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E-mail: jzus_b@zju.edu.cn

Review

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Roles of Wnt signaling pathway in cementum formation, cementum regeneration, and cementocyte function

Tiancheng LI[1,2*], Xinyi ZENG[1*], Shuxian YANG[1], Lynda Faye BONEWALD[3✉], Peipei DUAN[1✉]

[1]*State Key Laboratory of Oral Diseases / National Center for Stomatology / National Clinical Research Center for Oral Diseases / Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, China*
[2]*Department of Orthodontics, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, College of Stomatology, Shanghai Jiao Tong University, National Center for Stomatology, National Clinical Research Center for Oral Diseases, Shanghai Key Laboratory of Stomatology, Shanghai 200011, China*
[3]*Departments of Anatomy, Cell Biology & Physiology and Orthopaedic Surgery, Indiana Center for Musculoskeletal Health, Indiana University School of Medicine, Indianapolis, IN 46202, USA*

Abstract: Cementum, a mineralized connective tissue that covers the tooth root, is crucial in protecting the root from resorption, maintaining occlusal relationships, and supporting tooth function. Cementocytes are embedded within the cementum matrix and extend dendritic processes through the canaliculi. They are thought to be mechanosensitive, responding to changes in mechanical loading, and are physiologically responsive cells associated with the formation of cellular cementum in response to variations in functional demands on the tooth. The wingless and int (Wnt) signaling pathway, which controls cell fate and regulates growth, development, and homeostasis in the body, plays a pivotal regulatory role in normal biological development and disease progression. Currently, the mechanisms by which the Wnt signaling pathway influences cementogenesis and regeneration remain controversial. Research findings on the roles of Wnt/β-catenin signaling in cementoblast differentiation and function have been mixed. Some studies indicate that activating this pathway enhances cementoblast differentiation, while others suggest that Wnt signaling may inhibit it, favoring cell proliferation instead. This paper reviews the structure and physiological roles of cementum, focusing on how Wnt signaling influences the growth and differentiation of cementoblasts. We emphasize the pivotal role of the Wnt pathway in cementum formation and development, as well as in root resorption and repair, and hypothesize that maintaining low Wnt/β-catenin levels is crucial to achieving an optimal balance between cementoblast proliferation and differentiation. Finally, we propose periodontal regeneration treatment strategies based on the Wnt signaling pathway and suggest future research directions.

Key words: Dental cementum; Odontogenesis; Wingless and int (Wnt) signaling pathway; Cell differentiation; Biomineralization

1 Introduction

Dental cementum, a mineralized tissue covering the dentin of the tooth root, is histologically divided into two forms: acellular and cellular (Zhao et al., 2016a). Acellular cementum predominantly lines the root's cervical and middle regions, serving as a vital component in anchoring the tooth to the alveolar bone via the periodontal ligament (Zhao et al., 2016a). In contrast, cellular cementum, which lacks vascularization, forms along the apical portion of the root, where it plays an adaptive role in sustaining the tooth under occlusal forces (Xu et al., 2019). Notably, cellular cementum usually forms during healing, following the removal of diseased cementum, earning it the designation of reparative or regenerative cementum (Bosshardt and Sculean, 2009). Cementoblasts, specialized mesenchymal-derived cells, are responsible for this type of cementum formation by secreting extracellular matrix proteins that contribute to its mineralization and integration into the periodontal structure. As cementoblasts become embedded within the cementum matrix, they differentiate into cementocytes, which reside in lacunae and participate in

✉ Peipei DUAN, duanp@scu.edu.cn
Lynda Faye BONEWALD, lbonewal@iu.edu
* The two authors contributed equally to this work
Peipei DUAN, https://orcid.org/0000-0003-4111-8112
Lynda Faye BONEWALD, https://orcid.org/0000-0002-5536-9943
Tiancheng LI, https://orcid.org/0000-0001-9628-4316
Xinyi ZENG, https://orcid.org/0000-0002-3561-1720

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mechanosensing and mineral homeostasis. Cementum possesses distinct structural and functional traits that make it susceptible to pathologies like external root resorption. Unlike bone, cementum has a relatively low metabolic turnover and lacks an extensive vascular supply, impairing its capacity for resorption or rapid post-injury repair (Yamamoto et al., 2019). Critically, cementum lacks the cyclical remodeling seen in alveolar bone, rendering it prone to irreversible degradation once osteoclastic activation occurs. Consequently, it cannot counteract resorption under pathological stressors, such as infection, trauma, or orthodontic forces, particularly at the root apex (Ahangari et al., 2015). Cementum's structural integrity is therefore essential not only for preventing resorption but also for maintaining stable occlusal relationships and ensuring long-term tooth support (Morgenthal et al., 2021).

The wingless and int (Wnt) signaling pathway is crucial for the development and maintenance of multicellular organisms by regulating growth and differentiation and determining cell fate, including proliferation, apoptosis, and malignancy (Routledge and Scholpp, 2019). Notably, this pathway plays a central role in the formation and development of mineralized tissues such as bone and cementum, where Wnt/β-catenin signaling governs osteocyte survival, mechanotransduction, and metabolic homeostasis to sustain tissue integrity (Duan and Bonewald, 2016). Abnormal Wnt signaling has been linked to various diseases, such as metabolic disorders, bone diseases, and periodontal pathologies. Wnt signaling is generally divided into three key branches: the canonical Wnt pathway (Cadigan and Peifer, 2009), the noncanonical Wnt-planar cell polarity (Wnt-PCP) pathway (Jenny, 2010), and the Wnt-calcium (Wnt-Ca^{2+}) pathway (Kohn and Moon, 2005). Canonical Wnt signaling is activated when Wnt proteins, such as Wnt3a, bind to the co-receptor complex comprising frizzled (Fz) and lipoprotein receptor-related protein 5/6 (LRP 5/6) (Tamai et al., 2000). This interaction triggers the activation of dishevelled (DVL), which suppresses the destruction complex—comprising axis inhibition protein (Axin), adenomatous polyposis coli (APC), glycogen synthase kinase-3β (GSK-3β), and casein kinase 1α (CK1α)—allowing β-catenin to accumulate in the cytoplasm and subsequently move into the nucleus (Behrens et al., 1998). Within the nucleus, β-catenin partners with T-cell-specific factor/lymphoid-enhancing factor (TCF/LEF) to modulate the transcription of Wnt-responsive genes (Aoki et al., 1999). Without Wnt proteins, the destruction complex becomes active, phosphorylating β-catenin and targeting it for degradation via the proteasome (Karner and Long, 2017). Several extracellular inhibitors, including secreted frizzled-related protein (sFRP) (Karner and Long, 2017), dickkopf 1 (DKK1) (Shen et al., 2020), and sclerostin (Zhang et al., 2020), tightly regulate Wnt signaling by preventing pathway activation. These inhibitors are considered promising therapeutic targets for disease treatment and regenerative therapies (Fig. 1).

This narrative review focuses on the latest scientific knowledge regarding the association between cementum formation and the Wnt/β-catenin pathway, the most common type of Wnt signaling. We also discuss Wnt/β-catenin signaling's effect on the root resorption and repair process and the potential role of the noncanonical Wnt pathway in cementogenic differentiation. Wnt/β-catenin signaling plays a pivotal role in regulating cementum and periodontal tissue development. However, maintaining a balance in Wnt signaling is crucial, as both excessive and insufficient signaling can disrupt tissue homeostasis. We hypothesize that maintaining low levels of Wnt/β-catenin activity is critical for the proper differentiation of cementoblasts and the mineralization of cementum. Specifically, Wnt signaling must be finely tuned to promote cellular proliferation without driving excessive differentiation or mineralization, which could lead to pathological conditions such as cementum overgrowth or ankylosis.

