Review

Vitamin D_3 and Dental Mesenchymal Stromal Cells

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Featured Application: Vitamin D_3 might be locally activated and exert numerous physiological effects in dental tissues. These aspects should be considered for the application of vitamin D_3 at dentistry.

Abstract: Vitamin D_3 is a hormone involved in the regulation of bone metabolism, mineral homeostasis, and immune response. Almost all dental tissues contain resident mesenchymal stromal cells (MSCs), which are largely similar to bone marrow-derived MSCs. In this narrative review, we summarized the current findings concerning the physiological effects of vitamin D_3 on dental MSCs. The existing literature suggests that dental MSCs possess the ability to convert vitamin D_3 into 25(OH)D_3 and subsequently to the biologically active 1,25(OH)$_2D_3$. The vitamin D_3 metabolites 25(OH)D_3 and 1,25(OH)$_2D_3$ stimulate osteogenic differentiation and diminish the inflammatory response of dental MSCs. In addition, 1,25(OH)$_2D_3$ influences the immunomodulatory properties of MSCs in different dental tissues. Thus, dental MSCs are both producers and targets of 1,25(OH)$_2D_3$ and might regulate the local vitamin D_3-dependent processes in an autocrine/paracrine manner. The local vitamin D_3 metabolism is assumed to play an essential role in the local physiological processes, but the mechanisms of its regulation in dental MSCs are mostly unknown. The alteration of the local vitamin D_3 metabolism may unravel novel therapeutic modalities for the treatment of periodontitis as well as new strategies for dental tissue regeneration.

Keywords: Vitamin D_3; dental mesenchymal stromal cells; osteogenic differentiation; inflammatory response; immunomodulation

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1. Dental Mesenchymal Stromal Cells

Mesenchymal stem cells were first isolated from the bone marrow and were characterized as cells that can generate connective tissue-forming cells [1]. Later, cells with multipotent differentiation capacity, which were termed as mesenchymal stromal cells (MSCs), were found in almost all adult tissues. In 2006, the following minimal criteria for MSCs were proposed: Adherence to culture plastic under standard cell culture conditions; surface expression of mesenchymal markers CD73, CD90 and CD105 as well as lacking expression of hematopoietic markers CD11b, CD14, CD34, CD45, and HLA-DR; ability to differentiate into osteoblasts, adipocytes, and chondrocytes in vitro [2]. There is an ongoing discussion about the nature of these cells and if their classification as "stem cells" is appropriate [3]. The acronym MSCs is

widely used to define "mesenchymal stem cells" as "mesenchymal stromal cells". A recent consensus paper recommends to further use the abbreviation "MSCs" for identifying tissue-specific stromal cells, which should be supplemented by the tissue origin [4].

MSCs were found in different postnatal tissues [5], including numerous dental tissues: Dental pulp [6], human exfoliated deciduous teeth [7], periodontal ligament [8], apical papilla [9], dental follicle [10], gingival tissue [11], and periapical cyst [12]. Most dental tissue-derived MSCs are of neural crest origin and, therefore, they express several neural lineage markers [13,14]. Numerous previous studies were performed with fibroblasts-like cells isolated from specific tissues, e.g., human dental pulp cells (hDPCs), human gingival fibroblasts (hGFs), and periodontal ligament cells (hPDLCs). These cells exhibit largely similar properties to the corresponding "stem cell" populations isolated from these tissues [15–17]. For the sake of fairness, the cell names will be indicated in the present review as they are mentioned in the corresponding paper, but considered as tissue-specific MSCs. All these cells usually fulfill the minimal MSCs' criteria and also possess immunomodulatory potential [18,19]. This narrative review aimed to summarize the functional effects of vitamin D_3 on MSCs-like cells isolated from different dental tissues and to estimate their potential physiological relevance. This review mainly focused on studies conducted with primary human cells. The effects of vitamin D_3 on systemic level as well as on specialized immune cells are reviewed elsewhere [20–23] and go beyond the scope of this review.

2. Vitamin D_3

The term "vitamin D" comprises a group of fat-soluble prohormones, of which vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are the major inactive precursors [24]. In the human body, vitamin D_3 is naturally produced in the skin upon exposure to ultraviolet B radiation (280–320 nm). Chemically, this reaction includes photolyzation of 7-dehydrocholesterol into previtamin D_3, which is further converted into vitamin D_3 [25]. Additionally, vitamin D_3 can be acquired from some food or nutritional supplements [26]. Vitamin D_3 is transported to the liver, where it is metabolized into the most abundant circulating vitamin D_3 form called 25-hydroxyvitamin D_3 ($25(OH)D_3$). This reaction is catalyzed by the cytochrome P450 family member CYP2R1, also known as 25-hydroxylase [27]. The $25(OH)D_3$ is further converted by CYP27B1 (1α-hydroxylase) into the biologically most active metabolite, 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), which occurs predominantly in the kidneys [28]. The $1,25(OH)_2D_3$ exerts its multiple functions via binding to the intracellular vitamin D receptor (VDR), which is expressed in the majority of cell types and is responsible for the regulation of more than 200 genes [29]. After binding of $1,25(OH)_2D_3$, VDR forms a heterodimer with the retinoid x receptor. This complex binds to vitamin D-responsive elements, inducing the transcription of VDR-regulated genes [30]. Activation of VDR by $1,25(OH)_2D_3$ moreover up-regulates the expression of cytochrome P450 member CYP24A1, which catalyzes 24-hydroxylation of $1,25(OH)_2D_3$ and leads to its inactivation in the manner of a negative feedback loop [31]. Thus, activation of vitamin D_3 signaling activates not only a biological response, but also the mechanisms leading to vitamin D_3 inactivation.

