VITAMIN K2

VITAL FOR HEALTH AND WELLBEING

CHAPTER 7 **Vitamin K2 and its Impact on Tooth Epigenetics**

by Jan Oxholm Gordeladze, Maria A. Landin, Gaute Floer Johnsen, Håvard Jostein Haugen and Harald Osmundsen

CHAPTER PUBLISHED BY
INTECH open science | open minds
WORLD'S LARGEST
Science, Technology & Medicine Open Access book publisher.
kaqpa BIOSCIENCE

CHAPTER 7

Vitamin K2 and its Impact on Tooth Epigenetics

by Jan Oxholm Gordeladze, Maria A. Landin, Gaute Floer Johnsen, Håvard Jostein Haugen and Harald Osmundsen

Abstract

The impact of nutritional signals plays an important role in systemic-based «models» of dental caries. Present hypotheses now focus both on the oral environment and other organs, like the nervous system and brain. The tooth is subjected to shear forces, nourishing and cleansing, and its present "support system" (the hypothalamus/parotid axis) relays endocrine signaling to the parotid gland. Sugar consumption enhances hypothalamic oxidative stress (ROS), reversing dentinal fluid flow, thus creating an enhanced vulnerability to the oral bacterial flora. The acid, produced by the oral bacterial flora, then leads to erosion of the dentine, and an irreversible loss of dental enamel layers. This attack brings about inflammatory responses, yielding metalloproteinase-based "dissolution". However, vitamin K2 (i.e. MK-4/MK-7) may come to the rescue with its antioxidant property, locally (mouth cavity) or systemically (via the brain), thus sustaining/preserving hormone-induced dentinal fluid flow (encompassing oxidative stress) and boosting/magnifying bodily inflammatory responses. However, sugars may also reduce the tooth's natural defences through endocrine signaling, thus enhancing acid-supported enamel dentine erosion. Vitamin K2 sustains and improves the salivary buffering capacity via its impact on the secretion/flow of calcium and inorganic phosphates. Interestingly, primitive cultures' diets (low-sugar and high-K2 diets) preserve dental health.

Keywords: K2, SXR, endocrine interaction, deiodinases, TH receptors

1. Introduction

The "perfect" and healthy tooth is properly designed to cope with the rough environment in the oral cavity, since virtually cleanses itself in an inside-out manner. Dental caries is supposed to be a result of the phenomenon that a tooth's fluid flow has been halted or even reversed, thus compromising tooth's defense system. It is well known that the local enamel demineralization process, aided by bacterial acid, is vastly stimulated by nutritional conditions, specifically by today's refined carbohydrates (sucrose and corn syrup = free glucose + fructose). Hence, the ensuing process renders the tooth vulnerable, and part of this process begins in the hypothalamus, resulting in alterations of the fluid flow passing through the dentine layer [1]. Not to forget: in this context, nutritional phenomena play very important roles (both systemically and locally).

In the aftermath of acid-induced enamel demineralization of the breakdown of the dentin layer is accomplished by the body's own matrix metalloproteinase (MMP) enzymes [2, 3], a process which follows as a result of an untoward and galloping inflammatory response to an enhanced acid secretion. The present caries process begins as a more or less dormant, but reversible inflammation ("dentinitis"), while developing into a nonreversible dentin phase of caries after a while. This biological phenomenon is very much like reversible versus irreversible pulpitis and the terms gingivitis and periodontitis, while referring to the periodontium only [4].

However, there is a consensus that the process of dental caries recognization is multifactorial, as well as systemically based. It may not be sufficient to decrease the detrimental process rendered by the sugar intake with the ensuing enhancement of bacterial number and strains, but also boost the body's defense mechanisms with an antioxidant-rich diet that may be composed of fruits and vegetables, as well as vitamin K2.

Some research reports document that vitamin K2 can assist in significantly reducing dental caries [5, 6]. A larger body of research, however, is necessary in order to establish the mode, by which this vitamin may augment local defense mechanisms by altering saliva composition, while also systemically, via influencing the hypothalamus, as well as endocrine aspects of the parotid gland.

