A mixed cell sheet was designed in 2011 by coculturing human PDL stem cells with human bone marrow mesenchymal stem cells (BMMSCs) and mixed by ceramic bovine powder in a mixed-type stem cell-pellet cultivation system [65]. Cells contracted into a pellet when they were detached from the system. Transplantation of this pellet into immunocompromised mice resulted in the formation of cementum and PDL-like tissues in-vivo with neovascularization. This pellet can therefore potentially be used to repair periodontal defect as it mimics the microenvironment of PDL [65].

Periapical follicle SCs were found to generate cementum/ PDL- like tissues in-vivo and hence they can be regarded as a promising candidate for cementum/ PDL regeneration [66]. These cells were isolated from the apical end of developing human third molars [66].

- Other scaffolds for cementum regeneration:

Cloned cementoblasts were seeded onto three-dimensional polymer scaffolds for cementum engineering in 2003 [81]. In-vitro, these polylactic-co-glycolic acid (PGLA) scaffolds were shown to enhance cell attachment. In-vivo, when subcutaneously implanted in immunocompromised mice these scaffolds demonstrated mineral formation in these implants after retrieval. These implants showed presence of OCN and BSP [81].

Biomimetic hybrid scaffolds were made in 2010 by combining polycaprolactone (PCL) and polyglycolic acid (PGA) [60]. These polymeric scaffolds were used for targeted delivery of genetically modified cells in-vivo. When transplanted into the socket, new tissues formed within the construct of the scaffold depicting cementum, PDL and bone-like structures [60].

Cell delivery system with the help of PDL cell sheets and biphasic scaffold was developed in 2012 [40]. It made use of a Fused Deposition Modeling scaffold for bone compartment and electrospun [63] membrane for PDL. Not only did this scaffold was able to deposit mineralized cementum-like tissue on dentine in-vivo in rats but it also showed better attachment to dentine. *CEMP1* was localized at the cementum-dentine interface with immunohistochemistry [40].

Polycaprolactone scaffolds with growth factors such as connective tissue growth factor and bone morphogenetic proteins 2 and 7 were seeded with PDL cells in 2016 [44]. These protein- releasing scaffolds were placed on exposed surface of dentine in-vitro. This resulted in the formation of cementum-like layer on dentine surface along with the expression of *CEMP1* [44]. Efforts have been made to specifically design cementum-

dentin interface with compartmentalized triphasic scaffolds. In the presence of growth factors, PDL and alveolar bone stem cells, in-vivo implantation of these scaffolds produced *CEMP1*-positive and dentin sialophosphoprotein-positive dentine/cementum-like tissues [58].

A novel scaffold was made by Chung et al., group by combining degradable polymer, Poly-DL-lactide (PDLLA) and degradable bioceramics, toothapatite (TA) and β-tricalciumphosphate (TCP). When loaded with PDL stem cells and dental follicle stem cells (DFSCs), it resulted in the formation of cementum-like mineralized tissue in-vivo. Thus, this is another potential scaffold for cementum regeneration [57].

Kuboki et al., 2014 used bovine bone morphogenetic protein (BMP) incorporated in a double layer of fibrous collagen membrane [71]. This double layer technique regenerated cementum in furcation defects in monkey molars with collagen fibers arranged perpendicular to the cementum surface [71].

A study done in 2017 demonstrated periodontal regeneration using a porous tri-layered nanocomposite hydrogel scaffold [62]. The cementum layer was composed of chitin-polylactic-co-glycolic acid (PGLA), nanobioactive glass ceramic and *CEMP1*. PDL and alveolar bone layers were composed of PGLA and growth factors. Histological analysis revealed formation of new cementum, PDL and alveolar bone at the site of the periodontal defect where the scaffold was placed in rabbit molars [62]. Such multiphasic scaffolds allow compartmentalized tissue healing which is then integrated into one cohesive structure. These compartments include a bony compartment and the PDL compartment. The main focus with regards to PDL compartment is to first facilitate cementum formation onto the root surface, then PDL fiber formation and then insertion of these fibers into cementum on one end and bone on the other [64].

5. <u>Miscellaneous products for cementum regeneration:</u>

- Emdogain:

  Hertwig's epithelial root sheath is an extension of dental organ that forms enamel [68]. The cells of the sheath secrete enamel related matrix proteins onto the dentin which are temporarily deposited on the surface of root [69]. This provides a surface for the expression of cementum-forming cells, which forms the initial step in the formation of acellular cementum [69]. When matrix lays down on the surface of these proteins then the attachment apparatus develops [68].

  This enamel matrix layer that exists between dentin and the developing cementum laid down the basis for the development of enamel matrix derivative (EMD) or Emdogain. This was then used for EMD-supported PDL tissue engineering [68].