The vitamin D_3 status is usually assessed by measuring serum levels of $25(OH)D_3$, whereby concentrations of 75–125 nmol/L are considered as optimal [32,33]. The $25(OH)D_3$ serum concentrations <30 nmol/L indicates a risk of vitamin D_3 deficiency and $25(OH)D_3$ > 50 nmol/L are indicated to be sufficient [34]. In contrast, $1,25(OH)_2D_3$ levels are much lower, ranging between 0.035 and 0.2 nmol/L [35]. The prevalence of vitamin D_3 deficiency reaches a pandemic extent and can be observed in every age, gender, and ethnicity group, and is most commonly associated with inadequate exposure to sunlight [36].

3. Effects of Vitamin D_3 on Dental MSCs

3.1. Vitamin D_3 Metabolism in Dental MSCs

The exact role of vitamin D_3 in the metabolism of MSCs is currently under investigation [37]. Earlier studies showed that MSCs' differentiation into osteoblasts is enhanced by 1,25$(OH)_2D_3$ [38]. Later, it was demonstrated that osteoblastic differentiation of MSCs is also stimulated by 25(OH)D_3, which implies that MSCs can convert this vitamin D_3 form into biologically active 1,25$(OH)_2D_3$ [39]. Moreover, MSCs express several proteins involved in vitamin D_3 metabolism: VDR, vitamin D_3 hydroxylases CYP27B1, CYP27A1, and CYP24A1. The data about the regulation of CYP27B1 in MSCs are limited and contradictory. There is some evidence that the expression and activity of CYP27B1 in MSCs seems to be regulated by parathyroid hormone (PTH), 1,25$(OH)_2D_3$, and insulin-like growth factor I [37]. MSCs isolated from individuals with decreased serum 25(OH)D_3 levels, like aged or chronic kidney disease patients, exhibit impaired differentiation ability and lower CYP27B1 expression [40]. In contrast, van der Meijden et al. reported that CYP27B1 expression in primary human osteoblasts is regulated by PTH, fibroblast growth factor 23, and calcium, but not phosphate [41]. Similarly to other cell types, the expression of CYP24A1 in MSCs is strongly up-regulated by 1,25$(OH)_2D_3$ and 25(OH)D_3 [42,43]. Lou et al. implied that there are some differences in the expression of vitamin D_3 metabolism in pediatric MSCs depending on the gender [43].

All proteins involved in the conversion and activation of vitamin D_3 responses have been detected in dental MSCs. The expression of VDR has been reported in MSCs that originated from different dental tissues [44–48]. The existence and functionality of CYP27B1 in dental MSCs was first reported by Khanna-Jain et al., who detected this enzyme in hDPCs and human dental follicle cells (hDFCs) [48]. These cells produced 1,25$(OH)_2D_3$ upon stimulation with 25(OH)D_3, which was inhibited by cytochrome P450 inhibitor ketoconazole [48]. Later, Liu et al. showed that hGFs and hPDLCs express CYP27B1 and CYP24A1 and can convert 25(OH)D_3 into 1,25$(OH)_2D_3$ [49]. The functional effects of 25(OH)D_3 have been shown in hGFs and hPDLCs [45,47], which confirms that these cells express functionally active CYP27B1. Liu et al. reported that hGFs and hPDLCs also express CYP27A1, which might convert vitamin D_3 into 25(OH)D_3 via 25 hydroxylation [50]. Thus, dental MSCs seem to possess all enzymes and proteins involved in the activation and degradation of vitamin D_3, which implies the existence of a local vitamin D_3 metabolism (Figure 1).

The data about the regulation of the proteins involved in the local vitamin D_3 metabolism in dental MSCs are scarce. The effect of vitamin D_3 metabolites on the expression of VDR has been investigated in various dental MSCs [44,47–49]. The expression of VDR is increased upon stimulation with 1,25$(OH)_2D_3$ or 25(OH)D_3 in hDPCs, but not in hDFCs [48]. Gao et al. showed that VDR expression in hGFs and hPDLCs is up-regulated by both 1,25$(OH)_2D_3$ and 25(OH)D_3 [47]. A stimulating effect of 1,25$(OH)_2D_3$ on the expression of VDR in hPDLCs was reported by Hong et al. [44]. The expression of VDR in hGFs and hPDLCs does not seem to be affected by inflammatory stimuli, particularly by *Porphyromonas gingivalis* lipopolysaccharide (LPS) in hGFs and hPDLCs [49].

Only two studies were occupied with the regulation of CYP27B1 expression in dental MSCs [48,49]. According to Khanna-Jain et al., CYP27B1 expression in hDPCs and hDFCs is enhanced by 25(OH)D_3, but not affected by 1,25$(OH)_2D_3$ [48]. Liu et al. reported that CYP27B1 expression in hGFs and hPDLCs is up-regulated by interleukin (IL)-1β and sodium butyrate, but not affected by *P. gingivalis* LPS, PTH, calcium chloride, and 1,25$(OH)_2D_3$ [49]. Similarly to other cells, the basal expression of CYP24A1 in the dental MSCs is rather low and has been shown to be enhanced by 1,25$(OH)_2D_3$ and 25(OH)D_3 in hDPCs and hDFCs [48], as well as in hGFs and hPDLCs [49].