2 Roles of Wnt/β-catenin signaling in cementoblast cell growth and differentiation

2.1 Cementoblasts/cementocytes

The role of Wnt signaling in modulating the biological activities of cementoblasts and cementocytes was first recognized in the early 2000s through a study investigating the effect of extracellular phosphate on gene expression in an immortalized mouse cementoblast cell line over time. Employing DNA microarrays, altered expression of genes across multiple Gene Ontology (GO) categories was found, including those related to transcription factor activity and Wnt signaling (Rutherford et al., 2006). Subsequent transcriptomic assessments using next-generation RNA sequencing (RNA-seq) identified Wnt signaling, along with cadherin signaling and "anatomical structure development" as key biological

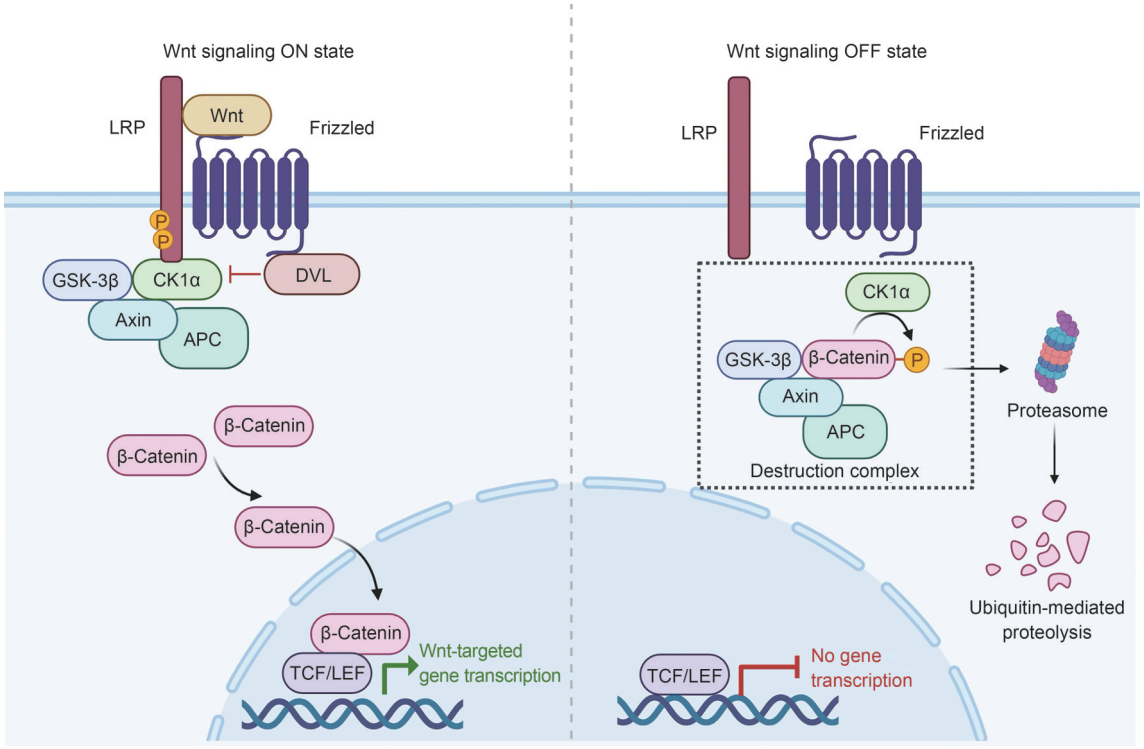

Fig. 1 Wingless and int (Wnt) signaling cascade, along with its regulatory components. Wnt ligands bind to the secreted frizzled-related protein–lipoprotein receptor-related protein 5/6 (sFRP–LRP 5/6) receptor complex, activating dishevelled (DVL) while suppressing the destruction complex. This releases β-catenin, which translocates to the nucleus and interacts with T-cell-specific factor/lymphoid-enhancing factor (TCF/LEF) to control Wnt-targeted gene expression. GSK-3β: glycogen synthase kinase-3β; CK1α: casein kinase 1α; Axin: axis inhibition protein; APC: adenomatous polyposis coli.

processes in periodontal ligament cells (PDLCs) with a higher potential of differentiating into osteo/cementoblastic (O/C) phenotypes (Saito et al., 2020).

However, investigations of the role of canonical Wnt signaling in cementoblastic cell growth and differentiation have produced controversial and inconsistent results. While some studies suggest that canonical Wnt/β-catenin signaling promotes cementoblast differentiation, others indicate that it may inhibit differentiation in favor of cell proliferation (Nemoto et al., 2009; Cao et al., 2015; Kunimatsu et al., 2022; Liu et al., 2022). These discrepancies may arise from variations in the timing and intensity of Wnt activation during differentiation, differences in the cellular context (e.g., primary vs. immortalized cell lines), and crosstalk with other signaling pathways like bone morphogenetic protein (BMP) or peroxisome proliferator-activated receptor γ (PPARγ). To address these inconsistencies, we propose utilizing conditioned media or genetically modified models to isolate the effects of specific Wnt ligands, as well as in vivo studies to better understand the effects of Wnt signaling under physiological conditions.

2.1.1 Wnt signaling in cementoblast/cementocyte differentiation

Baicalin, a naturally occurring bioactive flavonoid derived from *Scutellaria baicalensis* Georgi, has been shown to enhance the expression of osteoprotegerin (OPG) in human cementoblast cell line (HCEM) cells via activation of the Wnt/β-catenin signaling pathway (Kunimatsu et al., 2022). Baicalin also elevated alkaline phosphatase (ALP) protein levels, Runt-related transcription factor 2 (Runx2) expression, ALP activity, and calcium deposition, while DKK1 inhibited these effects (Kimura et al., 2018). Another regulatory mechanism involves brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1 (Bmal1), which promotes both cementoblast differentiation and cementum mineralization via the Wnt/β-catenin pathway (Liu et al., 2022). Fibroblast growth factor-2 (FGF-2) and dexamethasone synergistically enhance each other's effects on cementoblast proliferation and differentiation through the Wnt signaling pathway in human cementoblasts (Xie and Shen, 2018). Similarly, the combined

application of recombinant human BMP-2 (rh-BMP-2) and dentin sialoprotein (rh-DSP) synergistically stimulates cementoblastic growth and differentiation in vitro while simultaneously activating the Wnt/β-catenin pathway (Lee et al., 2014). Intermittent parathyroid hormone (PTH) increases Wnt10b expression in cluster of differentiation 8-positive ($CD8^+$) T cells, and co-culture experiments indicate that this T-cell-induced Wnt10b expression may play an essential role in stimulating cementoblast activity (Lyu et al., 2019).

Another study found that murine cementoblasts-30 (OCCM-30, an osteoblast cell line) cells plated on fibrin matrices enhanced canonical Wnt signaling and expressed higher levels of biomineralization-associated markers than those grown on standard tissue culture dishes. These marker levels were further elevated by treatment with lithium chloride (LiCl), a known activator of Wnt signaling and Runx. Wnt/β-catenin signaling could be blocked by the downregulation of the nuclear factor of activated T-cell 5 (Nfat5) or the forced expression of miR-361-3p, which targets Nfat5. Notably, miR-361-3p overexpression impaired cementoblast differentiation (Liao et al., 2019). Collectively, these findings highlight that the activation of the Wnt/β-catenin pathway promotes the osteogenic differentiation of cementoblasts.