A study done in 2014 showed that when diseased cementum was conservatively treated with EMD, it showed an increase in cementoblastic differentiation in-vitro [70]. Human PDL stem cells inoculated onto root slices were transplanted into nude mice. These cells formed cementum-like tissue and this in-vivo effect of these cells was enhanced in the presence of EMD [70].

- Osteogain:

  Recently a liquid carrier system on an absorbed collagen sponge was developed for EMD [72]. It was named Osteogain. It resulted in better connective tissue attachment, cementum formation and bone area in furcation defects in monkey molars than Emdogain [72].

- Cyclosporin A:

  Cyclosporin A is an immunosuppressive drug that was found to induce deposition of new cementum in-vivo in rats [73] and in-vitro in human cementoblastoma cells [74]. In another study it was found that after the termination of treatment in rats, the cementum thickness did not decrease. It was concluded that this cementum deposition which occurred as a result of Cyclosporin A treatment was therefore not reversible [75].

Conclusion:

To achieve the appropriate cementum regeneration, we will have to therefore take in to account the imperative roles that its mineral surface topography and molecular environment play in maintaining the integrity of the periodontal ligament complex [52]. From the discovery of cementum in 1800s to understanding its functional importance in the late 19th and early 20th centuries to understanding its ultrastructure and genetics and now repair and regeneration in the 21st century [117], we have indeed come a long way! Only the time will tell how far we still have to go until we will be able to successfully regenerate a fully functional cementum along with its surrounding hard and soft tissues.

References:

1 Salvi GE, Mischler DC, Schmidlin K, Matuliene G, Pieturrson BE, Bragger U, Lang NP. Risk factors associated with the longevity of multi-rooted teeth. Long term outcomes after active and supportive periodontal therapy. J Clin Periodontol 41(7):701-7, doi:111/jcpe.12266 (2014).

2 Kassebaum NJ, Bernabe E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. Journal of dental research, 93(11):1045-1053, doi:10.1177/0022034514552491 (2014).

3 Giannobile WV. Commentary: treatment of periodontitis: destroyed periodontal tissues can be regenerated under certain conditions. J Periodontol 85(9):1151-4, doi:10.1902/jop.2014.140254 (2014).

4 Bosshardt DD, Selvig KA. Dental cementum: The dynamic tissue covering of the root. Periodontal 13:41-75 (2000).

5 Willa Yeretsky. Gingivitis: Its causes and treatment. Jour. A.D.A 30(9):729-737, doi:10.14219/jada.archive.1943.0135 (1943).

6 Wojciech J. Grzesik, A.S. Narayanan. Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med 13(6):474-484 (2002).

7 Brian L. Foster, Kanako J. Nagatomo, Francisco H. Nociti Jr., Hanson Fong, Daisy Dunn, Anne B. Tran, Wei Wang, Sonoko Narisawa, Jose Luis Millan, Martha J. Somerman. Central role of pyrophosphate in acellular cementum formation. Plos one, 7(6):e38393, doi:10.1371/journal.pone.0038393 (2012).

8 Eva Matalova, Vlasta Lungova, Paul Sharpe. Development of tooth and associated structures. Stem cell biology and tissue engineering in dental sciences 335-346, doi:10.1016/B978-0-12-397-157-9.00030-8 (2015).

9 Lia Hoz, Enrique Romo, Margarita Zeichner-David, Mariano Sanz, Javier Nunez, Luis Gaitan, Gabriela Mercado, Higinio Arzate. Cementum protein 1 (CEMP1) induces differentiation by human periodontal ligament cells under three-dimensional culture conditions. Cell biology international 36(2):129-136, doi:10.1042/CBI20110168 (2012).

10 Marco Antonio Alvarez-Perez, Sampath Narayanan, Margarita Zeichner-David, Higinio Azrate, Bruno Rodriguez Carmona. Molecular cloning, expression and immunolocalization of a human cementum-derived protein (CP-23). Bone 38(3):409-19, doi:10.1016/j.bone.2005.09.009 (2006).

11 Song A, Cai J, Pan K, Yang P. Pre-existing root cementum may promote cementoblast differentiation of human periodontal ligament cells. Cell Prolif 45(3):249-58, doi:10.1111/j.1365-2184.2012.00815.x. (2012).

12 S. Pitaru, S.A. Narayanan, S. Olson, N. Savion, H. Hekmati, I. Alt, Z. Metzger. Specific cementum attachment protein enhances selectively the attachment and migration of periodontal cells to root surfaces. Journal of periodontal research 30(5):360-368, doi:10.1111/j.1600-0765.1995.tb01288.x (1995).

13 MacNeil RL, Berry J, D'Errico J, Strayhorn C, Piotrowski B, Somerman MJ. Role of two mineral-associated adhesion molecules, osteopontin and bone sialoprotein during cementogenesis. Connect Tissue Res 33(1-3):1-7, PMID:7554941 (1995).