Vitamin D_3 might be locally converted to 25(OH)D_3 and 1,25$(OH)_2D_3$ by dental MSCs. Both systemically and locally produced 1,25$(OH)_2D_3$ might exert the biological effects in dental tissues.

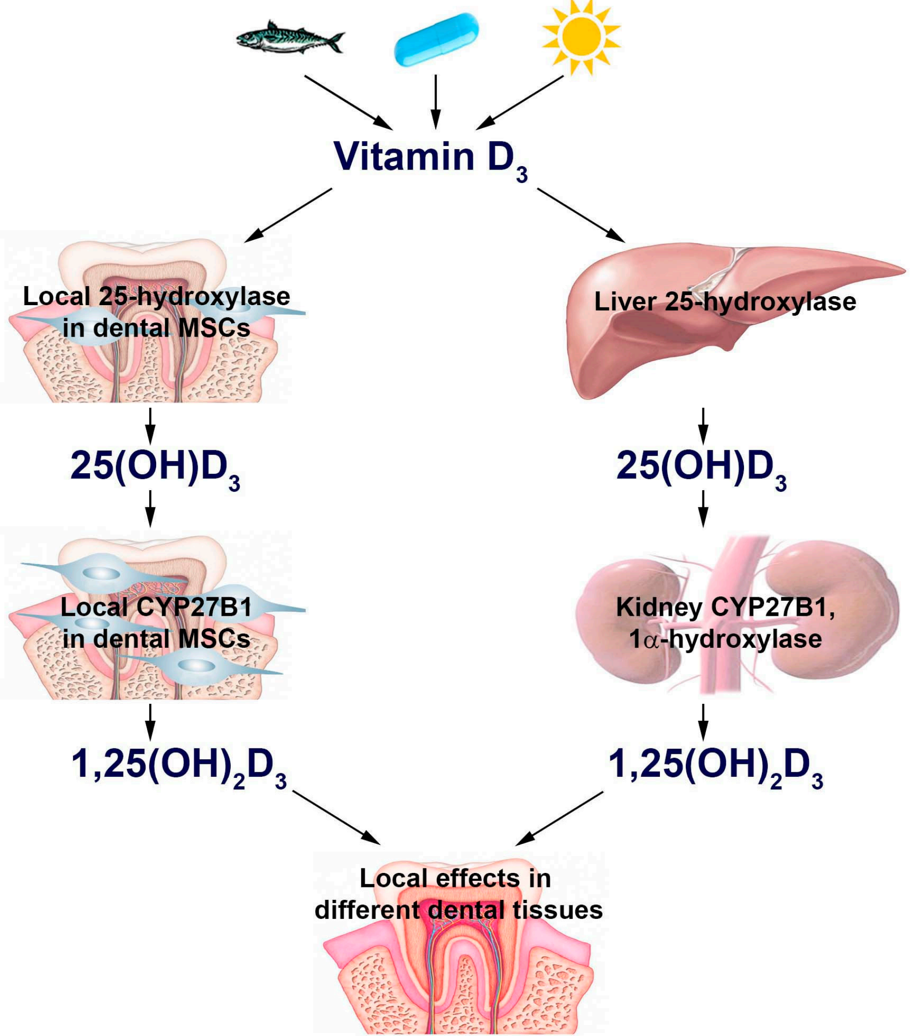

Figure 1. Systemic and local vitamin D_3 metabolism.

3.2. Effect of Vitamin D_3 on the Differentiation Potential of Dental MSCs In Vitro

One of the most prominent biological effects of vitamin D_3 is its ability to stimulate osteogenic differentiation of different MSCs in vitro [37]. This process is usually achieved by culturing cells for 3–4 weeks in media containing specific supplements, such as dexamethasone, β-glycerophosphate, and L-ascorbic acid [51]. Besides, the osteogenic medium is supplemented with high amounts of fetal bovine serum (20%). Osteogenic differentiation is usually assessed by staining extracellular calcium deposits with Alizarin red or von Kossa [51]. Alternatively, it can be evaluated by measuring the expression of specific osteogenesis markers, including alkaline phosphatase (ALP), osteocalcin (OCN), osteopontin (OPN), collagen 1 (Coll-1), bone sialoprotein (BSP), and runt-related transcription factor 2 (runx-2) [52].

Vitamin D_3 has been shown to stimulate osteogenic differentiation of MSCs from various dental tissues in vitro [44,48,53–61]. Khanna-Jain et al. reported that $1,25(OH)_2D_3$ and $25(OH)D_3$ promote mineralization of hDPCs and hDFCs in the presence of osteogenic supplements, but fail to induce mineralization in their absence. However, the gene expression of OCN and OPN has been shown to be enhanced by both vitamin D_3 metabolites, which occurred independently of the presence of osteogenic media [48]. A later study of this group showed that $1,25(OH)_2D_3$ enhances the ALP expression, as well as the gene expression of osteocalcin

and osteopontin in dental pulp cells cultured on a three-dimensional scaffold [55]. Woo et al. reported that $1,25(OH)_2D_3$ promotes mineralization and stimulates alkaline phosphatase activity in human dental pulp stem cells (hDPSCs) [60]. The effects of $1,25(OH)_2D_3$ were accompanied by the activation of extracellular signal-regulated kinases (ERKs) and abolished by a specific ERKs' inhibitor [60]. In dental bud stem cells, $1,25(OH)_2D_3$ has been observed to stimulate mineralization, alkaline phosphatase activity, and the expression of Coll-1, runx-2, BSP, and OPN [58,61]. Wang et al. reported that $1,25(OH)_2D_3$ promotes the mineralization, as well as the gene expression of ALP, BSP, Coll-1, and OCN of MSCs derived from alveolar periosteum [59]. A recent report by Bordini et al. showed that $1,25(OH)_2D_3$ stimulates mineralization and alkaline phosphatase activity of hDPCs grown on calcium–aluminate–chitosan scaffolds [53].

