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Review Article

Dentistry Section

# R-Spondins and Wnt Signalling Pathway: A Periodontal Perspective

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## ABSTRACT

Periodontitis is a multifactorial chronic condition associated with the formation of a dysbiotic biofilm, leading to inflammation that can modulate cell signaling. R-spondins (Rspos) are cysteine-rich secreted glycoproteins that control a variety of functions essential for embryonic development and tissue homeostasis. Numerous studies have explained about their pivotal role in critical developmental regulators, the most important finding being Rspos synergise Wingless/Integration (Wnt) signaling. Novel receptors for Rspos, the Leucine-rich repeat containing G protein-coupled (LGR) receptors, proposed that Rspos potentiate canonical Wnt signaling via these receptors. In this paper, the authors discuss Wnt signaling, outline the biological role of Rspos in tissue development and homeostasis, and explore the possibility of using Rspos as therapeutic targets. Wnt5a is important for promoting periodontal ligament remodeling and impairing regenerative responses modulated by the Wnt/β-catenin pathway, for alveolar bone formation.


**Keywords:** Osteoprotegerin, Periodontal disease, Receptors, Wingless/Integration

## INTRODUCTION

Oral diseases are a worldwide public health issue [1]. It is believed that 99% of the adult population presents inflammatory gingival disease, while the prevalence of severe periodontitis might reach 10-12% [2,3]. Furthermore, periodontitis is a significant financial burden and is typically related with systemic disorders [4]. Therefore, in order to address periodontitis' systemic implications and create new pharmaceutical strategies, we need a better understanding of the molecular mechanisms of the disease. In this regard, research into signalling pathways and how they are altered during tissue development, maintenance, and disease can help us better understand periodontitis and identify potential targets for treatment.

The Wnt signaling pathway comprises a family of secreted glycoproteins that modulate processes such as embryonic development and cell differentiation, proliferation and survival [5]. The management of periodontal destruction and periodontal regeneration are current focus in the field of periodontics. The progression of periodontitis and the maintenance of the homeostasis of periodontal tissues are all likely influenced by Wnt signalling, according to mounting evidence [5,6].

A single thrombospondin Type I Repeat (TSR) domain is found in the four cysteine-rich secreted proteins known as Rspos [7]. Significant advancements have been achieved in Rspo research over the past few years, particularly with regard to their functions in the activation of the Wnt signalling pathway. The recent discovery of numerous Rspo protein receptors raises the possibility that this family of proteins can influence various signalling cascades by making use of various receptors. Understanding the functional roles of Rspos in various biological processes and figuring out the molecular mechanisms by which Rspos regulate the Wnt signalling pathway have garnered significantly more interest in light of the broad spectrum of Wnt signalling functions in both healthy biological processes and disease conditions [8,9]. This review outlines the role of Wnt signalling in periodontal tissues, how R-spondin modulates the Wnt signalling pathway and their potentials for therapeutic applications in periodontal diseases in future.

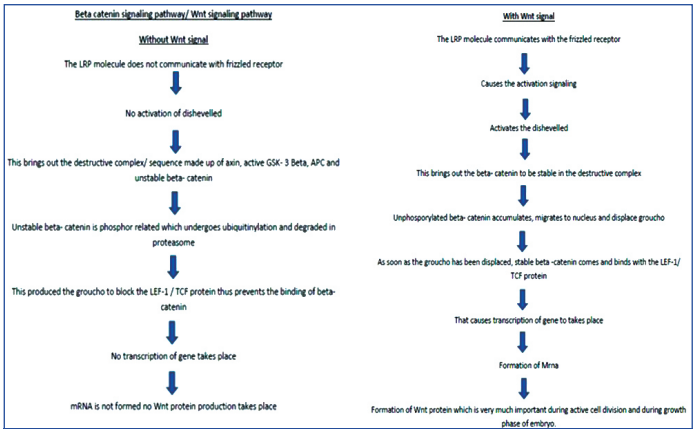
### What are R-spondins

Four secreted glycoproteins make up the Rspo family of proteins (Rspo1-4.7). According to several studies, Rspo proteins interact with Frizzled (FZD) 8 and the low-density lipoprotein receptor-related proteins 5 and 6 to activate the Wnt/β-catenin signalling pathway [10,11]. Recent investigations have revealed that the G protein-coupled receptors 4, 5 and 6 are the receptors for Rspos (LGR4, LGR5, and LGR6) [12]. Rspo2 plays crucial functions in a variety of biological processes, including skeletal development [13]. Furthermore, prior research has demonstrated that Rspo2 stimulates osteogenesis in MC3T3-E1 cells by triggering the Wnt/catenin signalling pathway [14].