14 M.J. Somerman, J. J. Sauk, R. A. Foster, K. Norris, K. Dickerson, W. S. Argraves. Cell attachment activity of cementum: bone sialoprotein_identified in cementum. Journal of periodontal research 26(1):10-16, doi:10.1111/j.1600-0765.1991.tb01620.x (1991).

15 Boyd DH, Kinirons MJ, Gregg TA. A prospective study of factors affecting survival of replanted permanent incisors in children. Int J Paediatr Dent 10:200-205 (2000).

16 Liu HW, Yacobi R, Savion N, Narayanan AS, Pitaru S. A collagenous cementum-derived attachment protein is a marker for progenitors of the mineralized tissue forming cell lineage of the periodontal ligament. J Bone Miner Res 12:1691-1699 (1997).

17 Bar-Kana I, Savion N, Narayanan AS, Pitaru S. Cementum attachment protein manifestation is restricted to the mineralized tissue forming cells of the periodontium. Eur J Oral Sci 106 (Suppl 1):357-364 (1998).

18 Saito M, Narayan AS. Signalling reactions induced in human fibroblasts during adhesion to cementum-derived attachment. J Bone Miner Res 14:242-248 (1999).

19 Komaki M, Kang M, Narayanan AS. Role of MAP kinases $p42_{erk-2}$ /$p44^{erk-1}$in cementum-derived attachment protein mediated cell attachment. J Dent Res 79:1789-1793 (2000).

20 Hans Clevers. Wnt/β-Catenin signalling in development and disease. Cell 127(3):469-480, doi:10.1016/j.cell.2006.10.018 (2006).

21 Lim WH, Liu B, Cheng D, Williams BO, Mah, SJ, Helms JA. Wnt signalling regulates homeostasis of the periodontal ligament. J Periodontal Res 49(6):751-9, doi:1111/jre.12158 (2014).

22 Shuigen Li, Jin Shao, Yinghong Zhou, Yin Xiao, Jiangwu Yao, Bin Shi, Yin Xiao. The impact of Wnt signalling and hypoxia on osteogenic and cementogenic differentiation in

human periodontal ligament cells. Molecular Medicine reports 14:4975-4982, doi:10.3892/mmr.2016.5909 (2016).

23 Zhou Y, Fan W, Xiao Y: The effect of hypoxia on the stemness and differentiation capacity of PDLC and DPC. Biomed Res Int 890675, doi:10.1155/2014/890675 (2014).

24 Bruno Carmona-Rodriguez, Marco Antonio Alvarez-Perez, A. Sampath Narayanan, Margarita Zeichner-David, Jose Reyes-Gasga, Juan Molina-Guarneros, Ana Lilia Garcia-Hernandez, Jose Luis Suarez-Franco, Ivet Gil Chavarria, Eduardo Villarreal-Ramirez, Higinio Arzate. Human Cementum Protein 1 induces expression of bone and cementum proteins by human gingival fibroblasts. BBRC 358(3):763-769, doi:10.106/j.bbrc.2007.04.204 (2007).

25 Hoz L, Romo E, Zeichner-David M, Sanz M, Nunez J, Gaitan L, Mercado G, Arzate H. Cementum protein 1 (CEMP1) induces differentiation of human periodontal ligament cells under three-dimensional culture conditions. Cell Biol Int 36(2):129-36, doi:10.1042/CBI20110168 (2012).

26 Chen X, Liu Y, Miao L, Wang Y, Ren S, Yang X, Hu Y, Sun W. Controlled release of recombinant cementum protein 1 from electrospun multiphasic scaffold for cementum regeneration. Int J Nanomedicine 11:3145-58, doi:10.2147/IJN.S104324 (2016).

27 Yinghong Zhou, Chengtie Wu, Xufang Zhang, Pingping Han, Yin Xiao. The ionic products from bredigite bioceramics induced cementogenic differebtiation of periodontal cells via activation of the Wnt/β-Catenin signalling pathway. Research gate, doi:10.1039/C3TB20445F (2013).

28 Guarino V, Causa F, Ambrosio L. Bioactive scaffolds for bone and ligament tissue. Expert Rev Med Devices 4(3):405-18, doi:10.1586/17434440.4.3.405 (2007).

29 Scott Stratton, Namdev B. Shelke, Kazunori Hoshino, Swetha Rudraiah, Sangamesh G. Kumar. Bioactive polymeric scaffolds for tissue engineering. Bioactive material 1(2016):93-108, doi:10.1016/j.bioactmat.2016.11.001 (2016).