2.1.2 Wnt pathway-mediated inhibition of cementoblast/cementocyte differentiation and enhancement of cell proliferation

Contrary to earlier findings, growing evidence suggests that activating the Wnt/β-catenin pathway may inhibit the mineralization and differentiation of cementoblasts and cementocytes. A recent investigation employing OCCM-30 cells stably transfected with Wnt1-hemagglutinin (HA) demonstrated that Wnt1 enhances cementoblast proliferation but not differentiation (Nottmeier et al., 2021). Similarly, activating canonical Wnt signaling through high-dose (200 ng/mL) Wnt3a reduced Runx2, ALP activity, and the expression of key cementogenic genes (Li et al., 2022). Wnt3a also elevated cyclin D1 expression, further promoting cell proliferation. Notably, pretreatment with DKK1, a Wnt pathway antagonist, mitigated the inhibitory effects of Wnt3a on *Runx2* and bone γ carboxyglutamate protein (*Bglap*) expression, indicating that canonical Wnt signaling suppresses cementoblast differentiation by modulating specific transcription factors (Nemoto et al., 2009). Research has shown that PPARγ could also enhance cementoblast mineralization and differentiation by suppressing the Wnt/β-catenin pathway (Hu et al., 2020). Furthermore, one study reported that osterix (Osx) overexpression in cementoblasts upregulated key cementogenic markers and enhanced ALP activity but unexpectedly resulted in a significant rise in DKK1 and reduced Wnt/β-catenin signaling (Cao et al., 2015). The activation of canonical Wnt signaling by LiCl or Wnt3a elevated hepatocyte nuclear factor 1α (HNF1α) or TCF1 expression while suppressing the expression of osteopontin (OPN), osteocalcin (OCN), and bone sialoprotein (BSP), ALP activity, and extracellular mineralized nodule formation. In the same study, in vivo analyses further demonstrated that Osx deletion reduced DKK1 expression and concurrently increased β-catenin levels in *Osx* conditional knockout (*Osx* cKO) mice. This study utilized *Osx-lacZ* knockout mice, with the *Osx* gene replaced by a *lacZ* reporter, to trace *Osx* expression in the periodontal ligament and cementum at six weeks of age. To prevent neonatal lethality and investigate early-stage cementogenesis, mice with a floxed *Osx* allele were crossed with 2.3 kb type I collagen (*Col1*)-Cre mice, enabling tissue-specific deletion of *Osx* during embryonic development. The authors of this study concluded that *Osx* is crucial for regulating cementoblast proliferation and differentiation by directly increasing DKK1 levels to maintain low Wnt/β-catenin activity (Cao et al., 2015).

With respect to noncoding RNA-related mechanisms, increased miR-155-3p was found in tumor necrosis factor-α (TNF-α)-stimulated OCCM-30 cells. miR-155-3p overexpression inhibited cementoblast mineralization, partially due to increased canonical Wnt signaling (Wang et al., 2017). Another study demonstrated that DKK1 reversed the suppression of miR-3064-3p-mediated cementoblast differentiation (Wang et al., 2018). Additionally, investigations into long noncoding RNAs (lncRNAs) revealed that intermittent PTH treatment repressed essential components of the Wnt/β-catenin pathway in OCCM-30 cells, suggesting that lncRNAs negatively regulate Wnt signaling during PTH-induced cementogenesis (Li et al., 2021, 2023). In summary, these findings highlight that Wnt/β-catenin signaling supports cementoblast proliferation but inhibits its differentiation. Maintaining low Wnt/β-catenin activity appears to be key to balancing cementoblast proliferation and differentiation, ensuring optimal cementum formation and function.

2.1.3 Mechanical stress and Wnt signaling

Under physiological conditions, periodontal tissues are subjected to intermittent occlusal stress, making canonical Wnt signaling particularly significant in the responses of cementoblasts and cementocytes under external forces. A recent study found that applying light compressive forces (1.2, 2.4, and 3.6 gf/cm^2, 1 gf/cm^2= 98.0665 Pa) using glass cylinders activated both β-catenin and mitogen-activated protein kinase (MAPK) signaling pathways in OCCM-30 cells. In addition, MAPK inhibition reduced β-catenin expression, suggesting that MAPK is critical in stabilizing β-catenin during cementogenesis (Yong et al., 2021). Recent research revealed that murine cementoblasts exhibit impaired mineralization under a compressive force of 1.5 gf/cm^2 for 12 h and that knockdown of periostin, a mediator of autophagy, further inhibited mineralization by destabilizing β-catenin and suppressing Wnt signaling (Yang et al., 2023). These observations highlight the essential role of autophagy in cementoblast mineralization via the periostin/β-catenin pathway in response to mechanical stress. Further investigations showed that uniaxial compressive stress (2000 με at 0.5 Hz, 1 με=10^{-6}) inhibited the mineralization of OCCM-30 cementoblasts by suppressing osteogenic markers (Runx2, Bglap, and integrin-binding sialoprotein (Ibsp)) and increasing the receptor activator of nuclear factor-κB ligand (RANKL)/OPG ratio through BMP signaling (Bai et al., 2019). Mechanical tension (2500 με) using a four-point bending system inhibited OCCM-30 cementoblast differentiation activity in vitro; however, this inhibition was reversed by activating Wnt/β-catenin signaling using LiCl (Ge et al., 2019). Mechanical stress, whether applied by four-point bending or tension, significantly elevated *Runx2* messenger RNA (mRNA) and β-catenin levels in OCCM-30 cementoblasts. DKK1 significantly reduced *Runx2* mRNA expression in OCCM-30 cementoblasts (Shuqin et al., 2015). The above studies demonstrate that Wnt/β-catenin signaling could mediate the anabolic effects of both compressive and tension stress in OCCM-30 cementoblasts.

Our studies utilized the immortalized murine cementocyte cell line IDG-CM6 (immortomouse/Dmp1-GFP-CM6), generated by crossing the 8-kb expressing green fluorescent protein (GFP) directed by the dentin matrix protein 1 (*Dmp1*) promoter (*Dmp1*-GFP) transgenic mouse line with the immortomouse (Fig. 2) (Zhao et al., 2016b). We found that, under compressive

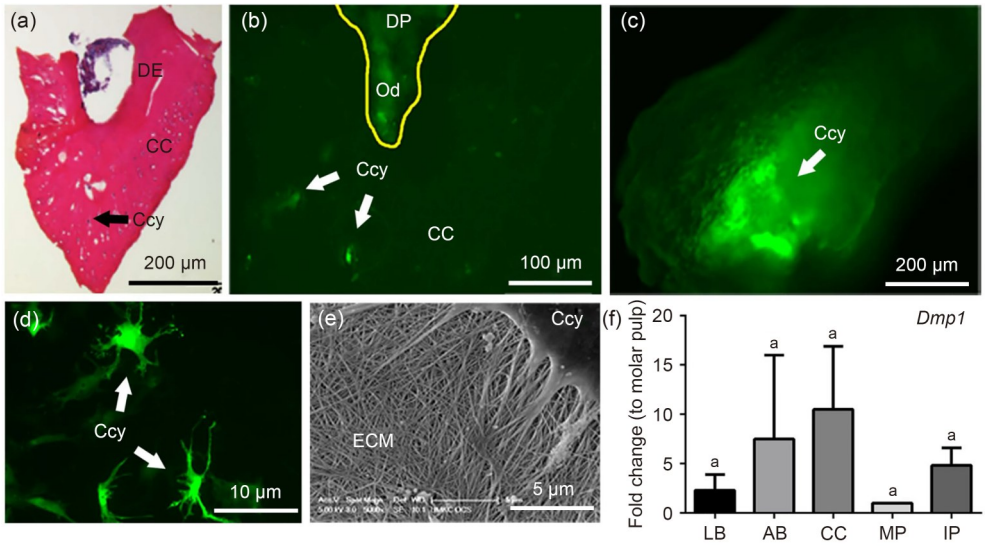

Fig. 2 Isolation of dentin matrix protein 1 (Dmp1)-expressing cementocytes and establishment of the IDG-CM6 (immortalized murine dentin-derived glucocorticoid-responsive cementocyte cell line 6) cell line. (a) Extracted molars provided apical root segments containing dentin (DE) and cellular cementum (CC). (b, c) Green fluorescent protein (GFP)-positive cells, cementocytes and odontoblasts, were isolated using enzymatic digestion, and then cloned and cultured to produce the IDG-CM6 cell line. (d, e) This cell line exhibits elevated Dmp1 expression and dendritic morphology and generates a collagen-like extracellular matrix. (f) Quantitative polymerase chain reaction (qPCR) of RNA from long bone (LB), alveolar bone (AB), CC, molar pulp (MP), and incisor pulp (IP) revealed no significant differences (the same letter above the column). Ccy: cementocytes; DP: dental pulp; Od: odontoblasts; ECM: extracellular matrix. Reprinted from Zhao et al. (2016b), Copyright (2016), with permission from Oxford University Press.

stress (2000 με at 0.5 Hz) using the SXG4201 four-point bending device, sphingosine-1-phosphate (S1P) signaling modulated key transcription factors, including prostaglandin E2 (PGE2) and β-catenin, alongside markers involved in cementogenesis and cementoclastogenesis (Wang et al., 2020). These results underscore the pivotal role of Wnt/β-catenin signaling in orchestrating the responses of cementoblasts and cementocytes to mechanical stimuli.