## ROLE OF WNT SIGNALING PATHWAY IN PERIODONTAL DISEASE

The Wnt/β-catenin (or canonical) pathway and the non canonical (or Wnt/β-catenin independent) pathway are the two primary branches of the Wnt pathway. Through the regeneration of the periodontal ligament and cementum, as well as the alveolar bone by regulating the Receptor Activator of Nuclear Factor-Kb Ligand (RANKL)/Receptor Activator of Nuclear factor-kB (RANK)/Osteoprotegerin (OPG) axis, the Wnt pathway contributes to the creation and maintenance of periodontal tissues [15]. Wnt ligands, FZD receptors, as well as a number of co-receptors and intracellular effectors, are necessary for this pathway. A certain pathway is selectively activated by Wnt ligands. For instance, whilst Wnt5a and Wnt11 activate the non canonical branch, Wnt1 and Wnt3a activate the Wnt/β-catenin pathway.

The Wnt/β-catenin pathway involves the binding of Wnt ligands to Low-density lipoprotein Receptor-related Protein (LRP5)/6 co-receptors and FZD receptors, which stabilises and translocates beta-catenin into the nucleus. While this is happening, the non canonical Wnt pathway is mediated by ligands like Wnt5a and Wnt11, FZD receptors, and co-receptors like Receptor Tyrosine Kinase like Orphan Receptor 1 (ROR1)/2 and Receptor-like Tyrosine Kinase (RYK), among others. This causes intracellular processes that are unrelated to beta-catenin stabilisation [16]. Importantly, there may be crosstalk between the two Wnt branches because they share proteins like FZD receptors and Dishevelled. Several secreted proteins have the ability to change Wnt signalling. These include the soluble Frizzled Related Proteins (sFRPs), the Wnt Inhibitory Factor 1 (WIF1), the Sclerostin (SOST), the Rspos proteins, and the Dickkopf (DKK) proteins [Table/Fig-1] [17].




**[Table/Fig-1]:** β-catenin signalling pathway without Wnt signal and with Wnt signal (self diagram) [17].

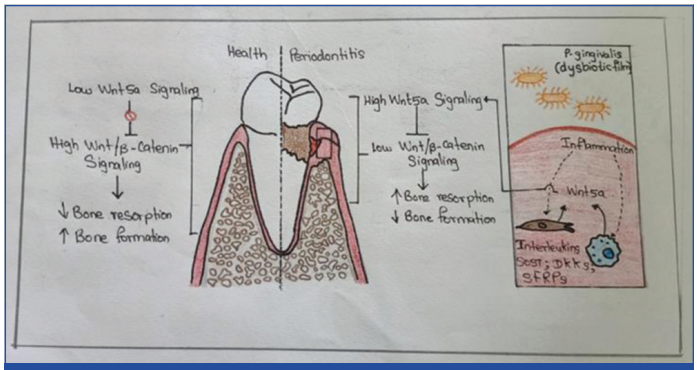
Based on a number of findings, a potential function in the stimulation of Wnt signalling in osteoblasts and osteoclasts emerged. The RANK/RANKL/OPG system acts as a link between osteogenesis and osteoclastogenesis. Moreover, it is believed that Wnt signalling via the RANK/RANKL/OPG pathway regulates osteoclastogenesis. OPG is an inhibitory factor in osteoclastogenesis, and interleukin-1-stimulated human gingival fibroblasts and Periodontal Ligament fibroblast Cells (PDLCs) produced less OPG when Wnt signalling was blocked by siRNA for catenin [18]. Additionally, treatment with amyloid peptide could prevent osteoclastogenesis in RAW264.7 cells by inhibiting the RANK/RANKL/OPG system. The impact was reduced by the Wnt signalling antagonist, but elevated by the Wnt signalling agonist. Furthermore, Wnt signalling promotes osteogenic differentiation while suppressing osteoclastogenesis via the RANK/RANKL/OPG system. As a consequence, Wnt signalling regulators may represent a significant therapeutic target for alveolar process reconstruction [19].

## WNT SIGNALING AND PERIODONTIUM

There is a gradient of Wnt/β-catenin activity across the PDL, with higher activity at the cementum/PDL interface, which correlates with higher cell proliferation. Lower Wnt/β-catenin activity, on the other hand, is detected at the interface between the PDL and the alveolar bone, corresponding to increased differentiation toward the osteogenic lineage. This shows that the Wnt/β-catenin pathway requires spatial finetuning for both proliferation and differentiation in the PDL [20].