30 Langer R, Vacanti JP. Tissue engineering. Science 260(5110):920-6 (1993).

31 R. Skalak, C.F. Fox, Eds., Tisuue engineering. Biomed. Eng. 19:529 (1988).

32 Chengtie Wu, Lei Chen, Jiang Chang, Lingfei Wei, Dong Chen, Yufeng Zhang. Porous nagelschmidtite bioceramic scaffolds with improved in vitro and in vivo cementogenesis for periodontal tissue engineering. RSC Advances 3(39):17843, doi:10.1039/c3ra43350a (2013).

33 Yufeng Zhang, Chengtie Wu, Shue Li. The in vitro and in vivo cementogenesis of $CaMgSi_2O_6$ bioceramic scaffolds. Journal of biomed mat res part a 102(1), doi:10.1002/jbm.a.34679 (2014).

34 Yinghong Zhou, Chengtie Wu, Yin Xiao. The stimulation of proliferation and differentiation of periodontal ligament cells by the ionic products from $Ca_7Si_2P_2O_{16}$ bioceramics. Acta biomaterilia 8(6):2307-16, doi:10.1016/j.actbio.2012.03.012 (2012).

35 Chengtie Wu, Yinghong Zhou, Chucheng Lin, Jian Chang, Yin Xiao. Strontium-containing mesoporous bioactive glass scaffolds with improved osteogenic/ cementogenic differentiation of periodontal ligament cells for periodontal tissue engineering. Acta Biomaterilia 8(10):3805-15, doi:10.1016/j.actbio:2012.06.023 (2012).

36 Pingping Han, Chengtie Wu, Jian Chang, Yin Xiao. The cementogenic differentiation of periodontal ligament cells via the cativation of Wnt/β-Catenin signalling pathway by Li+ ions release from bioactive scaffolds. Biomaterials 33(27):6370-9, doi:10.1016/j.biomaterials.2012.05.061 (2012).

37 Pingping Han, Mengchi Xu, Jiang Chang, Nishant Chakravorty, Chengtie Wu, Yin Xao. Lithium release from tricalcium phosphate inducing cementogenic and osteogenic differentiation hPDLCs and hBMSCs. Doi:10.1039/C4BM00111G (2014).

38 Xufang Zhang, Pingping Han, Anjali Jaiprakash, Chengtie Wu, Yin Xiao. A stimulatory effect of $Ca_3ZrSi_20_9$ bioceramics on cementogenic/ osteogenic differentiation of periodontal ligament cells. Journal of materials chemistry B 2:1415-1423, doi:10.1039/c3tb21663b (2014).

39 Gauthier P, Yu Z, Tran QT, Bhatti FU, Zhu X, Huang GT. Cementogenic genes in human periodontal ligament stem cells are downregulated in response to osteogenic stimulation while upregulated by Vitamin C treatment. Cell Tissue Res 368(1):79-92, doi:10.1007/s00441-016-2513-8 (2016).

40 Cedryck Vaquette, Wei Fan, Yin Xiao, Stephen Hamlet, Dietmar W. Hutmacher, Saso Ivanovski. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/ periodontal ligament complex. Biomaterials 33(22):5560-5573, doi:10.1016/j.biomaterials.2012.04.038 (2012).

41 Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 10-16:364(9429):149-55, doi:10.1016/S0140-6736(04)16627-0 (2004).

42 Zhengguo Cao, Hua Zhang Xin Zhou, Xianglong Han, Yinshi Ren, Tian Gao, Yin Xiao, Benoit de Crombrugghe, Martha J Somerman, Jian Q Feng. Genetic evidence for the vital function of Osterix in cementogenesis. JBMR 27(5):1080-92, doi:10.1002/jbmr.1552 (2012).

43 Zhengguo Cao, Rubing Liu, Hua Zhang, Haiqing Liao, Yufeng Zhang, Robert J Hinton, Jian Q. Feng. Osterix controls cementoblast differentiation through down-regulation of wnt-signalling via enhancing DKK1 expression. IJBS 11(3):335-334 doi:10.7150/ijbs.10874 (2015).

44 Cho H, Tarafder S, Fogge M, Kao K, Lee CH. Periodontal ligament stem/ progenitor cells with protein-releasing scaffolds for cementum formation and integration on dentin surface. Connect Tissue Res 57(6):488-495, doi:10.1080/03008207.2016.1191478 (2016).

45 Mao L, Liu J, Zhao J, Chang J, Xia L, Jiang L, Wang X, Lin K, Fang B. Effect of micro-nano-hybrid structured hydroxyapatite bioceramics on osteogenic and cementogenic differentiation of human periodontal ligament stem cell via Wnt signalling pathway. Int J Nanomedicine 10:7031-44, doi:10.2147/IJN.S90343 (2015).

46 D'Errico JA, MacNeil RL, Strayhorn CL, Piotrowski BT, Somerman MJ. Models for the study of cementogenesis. Connect Tissue Res 33(1-3):9-17 (1995).