In human periodontal ligament cells, Nebel et al. showed that $1,25(OH)_2D_3$ stimulates alkaline phosphatase activity and gene expression of OCN and OPNs [57]. Hong et al. observed that $1,25(OH)_2D_3$ stimulates mineralization of hPDLCs assessed by Alizarin red and von Kossa staining as well as the expression of ALP, OCN, and BSP [44]. Ji et al. showed that $1,25(OH)_2D_3$ stimulates the mineralization and alkaline phosphatase activity in human periodontal ligament stem cells (hPDLSCs) [54]. Besides, $1,25(OH)_2D_3$ has been reported to enhance the expression of transcriptional coactivator with PDZ-binding motif (TAZ) [54]. TAZ is the downstream effector of Hippo signaling, which regulates various cellular processes, including osteogenic differentiation. Therefore, there is a possibility that $1,25(OH)_2D_3$ might stimulate osteogenesis in hPDLSCs partially through TAZ [54].

Apart from osteogenesis, vitamin D_3 seems to enhance the differentiation of dental tissue-resident stem cells into other dental hard tissues, like dentin and cementum. Particularly, $1,25(OH)_2D_3$ stimulated the production of dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP-1) by hDPCs through the activation of ERKs [60]. The enhancing effect of $1,25(OH)_2D_3$ on the production of DSPP has also been reported in hDPSCs [56]. Bordini et al. showed that $1,25(OH)_2D_3$ promotes the expression of odontogenic factors, like DSPP and dentin matrix acidic phosphoprotein in hDPCs grown on calcium–aluminate–chitosan scaffolds [53]. In human periodontium cells, $1,25(OH)_2D_3$ induces the expression of cementum protein 1, which has been observed on both the gene and protein level [44].

3.3. Vitamin D_3 and Receptor Activator of Nuclear Factor κB Ligand Production by Dental MSCs

The processes of bone remodeling are, on the one hand, regulated by receptor activator of nuclear factor κB ligand (RANKL), which stimulates bone resorption, and, on the other hand, by osteoprotegerin (OPG), which is the natural RANKL antagonist [62]. In dental tissues, RANKL is produced by several cell types, including T-cells, B-cells, osteoblasts, osteocytes, and dental MSCs. However, the cellular source of RANKL, which is relevant for dental tissues, is a matter of debate [63]. Yang et al. showed orthodontic tooth movement in mice lacking RANKL in periodontal ligament cells, which suggests the functional importance of RANKL originating from dental MSCs [64]. The promoter of RANKL gene contains vitamin D-responsive elements and, therefore, RANKL production is up-regulated by vitamin D_3 [65].

Vitamin D_3 has been shown to affect the RANKL and OPG production by dental MSCs. Particularly, $1,25(OH)_2D_3$ has been reported to up-regulate RANKL expression and down-regulate OPG expression in hPDLCs [66,67]. Other studies showed that $1,25(OH)_2D_3$ stimulates RANKL production, but does not affect OPG production in hPDLSCs [68] and hDFCs [69]. Zhen et al. reported that $1,25(OH)_2D_3$ attenuates OPG expression in hDPCs [70]. Despite this clear evidence of a positive effect of $1,25(OH)_2D_3$ on RANKL production, its physiological importance is controversial. Bloemen et al. investigated osteoclast formation in the co-culture of human peripheral blood mononuclear cells and periodontal ligament fibroblasts in the presence or absence of $1,25(OH)_2D_3$ and found that osteoclastogenesis is strongly stimulated by periodontal ligament fibroblasts, while adding $1,25(OH)_2D_3$ has no further effect on the osteoclasts formation [71].

In contrast, Wang et al. revealed that 1,25(OH)$_2$D$_3$ stimulates hDFCs-induced osteoclastogenesis through a runx-2-dependent mechanism [69].

3.4. Vitamin D$_3$ and Inflammatory Immunomodulatory Properties of Dental MSCs

Vitamin D$_3$ is known to have an anti-inflammatory effect in different immune cells [72]. Dental MSCs are known to produce various inflammatory mediators and also possess immunomodulatory properties towards different immune cells [18,19,73–77]. Thus, dental MSCs are assumed to play an essential role in the inflammatory processes in the oral cavity.

The basal production of different pro-inflammatory mediators by dental MSCs is rather low. It is strongly enhanced by stimulation with bacterial components, such as LPS or inflammatory cytokines like IL-1β. Two studies investigated the effects of vitamin D$_3$ on the basal production of various inflammatory mediators. Tang et al. showed that 1,25(OH)$_2$D$_3$ inhibits the basal production of IL-8 by primary hPDLCs, but does not affect IL-6 production [78]. In contrast, Hong et al. reported that 1,25(OH)$_2$D$_3$ up-regulates the basal gene expression of IL-6 in human periodontium cells [44].