R-spondins proteins are released by cells from healthy PDL in rats as well as primary cultures of PDL cells, and they play an important role in controlling the osteoblastic differentiation of immature human PDL cells via the Wnt/catenin pathway. TGF-β may also trigger β-catenin to influence PDL cell differentiation [21].

Wingless/Integration pathway modulation is also possible in an inflammatory context [Table/Fig-2]. The Wnt/β-catenin balance may change in an inflammatory scenario, reducing the differentiation between osteogenic and cementogenic cells. The expression of Wnt5A and SFRP5 is variably modulated when human gingival cells are exposed to LPS from *Porphyromonas gingivalis* (*P.gingivalis*). Wnt5A levels were higher in periodontitis patients and in cells treated with Lipopolysaccharide (LPS), whereas SFRP5 levels were higher in healthy participants. However, LPS administration resulted in a decrease in SFRP5 levels [19]. In the cell line, Tamm-Horefall protein-1, LPS from *P.gingivalis* also raises Wnt5A levels. All of these findings point to a change in the relative importance of the Wnt pathways in a sick environment, favouring Wnt5a mediated signalling. Due to its capacity to encourage both the turnover of focal adhesions and the production of proteins like Laminin-2, Wnt5a plays a part in cell invasion and migration. It is interesting to note that periodontopathogens and Wnt5a exposure greatly boosts the expression of genes including POSTN, COL1A1, and FBN1 as well as proliferation and migration in PDL cells. As a result, the pathogenic challenge in the PDL may affect progenitor cell differentiation and extracellular matrix remodelling via Wnt5a [22].




**[Table/Fig-2]:** Role of Wnt signalling in health and periodontitis [22].
In a healthy environment, the Wnt/β-catenin pathway may play an important role in periodontal ligament homeostasis and bone and cementum maintenance. However, the challenge provided by periodontopathic bacteria may stimulate local production of Wnt5a, which may contribute to the maintenance of an inflammatory loop due to its propensity to induce the expression of Monocyte Chemoattractant Protein-1 (MCP-1), hence increasing macrophage recruitment. This may contribute to an imbalance between the branches of the Wnt pathway, favouring the non canonical Wnt pathway. Wnt5a may thereby increase bone tissue loss

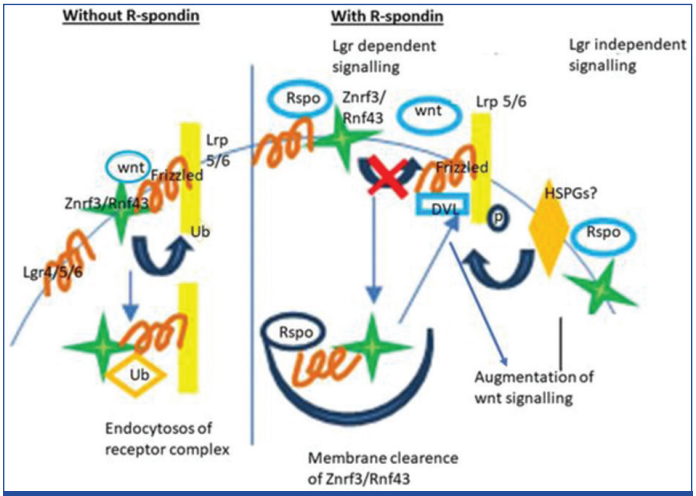
### Wnt Signaling in Alveolar Bone and Root Cement Maintenance

Negative modulation of the Wnt/β-catenin pathway in PDL cells may positively influence cementum regeneration. However, there is an increase in cementum deposition in cell lines with higher levels of Wnt/β-catenin activity. The Wnt/β-catenin signalling pathway stimulates the expression of Osterix (Osx), which leads to DKK1 expression, which dampens Wnt/β-catenin signalling. As a result, adequate fine tuning of the Wnt/β-catenin pathway may be required to increase cementum production [23]. Wu Y et al., discovered that in DacatOt mutant mice, there is a progressive proliferation of Wnt sensitive cells, which has a disastrous effect on the periodontium. Normally, Wnt/β-catenin signalling is localised to alveolar bone osteocytes and cementocytes, but in DacatOt mutant mice, Wnt signalling spreads to surround the whole PDL. Both osteoblasts and cementoblasts generally create and respond to Wnt signals, thus it is plausible to believe that when the PDL space narrows, Wnt signals emerging from the mineralising tissues begin to impact cells in the middle of the PDL space, promoting cementum and pdl development [24]. Gopinathan G et al., document the highly specific role of the Wnt inhibitor SFRP1 in maintaining the nonmineralised state of PDL progenitors [25].