47 Komaki M, Iwasaki K, Arzate H, Narayanan AS, Izumi Y, Morita I. Cementum protein 1 (CEMP1) induces a cementoblastic phenotype and reduces osteoblastic differentiation in periodontal ligament cells. J Cell Phyiol 227(2):649-57, doi:10.1002/jcp.22770 (2012).

48 J.Massague. TGF-β Signal Transduction. Annual Rev of Biochem 67:753-791, doi:10.1146/annurev.biochem.67.1.753 (1998).

49 Hwajung Choi, Yu-Hyun Ahn, Tak-Heun Kim, Cheol-Hyeon Bae, Jeong-Chae Lee, Hyung-Keun You, Eui-Sic Cho. TGF-β Signalling regulates cementum formation through osteric expression. Sci Rep 6:26046, doi:10.1038/srep26046 (2016).

50 Bosshardt DD. Are cementoblasts a subpopulation of osteoblasts or a unique phenotype? J Dent Res 84(5):390-406, doi:10.1177/154405910508400501 (2005).

51 Kagayama M, Li HC, Zhu J, Sasano Y, Hatakeyama Y, Mizoguchi I. Expression of osteocalcin in cementoblasts forming acellular cementum. J Periodontal Res Apr;32(3):273-8 (1997).

52 Smit Jayant Dangaria, Yoshihiro Ito, Xianghong Luan, Thomas G.H. Diekwisch. Successful periodontal ligament regeneration by periodontal progenitor preseeding on natural tooth root surfaces. Stem Cells Dev 20(10): 1659-1668, doi:10.1089/scd.2010.0431 (2011).

53 M.D. McKee, S. Zalzal, A. Nanci. Extracellular matrix in tooth cementum and mantle dentin: Localization of osteopontin and other noncollagenous proteins, plasma proteins and glycoconjugates by electron microscopy. AR 245(2):293-312, doi:10.1002/(SICI)1097-0185(199606)245:2<293::AID-AR13>3.0.CO;2-K (1996).

54 Teruko Takano-Yamamoto, Teiji Takemura, Yukihiko Kitamura, Shintaro Nomura. Site-specifc expression of mRNAs for Osteonectin, Osteocalcin and Osteopontin revealed by in-situ hybridization in rat periodontal ligament during physiological tooth movement. The journal of histocemistry and cytochemistry 42(7):885-896 (1994).

55 Mark R. Wick, Jason L. Hornick. Immunohistology of soft tissue and osseous neoplasms. Theranostic and genomic applications 83-136 (2011).

56 Jessica M. Trombetta, Amy D. Bradshaw. SPARC/ Osteonectin functions to maintain homeostasis of the collagenous extracellular matrix in the periodontal ligament. J Histochem Cytochem 58(10):871-879, doi:10.1369/jhc.2010.956144 (2010).

57 Jong Hoon Chung, Pill-Hoon Choung, Ki-Taek Lim, Han-Wool Choung. Scaffolds for human dental stem cells to regenerate cementum. Stem cells and cancer stem cells 5:161-170, doi:10.1007/978-94-007-2900-1_16 (2012).

58 Lee CH, Hajibandeh J, Suzuki T, Fan A, Shang P, Mao JJ. Three-dimensional printed multiphasic scaffolds for regeneration of periodontal complex. Tissue Eng Part A 20(7-8):1342-51, doi:10.1089/ten.TEA.2013.0386 (2014).

59 Bartold PM, McCulloach CA, Narayanan AS, Pitaru S. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. Periodontal 2000 24:253-69 (2000).

60 Chan Ho Park, Hector F. Rios, Qimimg Jin, Megan E. Bland, Colleen L. Flanagan, Scott J. Hollister, Williamm V. Giannobile. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. Biomat 31(23):5945-5952 (2010).

61 Iwasaki K, Komaki M, Yokoyama N, Tanaka Y, Taki A, Kimura Y, Takeda M, Oda S, Izumi Y, Morita I. Periodontal ligament stem cells possess the characteristics of pericytes. J Periodontal 84(10):1425-33, doi:10.1902/jop.2012.120547 (2014).

62 Sowmya S, Mony U, Jayachandran P, Reshma S, Kumar RA, Arzate H, Nair SV, Jayakumar R. Tri-layered nanocomposite hydrogel scaffold for the concurrent regeneration of cementum, PDL and alveolar bone. Adv Heakthc Mater 6(7), doi:10.1002/adhm.201601251 (2017).

63 Li Zhang, Yosry Morsi, Yanying Wang, Yubao Li, Seeram Ramakrishna. Review scaffold design and stem cells for tooth regeneration. Jap Dent Sci Rev 49(1):14-26, doi:10.1016/j.jdsr.2012.09.001 (2013).