The 1,25(OH)$_2$D$_3$ inhibits *P. gingivalis*-induced production of IL-8 by primary hPDLCs without affecting IL-6 production [78]. Andrukhov et al. showed that both 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$ attenuate the production of IL-8 and monocyte chemoattractant protein 1 (MCP-1) in hPDLCs stimulated with *P. gingivalis* LPS or heat-killed *P. gingivalis* [45]. The data on IL-6 were somewhat controversial: Both vitamin D$_3$ metabolites diminished IL-6 production in a commercial cell line, but not by primary cells. This observation suggests that the response of dental cells to vitamin D$_3$ might depend on the cell source [45]. Nebel et al. reported that 1,25(OH)$_2$D$_3$ attenuates *Escherichia coli* LPS-induced IL-6 and chemokine ligand 1 (CXCL-1) production by human periodontal ligament cells, but did not affect that of IL-1β and MCP-1. Hosokawa et al. showed that 1,25(OH)$_2$D$_3$ inhibits the IL-1β-induced production of several inflammatory mediators like IL-6, IL-8, CC chemokine ligand (CCL) 20, CXC chemokine ligand (CXCL) 10, and matrix metalloproteinase (MMP)-3, but has no effect on the production of tissue inhibitor of metalloproteinases 1 [46]. This effect is associated with the suppression of c-jun N-terminal kinase (JNK) phosphorylation and inhibitor kappa B-α degradation [46]. Nastri et al. observed various effects of 1,25(OH)$_2$D$_3$ on the inflammatory response in hGFs and hPDLCs stimulated with *P. gingivalis* and *Streptococcus pyogenes*. Under these conditions, 1,25(OH)$_2$D$_3$ slightly increases IL-6 production, inhibits IL-8 and IL-12 production, and strongly promotes IL-10 production [79]. De Filippis et al. demonstrated that 1,25(OH)$_2$D$_3$ inhibits *P. gingivalis*-induced production of tumor necrosis factor (TNF)-α, IL-8, and IL-12 production by hPDLCs [80]. Elenkova et al. reported that 1,25(OH)$_2$D$_3$ attenuates the production of IL-6 and IL-8 in hGFs stimulated by glycated human serum albumin (HSA) or HSA + IL-1β or IL-1β + IL-17 [81].

Dental MSCs, similarly to MSCs of other tissues, possess immunomodulatory features and regulate the functional properties of various immune cells [18,19]. The effects of MSCs on immune cells are mainly immunosuppressive. Particularly, MSCs inhibit T-cell proliferation, stimulate the differentiation of regulatory T-cells (Tregs), inhibit the activity of Th17 cells, and promote the differentiation of macrophages towards an anti-inflammatory phenotype. This immunomodulatory activity of dental MSCs is strongly activated by the inflammatory cytokines IL-1β, TNF-α, and interferon (IFN)-γ, which are produced mainly by immune cells [3,76,82,83]. Thus, there is a complex interaction between dental MSCs and immune cells, in which these cell types regulate each other's activity reciprocally.

The studies of our group investigated the effect of vitamin D$_3$ on the immunomodulatory properties of hPDLCs [84,85]. We found that 1,25(OH)$_2$D$_3$ down-regulates cytokine-induced expression of indoleamine-2,3-dioxygenase, programmed cell death 1 ligand 1 (PD-L1), PD-L2, and prostaglandin synthase 2. These proteins mediate the immunosuppressive effects of dental MSCs, and, thus, this finding implies that the reciprocal interaction between dental MSCs and immune cells might be affected by

vitamin D_3. To study the exact impact of vitamin D_3, we used a co-culture model with transwell inserts. In this model, cells continuously affect each other through paracrine mechanisms but have no direct cell-to-cell contact. We found that the effect of vitamin D_3 on CD4+ T lymphocytes strongly depends on the presence of hPDLCs. While 1,25(OH)$_2$D$_3$ inhibits the CD4+T cell proliferation and stimulates the formation of Tregs in the absence of hPDLCs, it has no effect on the CD4+T lymphocytes proliferation and inhibits Tregs' formation in the presence hPDLCs [84]. Moreover, when the immunosuppressive properties are enhanced by IFN-γ, 1,25(OH)$_2$D$_3$ even stimulates CD4+ T lymphocytes' proliferation [84]. The effects of 1,25(OH)$_2$D$_3$ are partially abolished when the immunomodulatory properties of hPDLCs are pharmacologically inhibited. Thus, vitamin D_3 might diminish the immunosuppressive action of dental MSCs and exert a pro-inflammatory effect on CD4+ T-cells by this indirect mechanism.

Vitamin D_3 also modulates the immunomodulatory activity of hPDLCs towards macrophages. Behm et al. investigated the effect of hPDLCs primed with one of the inflammatory cytokines, IL-1β, TNF-α, or IFN-γ, in the presence or absence of 1,25(OH)$_2$D$_3$ [85]. Interestingly, hPDLCs primed with different cytokines stimulate the expression of both pro- and anti-inflammatory factors in co-cultured macrophages. Priming of hPDLCs with 1,25(OH)$_2$D$_3$ and inflammatory cytokines usually diminish the expression of pro-inflammatory factors TNF-α, IL-12, and MCP-1 in the co-cultured macrophages. The quantitative extent of this indirect 1,25(OH)$_2$D$_3$ effect depends on the type of inflammatory cytokine. Interestingly, 1,25(OH)$_2$D$_3$ enhances the stimulating effect of IL-1β primed hPDLCs on the expression of anti-inflammatory factors IL-10 and transforming growth factor β3 in macrophages and inhibits that of IFN-γ-primed macrophages [85]. Thus, the effect of vitamin D_3 on the interaction between hPDLCs and macrophages strongly depends on the inflammatory environment.