2.2 Periodontal ligament cells

Emerging evidence indicates that mesenchymal progenitor cells within the periodontal ligament can differentiate into cementoblasts to form cementum (Cao et al., 2012). Several animal models have demonstrated that PDLCs can generate new periodontal-cementum complexes, both with and without the aid of growth factors (Hasegawa et al., 2005; Iwata et al., 2009; Dan et al., 2014). However, the precise molecular mechanisms governing the differentiation of PDLCs into cementoblast-like cells remain largely unclear, and recently, molecular mechanisms associated with Wnt signaling have been reported to be responsible for this process.

In vitro experiments using human PDLCs (hPDLCs) demonstrated that stimulating the canonical Wnt signaling pathway, LiCl, LV-Ctnnb, or Wnt3a-conditioned medium, significantly promoted mineralization, ALP activity, and the levels of key bone and cementum markers, including OCN, OPN, and cementum protein 1 (CEMP1). These findings indicate that canonical Wnt signaling promotes the cementogenic differentiation of hPDLCs in vitro (Han et al., 2015). Connective tissue growth factor (CTGF) triggers the connexin 43 (Cx43)/β-catenin signaling pathway to promote cementogenesis, as evidenced by protein interactions and the membrane accumulation of both Cx43 and β-catenin in human periodontal ligament stem cells (hPDLSCs). Furthermore, CTGF-induced nuclear translocation of β-catenin is mediated by Cx43 since silencing Cx43 reverses this effect (Wu et al., 2022). In hPDLSCs with enamel matrix derivatives-induced differentiation into cementoblast-like cells, miR-30a upregulation increased cathepsin K expression and promoted hPDLSCs to form cementum-like tissues with high CEMP1 and cementum attachment protein expression. This regulatory effect of miR-30a on cathepsin K expression is found to rely on Wnt/β-catenin pathway activation (Liu et al., 2021). A recent study reported that direct cell interactions between PDLCs and cementoblasts promote PDLC differentiation into cementoblasts. Juxtacrine signaling via the canonical Wnt pathway plays a role in this interaction. Wnt3a stimulation significantly upregulated integrin-binding sialoprotein expression in hPDLCs, whereas inhibiting Wnt signaling suppressed the effects of co-culture (Sawada et al., 2024). Under hypoxic conditions, hPDLC proliferation was reduced, but the expression of both cementum-related genes and Wnt pathway components was elevated, suggesting that hypoxia regulates hPDLC proliferation and cementogenic differentiation via the Wnt/β-catenin pathway (Xiao et al., 2017).

Many studies have indicated that various biological materials promote the cementogenic differentiation of PDLCs via Wnt/β-catenin signaling. One investigation utilized a scaffold comprising polydopamine and collagen-coated electrospun poly(lactic-co-glycolic acid)/poly(epsilon-caprolactone) (PLGA/PCL) (PP) integrated with the small molecule PFI-2 (PP-PFI-pDA-Col-PFI). Compared with the control group, this scaffold significantly reduced alveolar bone defects, extended root length, and promoted the formation of regenerated acellular cementum. This effect appeared to be mediated by inhibiting the lysine methyltransferase SET domain-containing 7 protein (SETD7)-mediated β-catenin protein methylation and increasing β-catenin nuclear localization (Lyu et al., 2022). Hydroxyapatite bioceramics with a microrod-nanorod hybrid surface also stimulated LRP5 and β-catenin expression, promoting ALP activity and the production of key osteogenic and cementogenic markers such as ALP, OCN, CEMP1, cementum attachment protein, and Runx2. DKK1, a canonical Wnt signaling inhibitor, reversed these effects (Mao et al., 2015). The release of lithium (Li) from Li-β-tricalcium phosphate (β-TCP) powders enhanced bone and cementum-related gene expression compared to β-TCP extracts lacking Li, suggesting that Li-enriched β-TCP bioceramics hold promise for enhancing bone and cementum regeneration (Han et al., 2014). Similarly, another study demonstrated that mesoporous bioactive glass scaffolds, modified with Li^+ ions, exhibited improved properties for cell attachment, proliferation, and canonical Wnt signaling activation, promoting the cementogenic differentiation of PDLCs (Han et al., 2012). Furthermore, the ionic products from bioactive bredigite ($Ca_7MgSi_4O_{16}$) bioceramic powder

extracts enhanced PDLC proliferation and cementogenic differentiation in a concentration-dependent manner. However, these effects were diminished upon introducing cardamonin, a Wnt/β-catenin signaling inhibitor, validating that the cementogenesis triggered by bredigite relies on Wnt/β-catenin pathway activation (Zhou et al., 2013).

However, some research has shown that Wnt signaling can inhibit PDLC cementogenic differentiation. It was reported that a Wnt3a-conditioned medium suppressed PDLC cementogenesis, whereas hypoxia promoted cementogenesis by downregulating Wnt signaling (Li et al., 2016). In another study, transforming growth factor-β1 (TGF-β1) increased β-catenin activation, causing ligament-fibroblastic differentiation of hPDLCs. To enable cementoblastic differentiation in these cells, the suppression of TGF-β1 signaling was necessary (Lim et al., 2020).

2.3 Dental follicle cells

The dental follicle is an ectomesenchymal tissue that surrounds the developing tooth germ and is crucial in regulating tooth eruption (Wise and King, 2008). It is widely recognized that DFCs contribute to tooth root formation (Foster et al., 2007; Huang et al., 2010; Jung et al., 2011). As progenitor cells for cementoblasts, osteoblasts, and periodontal ligament cells, dental follicle cells (DFCs) interact with Hertwig's epithelial root sheath (HERS) cells, which are believed to initiate root development, including cementogenesis and dentinogenesis (Sonoyama et al., 2007).

Studies investigating the involvement of Wnt/β-catenin signaling in the differentiation of DFCs into cementoblasts and osteoblasts found that treatment with Wnt3a or LiCl increased the mRNA expression of osteogenic factors, alkaline phosphatase (*Alpl*), *Bglap*, and *Ibsp*, and the protein expression of Runx2, OCN, and COL1. However, pretreatment with DKK1, a Wnt inhibitor, significantly reduced the effects of Wnt3a on *Alpl* mRNA expression and ALP activity (Du et al., 2012; Nemoto et al., 2016). These findings indicate that Wnt/β-catenin signaling facilitates the cementoblastic and osteoblastic differentiation of DFCs. Conversely, another study reported that Wnt3a activation suppressed BMP2-induced maturation of DFCs (using murine submandibular gland fibroblast clone 4 (SVF4) cells), reducing the expression of *Runx2*, *Alpl*, and *Bglap* transcripts (Silvério et al., 2012). However, the knockdown of β-catenin demonstrated that the BMP2-induced expression of these markers depends on endogenous β-catenin (Silvério et al., 2012). These findings suggest that while Wnt3a-induced stabilization of β-catenin inhibits cementoblastic and osteoblastic differentiation in DFCs, BMP2 requires minimal endogenous Wnt/β-catenin activity to achieve optimal cell maturation.