64 S. Ivanovski, C. Vaquette, Ferracane and William V. Giannobile. Multiphasic scaffolds for periodontal tissue engineering. J Dent Res 93(12):1212-1221, doi:10.1177/0022034514544301 (2014).

65 Xie H, Liu H. A novel mixed-type stem cell pellet for cementum/periodontal ligament-like complex. J Periodontal 83(6):805-15, doi:10.1902/jop.2011.110267 (2012).

66 Han C, Yang Z, Zhou W, Jin F, Song Y, Wang Y, Huo N, Chen L, Qian H, Hou R, Duan Y, Jin Y. Periapical follicle stem cell: a promising candidate for cementum/ periodontal ligament regeneration and bio-root engineering. Stem Cells Dev 19(9):1405-15, doi:10.1089/scd.2009.0277 (2010).

67 Hidetoshi Shimauchi, Eiji Nemoto, Hiroshima Ishihata, Masatsugu Shimomura. Possible functional scaffolds for PDL regeneration. 49(4):118-130, doi:10.1016/j.jdsr.2013.05.001 (2013).

68 Heijl L, Heden G, Svardstrom G, Ostgren A. Enamel matrix derivative in the treatment of intrabony periodontal defects. J Clin Periodontal 24:705-714 (1997).

69 Hammarstrom L. Enamel matrix and cementum development, repair and regeneration. J Clin Periodontology 24:658-668 (1997).

70 Qi Y, Feng W, Cai J, Sun Q, Li S, Li M, Song A, Yang P. Effects of conservatively treated diseased cementum with or without EMD on in-vitro cementoblast differentiation and in-vivo cementum-like tissue formation of human periodontal ligament cells. Cell prolif. 47(4):310-7, doi:10.1111/cpr.12116 (2014).

71 Yoshinori Kuboki, Masaru Sasaki, Akira Saito, Hiroko Takita, Hiroshi Kato. Regeneration of periodontal ligament and cementum by BMP-applied tissue engineering. European Journal of Oral Sciences 106:S1, doi:10.1111/j.1600-0722.1998.tb02176.x (2014).

72 Shirkata Y, Miron RJ, Nakamura T, Sena K, Shinohara Y, Horai N, Bosshardt DD, Noguchi K, Sculean A. Effects of EMD liquid (Osteogain) on periodontal healing in class III furcation defects in monkeys. J Clin Periodontal 44(3):298-307, doi:10.1111/jcpe.12663 (2017).

73 Ayanoglou CM, Lesty C. New cementum formation induced by cyclosporin: a histological, ultrastructural and histomorphometric study in the rat. J Period Res 32(6):543-56 (1997).

74 Higino Arzate, Marco A Alvarez, A Sampath Narayanan. Cyclosporin A promotes mineralization by human cementoblastoma-derived cells in culture. J of Period Res 40(3):218-24, doi:10.1111/j.1600-0765.2005.00797.x (2005).

75 Spolidorio LC, Spolidorio DM, Holzhausen M, Nassar CA, Nassar PO. Cyclosprin A-induced new cementum formation: a morphometric evaluation in the periapical region of rats. Braz Dent J 18(1):24-8 (2007).

76 Joerg Huelsken, Juergen Behrens. The Wnt signalling pathway. Journal of Cell Science 115:3977-3978, doi:10.1242/jcs.00089 (2002).

77 Daniel Pinto, Alex Gregorieff, Harry Begthel, Hans Clevers. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes&Dev, 17:1709-1713, doi:10.1101/gad267103 (2003).

78 Van Es JH, Jay P, Gregorieff A, Van Gijn ME, Jonkheer S, Hatzis P, Thiele A, Van Den Born M, Beqthel H, Brabletz T, Taketo MM, Clevers H. Wnt signalling induces maturation of Paneth cells in intestinal crypts. Nat Cell Biol, 7(4):381-386, doi:10.1038/ncb1240 (2005).

79 Bo Hai, Zhenhua Yang, Sarah E. Millar, Yeon Sook Choi, Makoto Mark Taketo, Andras Nagy and Fei Liu. Wnt/β-Catenin signalling regulates postnatal development and regeneration of the salivary gland. Stem cells and Dev, 19:11, doi: 10.1089/scd.2009.0499 (2010).

80 Vitor C.M. Neves, Rebecca Babb, Dhivya Chandrasekaran, Paul T. Sharpe. Promotion of natural tooth repair by small molecule GSK3 antagonists. Scientific reports 7:39654, doi:10.1038/srep39654 (2017).

81 Qiming Jin, M Zhao, S.A. Webb, Janice Elizabeth Berry, Martha J Somerman, William V Giannobile. Cementum engineering with three-dimensional polymer scaffolds. Journal of biomedical mat Res Part A 67(1):54-60, doi:10.1002/jbm.a.10058 (2003).