3.5. Vitamin D_3 and Antimicrobial Activity of Dental MSCs

Vitamin D_3 is a well-known activator of antimicrobial peptides' (AMPs) production by different cells [86]. In the oral cavity, antimicrobial peptides are produced mainly by oral epithelial cells, and their production is regulated by the oral microbiota [87]. Different vitamin D_3 metabolites are potent inducers of AMPs' production and antibacterial activity of oral epithelial cells [80,88–92]. MSCs of different tissues also produce various AMPs, but this ability seems to be somewhat limited compared to epithelial and immune cells [93]. Only a few studies reported the expression and production of AMPs in hGFs and hPDLCs [47,80,94]. The effect of vitamin D_3 on the AMPs' production by dental MSCs was investigated by two studies. De Filippis et al. showed that 1,25(OH)$_2$D$_3$ enhances both basal and *P. gingivalis*-induced production of human beta-defensin 3 by hPDLCs [80]. Moreover, 1,25(OH)$_2$D$_3$-treated hPDLCs inhibit the growth of *P. gingivalis*, as well as its adhesion to a Matrigel-coated polystyrene surface [80]. Gao et al. demonstrated that both 25(OH)D$_3$ and 1,25(OH)$_2$D$_3$ stimulate the production of LL-37 by hGFs and hPDLCs [47].

3.6. Biological Activity of 24R,25(OH)$_2$D$_3$ Vitamin D_3 Metabolite

The biological role of 1,25(OH)$_2$D$_3$ has been intensively investigated and sufficiently clarified over the last 30–40 years [20,21,25]. However, there are other potential biologically active vitamin D_3 metabolites, and their physiological role remains obscure. One of these metabolites is 24R,25(OH)$_2$D$_3$, which is formed by 24-hydroxylation of 25(OH)D$_3$. Its serum levels are comparable to those of 25(OH)D$_3$ and are about 1000 times higher than those of 1,25(OH)$_2$D$_3$ [95], but the mechanisms of its biological effects are not entirely clear. Earlier studies on chicken egg formation, rachitic chicks, and chick models of fracture healing showed that these processes depend on 24R,25(OH)$_2$D$_3$ rather than on 1,25(OH)$_2$D$_3$ [96–98], challenging the prevailing dogma about the central role of 1,25(OH)$_2$D$_3$ in the vitamin D_3 activity. Further confirmation about the importance of 24R,25(OH)$_2$D$_3$ in fracture healing comes from a recent study on CYP24A1-/-

mice [99]. Here, impairment of fracture repair in CYP24A1-/- mice, which can be corrected by exogenous administration of 24R25$(OH)_2D_3$ and not by 1,25$(OH)_2D_3$, was reported [99].

Biological effects of 24R,25$(OH)_2D_3$ were observed in MSCs and other cells [82,95,100,101]. In bone marrow-derived MSCs, 24R,25$(OH)_2D_3$ induced osteogenic differentiation even in the absence of dexamethasone, which was not observed for 1,25$(OH)_2D_3$ [100]. Furthermore, 24R,25$(OH)_2D_3$ also inhibits CYP27B1 expression and reactive oxygen species' production in MSCs [100]. A tight regulatory relationship between the effects of 1,25$(OH)_2D_3$ and 24R,25$(OH)_2D_3$ was hypothesized [100]. There is some evidence that 24R,25$(OH)_2D_3$ might also influence the inflammatory response. A recent study showed that 24R,25$(OH)_2D_3$, but not 1,25$(OH)_2D_3$, blocks the effects of IL-1β in rat articular chondrocytes [82]. The mechanisms of 24,25$(OH)_2D_3$ bioactivity remain unclear. An early study on human osteoblasts shows that 24-hydroxylated vitamin D_3 metabolites enhance osteogenesis through the VDR-dependent mechanism, similarly to 1,25$(OH)_2D_3$ [101]. In contrast, the cellular effects of 24,25$(OH)_2D_3$ in corneal epithelial cells seem to be independent of VDR [102]. A recent study identified FAM57B2 as a mediator of 24R,25$(OH)_2D_3$ effects [41]. One report showed that 25$(OH)D_3$ also has some biological effects in cells originating from CYP27B1-deficient mice, which suggests that 25$(OH)D_3$ may act independently of 1α-hydroxylation [103].

4. Physiological Relevance of Vitamin D_3 Effects in Dental MSCs

The biological effects of vitamin D_3 in dental tissues are multifaceted and associated with bone metabolism, immune response, and antimicrobial peptides' production [104]. Most of the studies on the effect of vitamin D_3 on the dental MSCs support its ability to stimulate osteogenic differentiation and inhibit the inflammatory response (Figure 2). However, the physiological relevance of these effects is not so obvious. Most effects of the biologically active metabolite 1,25$(OH)_2D_3$ on the osteogenesis and inflammatory response were observed at concentrations of 10–100 nM, which are about 1000 times higher than its serum levels [35]. Nebel et al. showed no effect of 1,25$(OH)_2D_3$ on the LPS-induced response in hPDLCs at a concentration of about 0.7 nmol/L (3 ng/mL) [57]. Biological effects of 25$(OH)D_3$ are observed at concentrations of 10–500 nmol/L [45,47–49], which only partially reflect those reported in the blood serum [32,33]. However, it should be noted that the local concentration of vitamin D_3 metabolites in dental tissues and intercellular space might differ from that observed in serum.