### How R-spondins Regulate Wnt Signalling

Without Rspos, Znrf3/Rnf43 ubiquitinates FZD, resulting in receptor complex endocytosis and Wnt signalling suppression. Rspos connect to their Lgr4/5/6 receptors, forming a complex that binds to Znrf3/Rnf43. The membrane is then cleansed of these E3 ubiquitin ligases. This allows the receptor complex to initiate downstream signalling, which amplifies the Wnt signalling cascade. Moreover, it is currently thought that Rspo2/3 can accelerate Wnt signalling by using signalling that is independent of Lgr. Rspo2 and Rspo3 can, however, use membrane-bound heparin sulphate proteoglycans to boost canonical Wnt signalling without the help of Lgrs (HSPG) [Table/Fig-3] [26].

The Wnt/β-catenin pathway, which promotes cell proliferation, must be tuned for effective osteogenic differentiation. On the other hand, periodontitis might lead to a hyperactivation of the non canonical Wnt pathway, which is mostly controlled by Wnt5a, which might trigger a flip between opposing Wnt modes. Appropriate balance between Wnt modalities, rather than independent modulation of single pathways, might be a healthier approach to restore




**[Table/Fig-3]:** Schematic illustration of Rspo-induced Wnt signaling augmentation (self diagram) [26].

tissue balance in periodontitis. On a pharmacological level, the discovery of specific intracellular Wnt5a transducers may enable the development of tailored medications to inhibit the signalling pathways activated by this ligand [27].

Rspos proteins (Rspo1-4) are powerful stem cell growth factors that significantly enhance Wnt/β-catenin signalling [28]. Several studies have shown that Rspos proteins help to regulate tissue patterning and differentiation [14,29]. By modifying Wnt/β-catenin signalling, Rspo2 has been shown to promote osteoblast development in MC3T3 E1 cells [30], murine Bone Marrow Stem Cells (BMSC) [31], and human periodontal ligament cells [32]. BMSCs isolated from Rspo2Ftl mice which are deficient in Rspo2 showed reduced osteogenesis compared with BMSCs from wild-type litter mates [31]. Rspo2 is highly expressed by osteoblasts in vitro and sufficient for Wnt/β-catenin signaling activation and enhanced osteoblast maturation or mineralisation [30]. Gong Y et al., proposed in 2020 that Rspo2 was essential for hDPSC proliferation and odontogenic differentiation, and that Rspo2 knockdown reduced this activity. DKK-1 inhibited Wnt/-catenin signalling, which diminished Rspo2's odontogenic effect. During the activation of the Wnt/β-catenin signalling pathway, Wnt3a and Rspo2 had a synergistic effect [33].

## R-SPONDIN- FUTURE THERAPEUTIC ASPECTS IN PERIODONTICS

The available knowledge of Rspo proteins, their receptors on Lgr4/5/6, and receptor-like proteins on Znrf3/Rnf43 has improved our understanding of the molecular mechanism by which Rspos augment the normal Wnt signalling pathway. Rspos play a role in regulating a number of bone-related biological processes and clearly promote osteogenic differentiation [26]. Despite a lack of studies in this area, the receptor Lgr4/5/6 appears to be necessary for Rspos Wnt/β-catenin signalling in bone cells. The precise regulatory mechanism governing Rspos expression and secretion in bone and bone cells must first be understood. Second, further research is needed to understand the specific molecular mechanism by which Rspos regulates bone, as well as the involvement of Lrgs and Znrf3/Rnf43 in bone. In the future, bone formation might focus on controlling the amount of Lgr4/5/6 receptors in bone cells. Another difficult challenge is determining which of the three Lgr receptors and the four Rspo proteins is the most effective target for altering bone metabolism. Many questions remain unresolved before it can be correctly identified how therapeutic drug exploitation and clinical application based on Rspo signalling for diseases associated with bone loss might work. The relative balance between canonical and non canonical Wnt signalling is lost in periodontitis, suggesting that rather than independent control of a particular Wnt pathway, recovery of periodontal tissues may need restoration of this balance [27]. As a result, agents like R-spondin that modulate Wnt signalling can be used as an adjuvant therapy for periodontitis treatment in future. In the near future, we can focus on in vivo investigations to investigate the potential clinical applications of Rspos and Wnt ligands for the treatment of periodontitis. Further research should be conducted to address crucial concerns such as administrative methods (local or systemic), safety, long-term efficacy, and durability.

## CONCLUSION(S)

R-spondins secreted proteins clearly have a substantial impact on their own or via regulating Wnt signalling activity. Given that Wnt signalling is one of the most essential developmental signalling pathways that regulate cell fate decisions, tissue development, growth, and homeostasis, Rspos may play key roles in these processes and serve as potential therapeutic targets.

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