2.4 Adipose tissue-deprived stem cells

Adipose tissue-deprived stem cells (ADSCs) have been widely utilized in tissue engineering (Liu et al., 2011; Shiraishi et al., 2012; Declercq et al., 2013). One group has demonstrated that dental follicle cell-conditioned medium (DFC-CM) creates a cementogenic microenvironment that promotes the differentiation of ADSCs toward a cementoblastic lineage (Wen et al., 2011). A subsequent study found that ADSCs cultured in DFC-CM supplemented with DKK1 exhibited enhanced cementogenic differentiation, marked by the upregulation of 3-hydroxyacyl-coenzyme A dehydratase 1 (*Hacd1*), *Alpl*, *Ibsp*, and secreted phosphoprotein 1 (*Spp1*) gene expression. However, introducing 100 ng/mL Wnt3a into the DFC-CM abrogated this effect, suggesting that Wnt signaling may inhibit ADSC differentiation into cementoblasts under certain conditions (Liu et al., 2014). While the Wnt/β-catenin pathway appears to influence the cementogenic differentiation of ADSCs, the specific molecular mechanisms require further verification and investigation.

2.5 Conflicting evidence and experimental approaches

Despite extensive research, the results of cell experiments examining the role of β-catenin in cementogenesis are not consistent even for the same cell type (Table 1). Considering the crucial role of Wnt signaling in maintaining periodontal homeostasis and its complex crosstalk with other signaling pathways (Rooker et al., 2010; Lim et al., 2014; Bao et al., 2021), we propose that the regulatory influence of Wnt signaling may shift depending on the stage of cementum formation or regeneration. These inconsistencies may also arise from differences in experimental models, such as variations in the timing and intensity of Wnt signaling activation. To address these conflicts, future experimental designs could focus on (1) longitudinal studies assessing the dynamic regulation of Wnt signaling during cementogenesis, (2) conditional knockouts or activators in specific cell populations (e.g., cementoblasts and

Table 1 Dual roles of Wnt/β-catenin signaling in cementoblast proliferation and differentiation

Study context	Wnt signaling manipulation	Wnt/β-catenin signaling	Effects on differentiation	Effects on proliferation	References
Cementoblasts/cementocytes					
In vitro (HCEM)	Baicalin	↑ Wnt/β-catenin	Promote (↑ OPG, ALP, Runx2, calcium deposition)	Not significant	Kimura et al., 2018; Kunimatsu et al., 2022
In vitro (OCCM-30)	Bmal1	↑ β-Catenin, Tcf1, Lef1	Promote (↑ ALP, OPN, OCN, mineralized nodules)	Not assessed	Liu et al., 2022
	OCCM30-fibrin	↑ Wnt/β-catenin, Wnt3a, Tcf, TOP	Promote (↑ biomineralization markers BSP, OCN, Runx2)	Not assessed	Rahman et al., 2017
	Wnt1-HA	↑ Wnt1	Not significant	Promote (↑ cell culture)	Nottmeier et al., 2021
	Wnt3a	↑ Wnt/β-catenin	Inhibit (↓ ALP, Runx2, Bglap)	Promote (↑ cyclin D1)	Nemoto et al., 2009
	PPARγ	↓ β-Catenin	Promote (↑ ALP, Runx2, OCN, mineralized nodules)	Not assessed	Hu et al., 2020
	Osx overexpression	↓ β-Catenin, Tcf1	Promote (↑ OCN, OPN, BSP, ALP, DKK1)	Not assessed	Cao et al., 2015
	miR-155-3p overexpression	↑ Wnt/β-catenin	Inhibit (↓ mineralization)	Not assessed	Wang et al., 2017
In vivo (Osx cKO mice)	Osx knockout	↑ β-Catenin	Inhibit (↓ DKK1, cementum and bone formation)	Not assessed	Cao et al., 2015
PDLCs					
In vitro (hPDLCs)	LiCl, LV-Ctnnb	↑ Wnt/β-catenin, Wnt3a	Promote cementogenic differentiation (↑ ALP, OCN, OPN, CEMP1, mineralization)	Not assessed	Han et al., 2015
	CTGF	↑ β-Catenin	Promote cementogenic differentiation (↑ Cx43, ALP, Osx, Runx2, Col1)	Not assessed	Wu et al., 2022
	Hypoxia	↑ Wnt	Promote cementogenic differentiation (↑ cementum-related genes *OPN*, *ALP*, *CEMP1*, *CAP*)	Inhibit	Xiao et al., 2017
	Hypoxia	↓ Wnt/β-catenin	Promote cementogenic differentiation (↑ Col1, Runx2, CEMP1)	Not assessed	Li et al., 2016
	TGF-β	↑ β-Catenin	Promote ligament–fibroblastic differentiation rather than cementogenic differentiation	Not assessed	Lim et al., 2020
DFCs					
In vitro (DFCs)	Wnt3a, LiCl	↑ Runx2, ALP, OCN, Col1	Promote cementoblastic and osteoblastic differentiation	Not assessed	Du et al., 2012; Nemoto et al., 2016
	Wnt3a activation	↓ β-Catenin	Inhibit osteogenic differentiation (BMP2-induced Runx2, Alpl, Bglap)	Not assessed	Silvério et al., 2012

To be continued

Table 1 (continued)

Study context	Wnt signaling manipulation	Wnt/β-catenin signaling	Effects on differentiation	Effects on proliferation	References
ADSCs					
In vitro (ADSCs)	DFC-CM+DKK1	↓ Wnt/β-catenin	Promote cementogenic differentiation (↑ Hacd1, Alpl, Ibsp, Spp1)	Not assessed	Liu et al., 2014
	DFC-CM+Wnt3a	↑ Wnt/β-catenin	Inhibited cementogenic differentiation (↓ Hacd1, Alpl, Ibsp, Spp1)	Not assessed	Liu et al., 2014

Wnt: wingless and int; HCEM: human cementoblast cell line; OPG: osteoprotegerin; ALP: alkaline phosphatase; Runx2: Runt-related transcription factor 2; OCCM-30: murine cementoblasts-30; Tcf1: T-cell-specific factor 1; Lef1: lymphoid enhancer-binding factor 1; OPN: osteopontin; OCN: osteocalcin; TOP: optimal motif; BSP: bone sialoprotein; HA: hemagglutinin; Bglap: bone γ-carboxyglutamate protein; PPARγ: peroxisome proliferator-activated receptor γ; DKK1: dickkopf 1; Osx cKO: osterix conditional knockout; PDLCs: periodontal ligament cells; LiCl: lithium chloride; CTGF: connective tissue growth factor; Cx43: connexin 43; OSX: osterix; Col1: type I collagen; CEMP1: cementum protein 1; CAP: cementum attachment protein; TGF-β: transforming growth factor-β; DFCs: dental follicle cells; BMP2: bone morphogenetic protein 2; Alpl: alkaline phosphatase; ADSCs: adipose-derived stem cells; DFC-CM: DFC-conditioned medium; Hacd1: 3-hydroxyacyl-coenzyme A dehydratase 1; Ibsp: integrin-binding sialoprotein; Spp1: secreted phosphoprotein 1; ↑: upregulation; ↓: downregulation.

periodontal ligament stem cells) to pinpoint cell-specific roles of Wnt signaling, and (3) single-cell RNA-seq to analyze gene expression profiles and identify potential regulatory networks that modulate Wnt signaling in cementum formation. By refining these experimental approaches, researchers can better understand the complex roles of Wnt/β-catenin signaling in periodontal tissue development and repair.