82 Lenka Kibiczkova, Lenka Sedlarikova, Roman Hajek, Sabina Sevcikova. TGF-β- an excellent servant but a bad master. J of transl med 10:183, doi:10.1186/1479-5876-10-183 (2012).

83 Xin Zhou, Zhaoping Zhang, Jian Q. Feng, Vladmir M. Dusevich, Krishna Sinha, Hua Zhang, Bryant G. Darnay, Benoit de Crombrugghe. Multiple functions of Osterix are required for bone growth and homeostasis in postnatal mice. PNAS 107(29):12919-12924, doi:10.1073/pnas.0912855107 (2010).

84 Shang Gao, Yuzhuo Wang, Xiaolong Wang, Peng Lin, Min Hu. Effect of lithium ions on cementoblsts in the presence of lipopolysaccharide in vitro. Exp Ther Med 9(4):1277-1282, doi:10.3892/etm.2015.2276 (2015).

85 Orimo H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. J Nippon Med Sch 77(1):4-12 (2010).

86 Ellis E. Golub, Kathleen Boesze- Battaglia. The role of alkaline phosphatase in mineralization. Current opinion in orthopaedics 18:444-448 (2007).

87 E. Michael Lewiecki. Role of sclerostin in bone and cartilage and its potential as therapeutic target in bone diseases. Ther Adv Musculoskeletal Dis 6(2):48-57, doi:10.1177/1759720X13510479 (2014).

88 Xingfu Bao, Yuyan Liu, Guanghong Han, Zingang Zuo, Min Hu. The effect on proliferation and differentiation by using sclerostin as inhibitor. Int J Mol Sci 14(10):21140-21152, doi:10.3390/ijms141021140 (2013).

89 Glen M Blake, Ignac Fogelman. Strontium ranelate: a novel treatment for postmenopausal osteoporosis: a review of safety and efficacy. Clin Interv Aging 1(4):367-375 (2006).

90 Xingfu Bao, Xianjun Liu, Yi Zhang, Yue Cho, Jindan Yao, Min Hu. Strontium promotes cementoblasts differentiation through inhibiting sclerostin expression in-vitro. Biomed Res Int 487535, doi:10.1155/2014/487535 (2014).

91 Foster BL, Somerman MJ, Cementum. In: McCauley LK, Somerman MJ, editors. Mineralized tissues in oral and craniofacial science. Hoboken, NJ, USA: Wiley-Blackwell; 169-181 (2012).

92 Brya G Mathews, Hrvoje Roguljic, Tiziana Franceschetti, Emilie Roeder, Igor Matic, Ivana Vidovic, Pujan Joshi, Kee-Yeon Kum, Ivo Kalajzic. Gene expression analysis of cementoblasts and osteoblasts. J Periodontal Res 51(3):304-312, doi:10.1111/jre.12309 (2016).

93 M. Li, D. D. Thompson, V.M Paralkar. Prostaglandin E_2 receptors in bone formation. Int Orthop 31(6):767-772, doi:10.1007/s00264-007-0406-x (2007).

94 Mada Y, Miyauchi M, Oka H, Kitagawa M, Sakamoto K, Iizuka S, Sato S, Noguchi K, Somerman MJ, Takata T. Effects of endogenous and exogenous prostaglandin E_2 on the proliferation and differentiation of a mouse cementoblast cell line (OCCM-30). J Periodontol 77(12):2051-8 (2006).

95 Nemoto E, Darveau RP, Foster BL. Regulation of cementoblast function by P.gingivalis lipopolysaccharide via TLR2. J Dent Res 85(8):733-738 (2006).

96 MacNeil RL, Somerman MJ. Development and regeneration of periodontium: parallels and contrasts. Periodontal 19:8-20 (2000).

97 Dieter D. Bosshardt, Anton Sculean. Does periodontal tissue regeneration really work? Peridontology 51:208-219, doi:10.1111/j.1600-0757.2009.00317.x (2000).

98 Narayanan AS, Bartold PM. Biocemistry of periodontal connective tissues and their regeneration: a current perspective. Conect tissue res 34:191-20, doi:10.3109/03008209609000698 (1996).

99 Saygin NE, Giannobile WV, Somerman MJ. Molecular and cell biology of cementum. Perodontal 24:73-98, doi:10.1034/j.1600-0757.2000.2240105.x. (2000).

100 Yan XZ, Yang F, Jansen JA, de Vries Rob BM, van den Beucken Jeroen JJP. Cell-based approaches in periodontal regeneration: a systematic review and meta-analysis of periodontal defect models in animal experimental work. Tissue Eng Part B review. 21:411-426, doi:10.1089/ten.teb.2015.0049 (2015).