Local vitamin D_3 metabolism could be involved in the regulation of both bone formation and bone resorption. MSCs are a crucial factor for bone formation in the maxilla and mandible [105]. Most studies describe stimulating effects of vitamin D_3 on the osteogenic differentiation of dental MSCs, which implies its positive impact on bone formation. It should be noted that this effect is usually observed in osteogenic media, which contain several artificial components, including dexamethasone. In the absence of these supplements, no effect of vitamin D_3 on the osteogenesis is observed. Moreover, the mechanisms of osteogenic differentiation in vitro and in vivo are different. Most studies suggest that vitamin D_3 increases RANKL/OPG ratio, which might result in increased osteoclastogenesis and bone resorption. Interestingly, dental MSCs with periodontitis-associated VDR phenotype exhibit higher RANKL production upon the stimulation with 1,25$(OH)_2D_3$ [106], which argues the physiological importance of this effect. Vitamin D_3 might also inhibit bone resorption indirectly, for example, through inhibition of IL-6 production by different MSCs. Furthermore, the direct and indirect effects of vitamin D_3 on immune cells might modulate their impact on bone metabolism [107].

The 1,25$(OH)_2D_3$ enhances osteogenic differentiation and production of RANKL and antimicrobial peptides and diminishes inflammatory response and immunomodulatory ability of dental MSCs.

Local vitamin D_3 metabolism might also be involved in the regulation of local inflammatory processes. Resident dental MSCs play an essential role in the progression of inflammatory diseases, such as

periodontal disease and pulpitis [18]. Vitamin D_3 attenuates the response of different dental MSCs to inflammatory stimuli, which underlie its anti-inflammatory role under these conditions. However, besides its anti-inflammatory actions, vitamin D_3 might partially abolish the immunosuppressive effect of dental MSCs towards different immune cells [84,85]. Thus, vitamin D_3 seems to fine-tune the inflammatory response and functions either anti- or pro-inflammatorily, depending on the microenvironment. Besides, the expression and activity of local CYP27B1 in dental MSCs are regulated by IL-1β [49]. Our recent study showed that the responsiveness of hPDLCs to 25(OH)D_3 and 1,25$(OH)_2D_3$ is diminished under inflammatory conditions [108]. Summarizing, these observations suggest a reciprocal regulation of local vitamin D_3 metabolism and the inflammatory response.

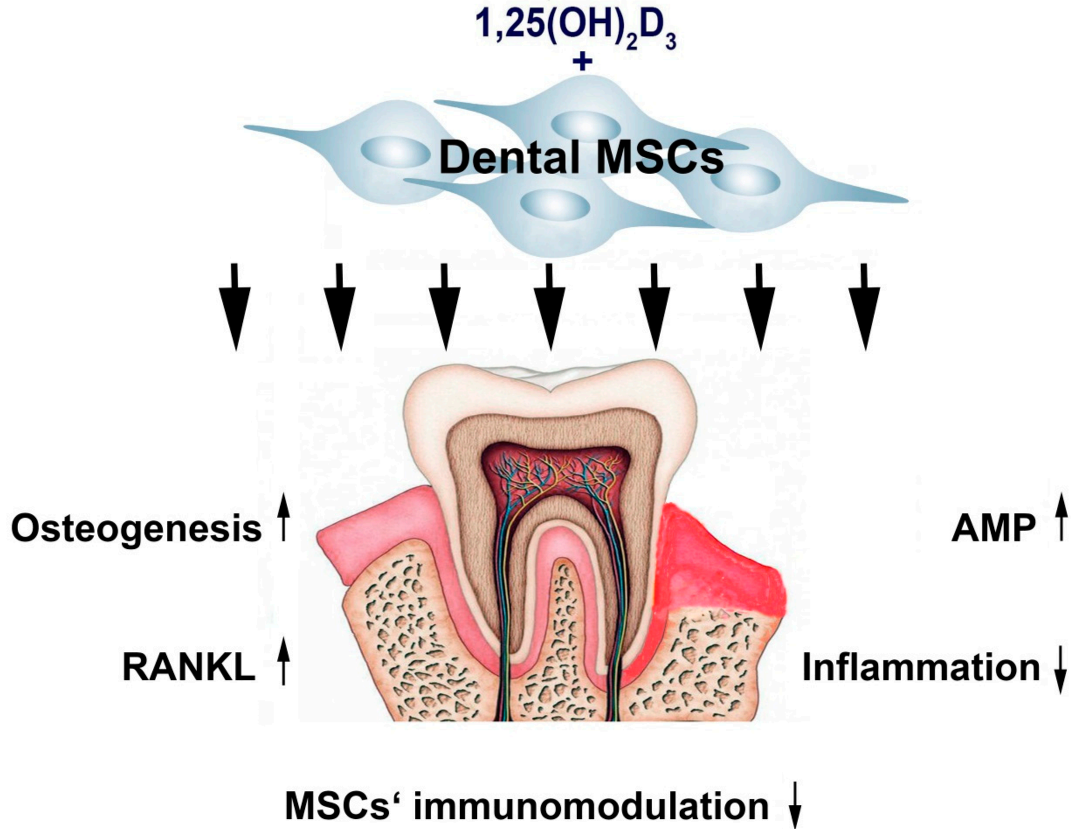

Figure 2. Effects of biologically active vitamin D_3 metabolite 1,25$(OH)_2D_3$ in dental mesenchymal stromal cells.