3 Roles of Wnt/β-catenin signaling in cementum formation and development

An early study investigated differences in gene expression between cementoblasts and osteoblasts by performing gene profiling on cell populations isolated from osteocalcin (*OC*)-GFP transgenic mice. Microarray analysis revealed that the Wnt signaling activity of cementoblasts and osteoblasts differed, along with variations in genes associated with skeletal development. Real-time polymerase chain reaction (PCR) further showed that the expression of Wnt inhibitors, Wnt inhibitory factor 1 (Wif1) and sFRP, was elevated in cementoblasts, highlighting the distinct role of Wnt signaling in cementum development and formation (Matthews et al., 2016).

Many researchers have explored the impact of constitutive β-catenin activation within the dental mesenchyme. Impaired root formation in *OC*-Cre;β-catenin *Ctnnb1*$^{lox(ex3)/+}$ mice resulted in malformed teeth with aberrantly formed dentin and excessive cementum deposition. These mice exhibited short molar roots covered with excess cementum, and, over time, their coronal pulp chambers and periodontal spaces became progressively narrowed due to excessive dentin and cementum deposition (Kim et al., 2011; Bae et al., 2013, 2017). Similarly, Daβcat (*Ot*) mice, produced by crossing *DMP1-8kb*-Cre mice with *Ctnnb1*$^{lox(ex3)/+}$ mice to express stabilized β-catenin in DMP1-positive osteocytes and cementocytes, exhibited excessive cellular cementum formation alongside calcified periodontal ligaments and alveolar bone, eventually leading to ankylosed teeth (Wu et al., 2019). Another study, employing a Wnt1-inducible transgenic mouse model (crossing *Col1a1*-rtTA mice with ptet-*Wnt1* mice on a mixed background), demonstrated that Wnt1 potently stimulates cementum and alveolar bone formation in vivo (Nottmeier et al., 2021). In another model, achieving conditional β-catenin activation by intercrossing *Ctnnb1*$^{lox(ex3)/+}$ mice with *Col1a1*-Cre mice led to hypoplastic cementum and periodontal ligament, accompanied by a narrowed periodontal space due to increased alveolar bone formation (Kim et al., 2012). A similar phenotype was observed in homozygous sclerostin (*Sost*)-knockout mice, which exhibited thicker cementum and a moderately reduced periodontal space without significant changes in tooth or pulp chamber volume (Kuchler et al., 2014). In a loss-of-function model, *Ctnnb1* or *Wls* (a chaperone protein essential for Wnt secretion) gene was conditionally knocked out within developing odontoblasts and cementoblasts using *OC*-Cre;*Ctnnb1*$^{flox/flox}$ or *OCN*-Cre;*Wls*$^{flox/flox}$ mice. Rootless molars, incomplete incisors, and disrupted cementoblast formation were observed in

the mice (Zhang et al., 2013; Lim et al., 2014). These in vivo findings suggest that localized β-catenin activation within osteoblasts and odontoblasts drives premature differentiation of odontoblasts and cementoblasts, excessive dentin and cementum formation, and defective dento-alveolar complex formation (Fig. 3). These findings highlight the need for precise temporal and spatial regulation of Wnt/β-catenin signaling to ensure the proper differentiation of odontoblasts and cementoblasts. Furthermore, inhibiting Wnt/β-catenin activity at appropriate levels appears to be essential not only for balanced cementum and dentin formation but also for maintaining the fibrous structure of periodontal ligaments during tooth development.

Wnt-responsive genes, such as *Axin2*, are expressed across various stem and progenitor cells (Lim et al., 2016; Maruyama et al., 2016; Usami et al., 2019). A recent investigation examined whether Wnt-responsive cells (Axin2$^+$-mesenchymal PDLCs) directly contribute to postnatal cementogenesis. Consistent with the above suggestions, the study found that these cells, along with their descendants, are crucial for the formation of acellular and cellular cementum. The expression levels of Axin2 and β-catenin in PDLCs were inversely correlated with the rate of cementum growth. Notably, ablation of these Axin2$^+$ cells in *Axin2*$^{Cre\text{-}ERT2/+}$; *R26R*$^{DTA/+}$ mice (ERT: estrogen receptor tamoxifen-binding domain; R26R: ROSA26 reporter; DTA: diphtheria toxin A fragment) led to severe hypoplastic cementum. Conversely, continuous β-catenin activation in these cells accelerated cementogenesis and induced a shift from acellular to cellular cementum (Xie et al., 2019). These findings highlight the necessity of precisely regulated Wnt signaling to maintain the specific apposition patterns of cellular and acellular cementum. The data suggest that maintaining low Wnt activity in mature cementoblasts is critical for preserving acellular cementum's unique structural properties. A subsequent study utilized triple transgenic mice to conditionally delete *β-catenin* within cells of the *Axin2* lineage by crossing *Axin2*$^{Cre\text{-}ERT2/+}$; *R26R*$^{tdTomato/+}$ mice with *β-catenin*$^{flox/flox}$ mice. The loss of *β-catenin* in *Axin2*$^+$ cells caused cementum hypoplasia, significantly reducing both acellular and cellular cementum formation (Ma et al., 2023). Conversely, activating Wnt signaling in *Axin2*$^+$ cells promoted osteodentin formation along with excessive cementum deposition (Shi et al., 2023). Collectively, these findings highlight Wnt signaling as a key driver of cementogenesis, while sustained low Wnt activity levels are essential to ensure balanced cementum growth, mineralization, and maintenance. By "low levels of Wnt/β-catenin," we refer to a finely tuned state of Wnt signaling activity that is not fully suppressed but kept at a level that prevents excessive cementoblast proliferation while promoting proper differentiation. These low levels are critical for balancing cementoblast proliferation and differentiation, thus ensuring appropriate cementum formation and preventing pathological cementum deposition. Maintaining this balance is key to the proper development of the dento-alveolar complex.

Other cell markers and signaling pathways linked to canonical Wnt/β-catenin signaling have also been explored in relation to tooth development. A recent study identified two distinct stem cell populations contributing to cementoblast differentiation at various developmental stages (Zhao et al., 2021). During postnatal growth, cementoblasts arise from perivascular-derived

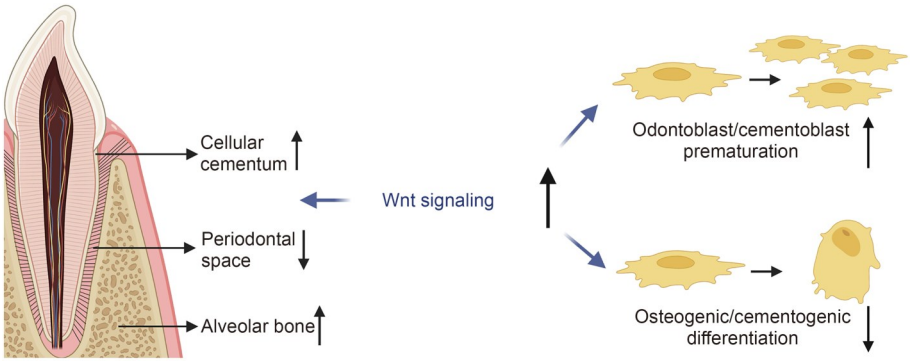

Fig. 3 Impact of wingless and int (Wnt)/β-catenin signaling on periodontal formation and development. Stimulating the Wnt/β-catenin pathway appears to promote alveolar bone formation, accelerate the deposition of dentin and cellular cementum, and decrease the periodontal space. The molecular mechanism may be the premature differentiation of odontoblasts and cementoblasts.