101 Chen FM, Sun HH, Lu H, Yu Q. Stem cell-delivery therapeutics for periodontal tissue regeneration. Biomaterials 33:6320-6344, doi:10.1016/j.biomaterials.2012.05.048 (2012).

102 Young CS, Abukawa H, Asrican R, Ravens M, Troulis MJ, Kaban LB, Vacanti JP, Yelick PC. Tissue-engineered hybrid tooth and bone. Tissue Eng. 11:1599-1610, doi:10.1089/ten.2005.11.1599 (2005).

103 Chen FM, Zhang J, Zhang M, An Y, Chen F, Wu ZF. A review on endogenous regenerative technology in periodontal regenerative medicine. Biomaterials, 31:7892-7927, doi:10.1016/j.biomaterials.2010.07.019 (2010).

104 C.A. Ramseier, G. Rasperini, S. Batia, W.M. Giannobile. Advanced reconstructive technologies for periodontal tissue repair. Periodontol 2000, 59(1):185-202, doi:1111/j.1600-0757.2011.00432.x. (2012).

105 Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL. Regeneration of periodontal tissue: bone replacement grafts. Dent Clin North Am, 54(1):55-71, doi:10.1016/j.cden.2009.09.003 (2010).

106 Richard N. Wang, Jordan Green, Zhongliang Wang, Youlin Deng, Min Qiao, Michael Peabody, Qian Zhang, Jixing Ye, Zhengjian Yan, Sahitya Denduluri, Olumuyiwa Idowu, Melissa Li, Christine Shen, Alan Hu. Bone morphogenetic protein (BMP) signalling in development and human diseases. Genes and diseases 1(1):87-105, doi:10.1016/j.gendis.2014.07.005 (2014).

107 Zhao M, Xiao G, Berry J. E., Franceschi R. T., Reddi A., Somerman M. J. Bone morphogenetic protein 2 induces dental follical cells to differentiate towards a cementoblast/ osteoblast phenotype. Journal of bone and min res 17(8):1441-1451, doi:10.1359/jbmr.2002.17.8.1441 (2002).

108 Zhao M., Berry J. E., Somerman M. J. Bone morphogenetic protein-2 inhibits differentiation and mineralization of cementoblasts in vitro. Journal of dental research 82(1):23-27, doi:10.1177/154405910308200106 (2003).

109 Yang Z., Hai B., Qin L., Cessation of epithelial BMP signalling switches the differentiation of crown epithelia to the root lineage in a β-Catenin dependant manner. Mol and Cel Biol 33(23):4732-4744, doi:10.1128/mcb.00456-13 (2013).

110 Bae C. H., Lee J. Y., Kim T. H. Excessive Wnt/ β-Catenin signalling disturbs tooth-root formation. Journal of Periodontal Res 48(4):405-410, doi:10.1111/jre.12018 (2013).

111 Kuchler U., Schwarze U. Y., Dobsak T. Dental and periodontal phenotype in sclerostin knockout mice. Intl J of Oral Sci 6(2):70-76, doi:10.1038/ijos.2014.12 (2014).

112 Lim W. H., Liu B., Hunter D. J., Cheng D., Mah S.J., Helms J. A. Downregulation of Wnt causes root resorption. American Journal of orthodontics and dentofacial orthopaedics. 146(3):337-345, doi:10.1016/j.ajodo.2014.05.027 (2014).

113 Jager A., Kunert D., Friesen T., Zhang D., Lossdorfer S., Gotz W. Cellular and extracellular factors in early root resorption repair in the rat. European Journal of orthodontics. 30(4):336-345, doi:10.1093/ejo/cjn012 (2008).

114 Zhang P., Wu Y., Jiang Z., Jiang L., Fang B. Osteogenic response of mesenchymal stem cells to continuous mechanical strain is dependent on ERK1/2-Runx2 signalling. Intl Journal of Mol Med. 29(6):1083-1089, doi:10.3892/ijmm.2012.934 (2012).

115 Diercke K, Kohl A, Lux CJ. IL-1 beta and compressive forces lead to a significant induction of RANKL-expression in primary human cementoblasts. J Orofac Orthop. 73(5):397-412 (2012).

116 Diercke K, Konig A, Kohl A. Human primary cementoblasts respond to combined IL-1 beta stimulation and compression with an impaired BSP and CEMP-1 expression. Eur J Cell Biol. 91(5):402-412 (2012).

117 Foster BL. On the discovery of cementum. J Periodontal Res. 52(4):666-685, doi:10.1111/jre.12444 (2017).

118 Boyera N, Galey I, Bernard BA. Effect of vitamin C and its derivatives on collagen synthesis and cross-linking by normal human fibroblasts. Int J Cosmet Sci. 20(3):151-8, doi:10.1046/j.1467-2949.1998.171747.x. (1998).