5. Vitamin D_3 and Periodontal Disease

A recent systematic review suggested a positive association between vitamin D_3 deficiency and the risk of periodontitis, although the data are still scarce and controversial [109]. Moreover, VDR polymorphisms also show an association with the susceptibility to periodontitis [110]. Local anti-inflammatory effects of vitamin D_3 in dental MSCs might partially underlie the association between vitamin D_3 deficiency and periodontitis. This statement is supported by studies using periodontitis animal models. Intraperitoneal injection of 25(OH)D_3 ameliorates periodontitis in diabetic mice through the modulation of the inflammatory response [111,112]. Bi et al. showed that 1,25$(OH)_2D_3$ supplementation in the oral gavage suppresses lipopolysaccharide-induced alveolar bone damage in rats by regulating T-helper-cell subset polarization [113].

The effect of vitamin D_3 supplementation during periodontitis treatment is investigated rarely. Civitelli et al. (and collaborators) reported that vitamin D_3 and calcium supplementation show a trend for better periodontal health compared to those not taking supplements, but these effects were only modestly positive after one year [114,115]. Furthermore, Bashutski et al. observed impaired periodontal surgery outcomes in vitamin D_3-deficient patients and showed that vitamin D_3 supplementation at the time of surgery fails to prevent those outcomes [116]. A randomized, double-masked, placebo-controlled clinical trial suggested a positive impact of short-term vitamin D_3 supplementation after non-surgical periodontal therapy [117].

6. Future Perspectives and Open Questions

The existing literature suggests that the functional properties of dental MSCs are affected by different vitamin D_3 metabolites (Figure 2). Furthermore, dental MSCs express the enzymes, which enable the conversion of vitamin D_3 into 25(OH)D_3 and subsequently to 1,25(OH)$_2$$D_3$, as well as 1,25(OH)$_2$$D_3$ inactivating enzyme. This suggests the existence of a local vitamin D_3 metabolism in various dental tissues. The conversion of vitamin D_3 by dental MSCs is assumed to be important in the local tissue homeostasis, although its exact role has still to be investigated. One of the most important questions is the regulation of local vitamin D_3 conversion by dental MSCs, which remains largely unexplored. Obviously, CYP27B1 is differently regulated in dental tissues than in the kidneys. Another critical question is the physiological relevance of 24R,25(OH)$_2$$D_3$: It is known that this metabolite possesses biological activity, but its effects in dental MSCs are not known so far.

Understanding of the local vitamin D_3 metabolism could open new perspectives in the treatment of periodontal disease. Vitamin D_3 deficiency is a recognized risk factor of periodontal disease. However, the effect of vitamin D_3 supplementation during periodontal therapy is almost not explored. It should be noted that an effective increase in the serum 25(OH)D_3 within a short time could be achieved only by high doses of vitamin D_3 supplementation. Mainly, supplementation with 1000 IU (25 μg) during 3–4 months is necessary to enhance the serum level of 25(OH)D_3 by 10 ng/mL [118]. Moreover, an increase in 25(OH)D_3 by vitamin D_3 supplementation does not always result in a clinically relevant effect [119,120]. High doses of vitamin D_3 might have a potentially harmful impact on bone fracture [121,122]. Therefore, further randomized clinical trials are necessary to determine the doses of vitamin D_3 supplements during periodontal therapy based on the balance of clinical effectiveness and safety [123,124].

Approaches for local vitamin D_3 delivery should be elaborated as an addendum for the dietary supplementation, and their therapeutic and regenerative potential should be tested in pre-clinical and clinical studies. Modern cell-based therapy is based on three pillars: Cells, scaffold, and growth factors [125–127]. Vitamin D_3-loaded scaffolds could be considered as potential carriers for local vitamin D_3 delivery [128] and this approach should be tested in the dental field. Posa et al. reported that 1,25(OH)$_2$$D_3$ enhances the expression of integrin αV and integrin β3 in human dental bud cells [61]. Integrins are transmembrane proteins mediating cell interaction with extracellular matrix and scaffolds and might facilitate dental tissue regeneration [129]. Thus, vitamin D_3 might modify the interaction of dental MSCs with a scaffold, but this question needs to be further investigated. Modern scaffolds are characterized by unique structural features of both micro- and nanoscale levels. Nanostructure features of scaffolds are known to influence cell response [130] and might hypothetically modulate vitamin D_3 biological activity. Furthermore, modulation of the expression and activity of the proteins involved in the local vitamin D_3 metabolism could also be considered as a potential therapeutic strategy. However, the mechanisms involved in the regulation of the local vitamin D_3 homeostasis in dental tissues need to be further explored.

An interesting perspective is the adjustment of the local vitamin D_3 homeostasis by optimizing environmental factors, such as lifestyle and food intake [131]. Some dietary supplements like

docosahexaenoic acid and curcumin are low-affinity VDR ligands [132]. A beneficial effect of curcumin in periodontal therapy has been reported [133,134], but a possible contribution of VDR has never been investigated. Soy progesterone genistein has been shown to inhibit the expression and activity of CYP24A1 in vitro and in vivo [135,136], and to regulate the expression of CYP27B1 [137]. This opens the perspective of the manipulation of vitamin D_3 metabolism enzymes via nutrition [138]. Understanding the dietary effect on the local vitamin D_3 homeostasis in dental tissues and particularly in dental MSCs could be essential for optimizing the clinical benefit of vitamin D_3.

7. Conclusion

Vitamin D_3 can be locally converted to $25(OH)D_3$ and $1,25(OH)_2D_3$ by dental MSCs and affect their biological functions. This local vitamin D_3 metabolism might play an essential role in several processes, like maintenance of tissue homeostasis, inflammatory diseases, and tissue regeneration.

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