cells marked by CD90 expression and perivascular-associated cells expressing *Axin2*. In adulthood, only Wnt-responsive *Axin2*$^+$ cells differentiate into cementoblasts. However, under experimentally induced periodontitis, CD90$^+$ cells are the primary contributors to cementoblast formation (Zhao et al., 2021). Gli1 (glioma-associated oncogene homolog 1), a critical transcriptional factor within Hedgehog (Hh) signaling, has been recognized as a mesenchymal progenitor marker in multiple tissues, including the periodontal ligament. Studies indicate that Gli1$^+$ progenitors are integral to forming both cellular and acellular cementum, with Wnt/β-catenin signaling promoting their differentiation into cementoblasts (Men et al., 2020; Xie et al., 2021). Recent research suggests that aberrant Wnt signaling and mechanosensitive Piezo2 channel activation occur following FGF signaling disruption in *Gli1-Cre*^{ER}; *Fgfr1*$^{flox/flox}$ (fibroblast growth factor receptor 1 floxed) mice. Piezo2 overexpression promotes osteoblastic differentiation, while its downregulation reduces Wnt signaling. Notably, decreased Wnt signaling rescues ankylosis in *Fgfr1*-mutant mice, revealing that the FGF/Piezo2/Wnt signaling axis governs the differentiation of Gli1$^+$ stem/progenitor cells (Pei et al., 2024). A marked reduction in cellular cementum apposition was observed in two transgenic mouse models designed to activate Hedgehog signaling by inactivating the suppressor of fused (Sufu), a negative regulator of Hh signaling (Sufu^{OC}), or by forced activation of Smo (SmoM2^{OC}) through OC promoter-driven Cre recombinase. Additionally, Wnt antagonists, including *Sostdc1* and *DKK1*, were also upregulated, reducing Osx expression and β-catenin activity. However, compound mutant mice with both stabilized β-catenin and activated Hh-Smo signaling restored defective cellular cementum, suggesting that Hh signaling modulates cementogenesis through a Wnt/β-catenin/Osx-dependent mechanism (Choi et al., 2020). To explore further interactions between Wnt/β-catenin signaling and Osx during cementogenesis, researchers developed transgenic mice carrying constitutively active β-catenin and inactivated Osx, specifically within the dental mesenchyme. Results showed that β-catenin activation requires Osx to induce the accumulation of excessive cellular cementum; conversely, the absence of Osx prevents this buildup, suggesting that the TCF/LEF-binding function of Wnt/β-catenin signaling depends on Osx (Choi et al., 2017).

The interplay between TGF-β and Wnt signaling is critical in regulating cementoblast differentiation and root lineage specification. BMP signaling, a TGF-β subfamily member, regulates root lineage differentiation by modulating Wnt/β-catenin activity. In a study where bone morphogenetic protein receptor type 1a (Bmpr1a) was depleted during the differentiation stage of epithelial tissues, the loss of BMP signaling led to ectopic cementum-like structures. This shift was accompanied by increased Wnt/β-catenin activity and the induction of epithelial–mesenchymal transition (EMT), indicating that the loss of Bmpr1a triggers ectopic cementogenesis through increased Wnt/β-catenin activity and EMT induction (Yang et al., 2013). Mechanistically, TGF-β/BMP signaling typically suppresses Wnt/β-catenin activity via transcriptional regulation to maintain cementoblast progenitor pools and prevent premature differentiation. For instance, BMP-activated small mother against decapentaplegic (Smad) proteins can inhibit Wnt-targeted genes by competing for co-activators like β-catenin or promoting the degradation of Wnt pathway components (Guo and Wang, 2009). Conversely, Wnt activation counteracts the inhibitory effects of TGF-β by phosphorylating Smad1/5 linker regions, preventing nuclear translocation and stabilizing β-catenin, thereby promoting cementoblast differentiation (Lin and Hankenson, 2011; Vlashi et al., 2023). This intricate crosstalk between Wnt, BMP, and TGF-β pathways suggests a finely tuned regulatory network essential for cementum homeostasis and regeneration.

These studies mentioned above further emphasize the pivotal role of Wnt/β-catenin in regulating cementoblast differentiation and cementum formation, interacting with various pathways such as Hedgehog, FGF, and BMP. This pathway is essential in guiding the fate of stem/progenitor cells during cementogenesis and contributes to both normal development and pathological processes, including the antagonistic regulation of root lineage differentiation and EMT.

4 Roles of Wnt/β-catenin signaling in root resorption and repair

Physiological root resorption is a common phenomenon in mammalian teeth, which can result from compressive strains that accumulate on the root surface in response to mastication (Turkkahraman et al.,

2020). In areas where resorption lacunae form, progeny from Wnt-responsive stem cell populations within the periodontal ligament directly contribute to cementum repair, indicating that this process relies on Wnt/β-catenin signaling (Turkkahraman et al., 2020). This suggests that a Wnt/β-catenin-dependent mechanism is responsible for physiological cementum repair. The involvement of Wnt/β-catenin signaling was also reported in pathological cementum repair using different damage models. In a rat periodontal model, local activation of canonical Wnt signaling promoted substantial cellular cementum deposition and the development of periodontal ligament fibers, which have not been observed in control groups (Han et al., 2015).

Under excessive orthodontic intrusive force, root resorption was induced, which promoted S1P signaling and decreased nuclear translocation of β-catenin. The inhibition of S1P signaling reduced excessive intrusive force-induced root resorption and increased the nuclear β-catenin levels (Wang et al., 2022). Another study simulated orthodontic biomechanics by applying 50 g of force using nickel-titanium coil springs between the upper incisors and maxillary first molars to simulate orthodontic biomechanics. During 14 d of orthodontic tooth movement, the rats were gavage-fed with LiCl, which attenuated orthodontically induced root resorption (Wang et al., 2014). In addition, a recent study leveraged lithium's therapeutic potential to develop porous silicon nanowires infused with Li (LipSiNs) that gradually release lithium and silicic acid over controlled timeframes ranging from days to weeks. This bioresorbable material exhibited a synergistic effect by stimulating osteogenesis and cementogenesis and activating the Wnt/β-catenin pathway, thereby promoting the regeneration of bone, cementum, and periodontal ligament fibers in periodontal defects (Kaasalainen et al., 2024). These studies demonstrate the benefits of using lithium in activating Wnt/β-catenin signaling to promote periodontal tissue regeneration.

5 Roles of the non-canonical Wnt pathway in cementogenic differentiation

To date, many studies have demonstrated the involvement of canonical Wnt/β-catenin signaling in the differentiation and mineralization of dental follicle cells, cementoblasts, and periodontal ligament cells during root formation, but the functional importance of non-canonical Wnt signaling remains poorly understood. Wnt5a, a key non-canonical Wnt ligand, was identified in tooth root lining cells (i.e., precementoblasts/cementoblasts) and dental follicle cells during mouse tooth development (Sakisaka et al., 2015). Silencing *Wnt5a* in a dental follicle cell line enhanced Wnt3a-mediated ALP expression, while recombinant Wnt5a treatment suppressed ALP expression, suggesting that Wnt5a negatively regulates Wnt3a-induced ALP activity in these cells. Additionally, Wnt5a did not influence the nuclear translocation of β-catenin or the β-catenin/TCF transcriptional activity induced by Wnt3a, indicating that Wnt5a acts downstream of β-catenin to inhibit canonical Wnt signaling (Sakisaka et al., 2015). Additionally, non-canonical Wnt signaling, particularly Wnt5a-receptor tyrosine kinase-like orphan receptor 2 (Ror2), has been implicated in regulating cytoskeletal remodeling and cell migration (Uehara et al., 2018). For instance, Wnt5a can activate small guanosine triphosphatases (GTPases) such as Ras homolog (Rho) and Ras-related C3 botulinum toxin substrate (Rac), leading to changes in the actin cytoskeleton and promoting cell migration and adhesion (Maeda et al., 2012; Uehara et al., 2017). This mechanism is crucial for the function of osteoclasts and the role of cementoblasts in cementum regeneration. Another study reported that both cytokines, interleukin-6 (IL-6) and TNF-α, were found to upregulate non-canonical Wnt/Ca^{2+} signaling-related genes and proteins while inhibiting cell proliferation, ALP activity, and the expression of bone- and cementum-related markers, as well as those related to canonical Wnt signaling in hPDLCs. Notably, blocking the Wnt/Ca^{2+} pathway with the Ca^{2+}/calmodulin-dependent protein kinase II inhibitor KN93 restored cementogenesis, even in the presence of IL-6 and TNF-α (Han et al., 2016). The above findings suggest that the non-canonical Wnt signaling pathways, particularly the Wnt5a and Wnt/Ca^{2+} pathways, negatively regulate cementoblast differentiation by inhibiting canonical Wnt signaling. Non-canonical Wnt signaling may play a multifaceted role in cementum regeneration by regulating cell migration and cytoskeletal organization. Non-canonical Wnt signaling may fine-tune cementoblast maturation and respond to external stressors like mechanical forces. We hypothesize that non-canonical signaling may complement or antagonize canonical Wnt signaling through crosstalk involving downstream

effectors such as β-catenin and c-Jun N-terminal kinase (JNK), balancing cementum formation and differentiation under varying physiological conditions.

6 Summary and future outlook

Although current research findings are not entirely consistent, it is evident that the canonical Wnt signaling pathway is crucial for the growth and differentiation of cementoblasts/cementocytes, PDLCs, DFCs, and ADSCs. During tooth development, the appropriate suppression of Wnt/β-catenin signaling is necessary to support the formation of dentin and cementum and maintain the fibrous nature of the periodontal ligament. Wnt-responsive stem cells are involved in physiological cementum repair, and the local activation of canonical Wnt signaling attenuates orthodontically induced root resorption (Fig. 4). The nature of the crosstalk between canonical and non-canonical Wnt signaling during cementum formation and differentiation remains to be elucidated.

Future research should focus on clarifying the following issues:

(1) How can we determine the timing, intensity, and duration of Wnt signaling during normal cementum development? What are the commonalities and differences in the roles of Wnt signaling among the different cell types in the periodontium?

(2) How can we determine the timing, intensity, and duration of Wnt signaling in cementum remodeling/resorption under physiological and pathological stress conditions?

(3) Can dynamic regulation of the Wnt signaling pathway be achieved locally in the periodontium? How can this be combined with existing periodontal regeneration methods to achieve better therapeutic outcomes?

(4) Can different magnitudes of Wnt signaling serve as diagnostic or prognostic markers for diseases such as root resorption?

Although several drugs targeting the Wnt pathway have been discovered and used to treat cancer, osteoporosis, and certain neurodegenerative diseases, their efficacy in periodontal regeneration and root resorption remains unknown. Most drugs utilize the Wnt/β-catenin pathway to promote the regeneration of various tissues, including bone, skin, and liver. Several Wnt-targeting drugs have been explored for therapeutic applications, including those designed to activate or inhibit the Wnt/β-catenin pathway. For example, small molecule activators such as LGK974 (a porcupine inhibitor) and ICG-001 (a selective inhibitor of β-catenin/CREB-binding protein (CBP) interaction) have shown promise in cancer therapies by modulating Wnt signaling. However, excessive Wnt signaling activation could lead to aberrant mineralization, as seen in disorders like osteosarcoma, or result in abnormal tissue overgrowth, which could be detrimental in periodontal therapies.

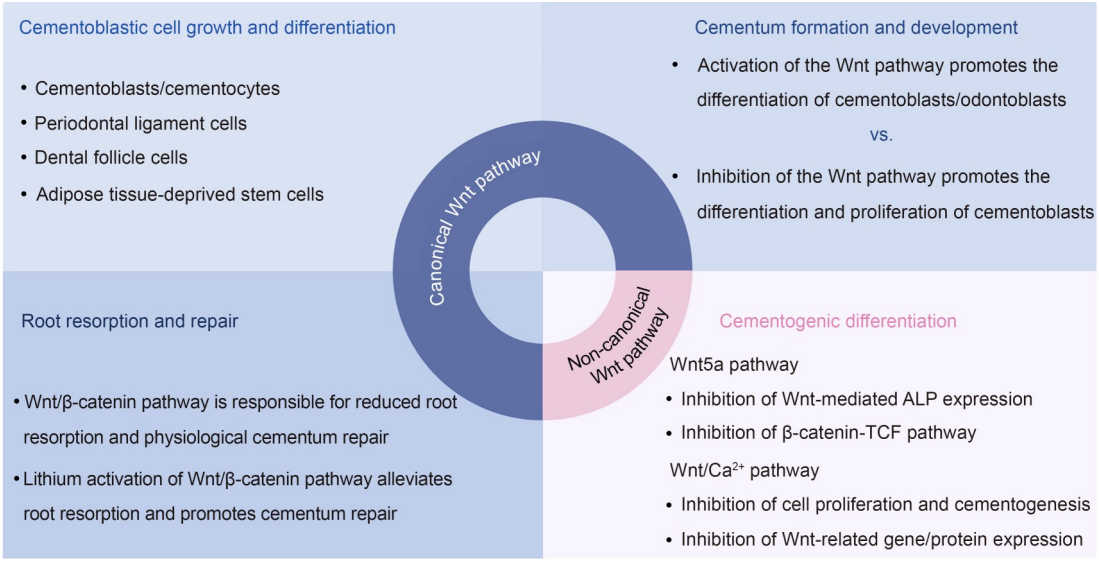

Fig. 4 Schematic diagram summarizing the impact of the canonical wingless and int (Wnt) pathway on cementoblastic cell growth and differentiation, cementum formation and development, root resorption and repair, and cementogenic differentiation, as discussed in this review. ALP: alkaline phosphatase; TCF: T-cell-specific factor.

In contrast, the inhibition of Wnt signaling could impair bone and cementum regeneration, leading to defects in periodontal tissue repair. Therefore, careful modulation of Wnt/β-catenin activity is essential to avoid such risks.

To achieve effective clinical outcomes, future therapies will need to focus on precise spatiotemporal regulation of Wnt signaling. This can be achieved by utilizing targeted delivery systems, such as biomaterials or gene-editing technologies, to locally modulate Wnt activity in the periodontal tissue. For instance, materials infused with small molecules or nanoparticles that control the release of Wnt activators or inhibitors could offer controlled modulation of Wnt signaling over time, allowing for more effective regeneration of cementum, bone, and periodontal ligament fibers. Additionally, biomarkers that reflect Wnt pathway activity could guide the use of Wnt-targeted therapies. Monitoring the levels of β-catenin or other Wnt-responsive proteins in patient samples may help determine the optimal timing and intensity of treatment, thereby minimizing the risk of Wnt signaling overactivation or misregulation, such as aberrant mineralization or other adverse effects. These biomarkers could be used not only to guide therapy but also as diagnostic or prognostic tools for diseases such as root resorption or periodontal degeneration.

In conclusion, while significant progress has been made in understanding the role of Wnt signaling in periodontal regeneration, translating these findings into clinical practice will require further refinement of delivery methods, as well as the identification of reliable biomarkers for therapeutic guidance. The dynamic regulation of Wnt signaling holds great potential for advancing the treatment of periodontal diseases and improving outcomes for patients suffering from root resorption and other periodontal disorders.

One limitation of this review lies in the potential subjectivity involved in selecting search terms and criteria for study inclusion, which may have resulted in the exclusion of some relevant literature. Given the complexity of the field, variations in findings and conclusions across studies are common. Our review of the molecular mechanisms by which Wnt signaling influences tooth development and root repair is still incomplete, with additional important regulators and molecules in this pathway yet to be identified. Additionally, clinical studies on Wnt pathway-targeted drugs for root resorption are limited, constraining our ability to comprehensively evaluate their therapeutic potential and safety.

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Author contributions

Tiancheng LI and Xinyi ZENG determined the topic of the article, proposed this program, and wrote and edited the manuscript. Shuxian YANG collected the literature. Lynda Faye BONEWALD and Peipei DUAN reviewed and edited the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Tiancheng LI, Xinyi ZENG, Shuxian YANG, Lynda Faye BONEWALD, and Peipei DUAN declare that they have no conflicts of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

Declaration on the use of generative AI tools

No generative AI tools were used in the preparation of this manuscript.

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