This concept of systemically delivered impact underscores an important shift in paradigm, from a traditional 'acid theory' explaining the development of dental caries that carries a plethora of implications for the prevention of dental decay in the future. Furthermore, it will necessary to document, via the mechanism of action of vitamin K2, how this small molecule affects gene regulation of the intimate play of osteoblast- and osteoclast-like cells in the organ layers constituting developing and full-frown teeth. The present chapter attempts to create a synthesis of current knowledge and recent research reports and ongoing research projects, with the intention of shedding new light on the impact of K2 on dental health.

2. Backing of a new concept

2.1. Oral and systemic stress responses with common denominators

The definition of the stress concept dates back some 60–70 years [7]. It was meant to feature the process of how irritants caused a bodily reaction, and how the body dealt with it. If a stressor was defined as local, e.g. acid-induced enamel demineralization or irritation of the periodontal tissues by plaques, the body might provoke a specific, but local reaction. This would be controlled inflammatory responses that will remain similar throughout the entire body. Hence, they are defined as "local adaptation syndromes" (LAS). In the dentin of a tooth, "dentinitis" represents this local inflammatory response.

A focal reaction is often mild, but resides rather quickly. However, this limited response may develop into a systemic and an exaggerated variant, thus "threatening" the entire body via the endocrine system. The present response is named "GAS" ("general adaption syndrome"), because its "attack" on the organism pretty much resembles the systemic type reaction, even though it represents a local type of stress reaction [7]. The hypothalamus/pituitary/adrenal axis serves as the mechanism in charge of the body's general, untoward reactions.

The essence of the problem resides with the ingestion of refined carbohydrates (i.e., mono and disaccharides in particular) that locally escalate the growth of microorganisms within the oral cavity and their production of acid. This inflammation causes a rapid loss of minerals, such as dental enamel, and it is named "dentinitis" and serves as a local adaptation, being a part of an LAS.

However, subsequent to this local reaction, small-molecular sugar entities (like sucrose, glucose, and fructose) exert a major impact on the body, when absorbed. Blood-sugar spikes are therefore counteracted by emerging dentinal fluid flows through the tooth by coordinated signals emanating from the hypothalamus. This adaptation (GAS) is chiefly endocrine, and affects the entire body. Hence, the present hypothalamic-parotid axis serves as the endocrine axis that is instrumental in maintaining the dental health [8]. The essence of it is the following: local irritation is magnified in the presence of a GAS response [7, 9]. Sugar molecules (mostly monosaccharides), with their marked effect on the whole organism, magnifies the local acid attack by triggering the GAS response. As a consequence, the tooth is rendered more vulnerable to the acid exposure [1], whether the acid is produced from sugar entities (via bacteria) or comes from various carboxylic acids in the diet.

2.2. How the tooth develops caries

The tooth is fed by the alimentary elements delivered by fluid flow through the dentin which may be halted or reversed, when impacted by a systemic stressor, like excessive sugar intake [10, 11]. This allows bacteria in the oral cavity to attach to the tooth, where they enhance the local concentration of acid, leading to the well-known demineralization of the enamel surface. Consequently, the flow of fluid through the tooth is enhanced by parotid hormone [8]. The secretion of parotid hormone, as well as the secretion of insulin [12, 13] is regulated by the hypothalamus, influencing GAS, and eventually affecting the adrenal glands. Finally,

a normal inflammatory reaction (LAS) occurs with a corresponding enhanced metabolism, i.e., (1) an increase in reactive oxygen species (ROS) production, and (2) activation of MMP's (e.g., collagenase). Normally, tissue inhibitors of metalloproteinases (TIMP's) serve to neutralize activated MMP's, when the body has regained its control over the inflammatory process. Antioxidants aid in the control inflammation by hampering an ROS activity, thus minimizing the necessity for stimulating MMP activation. It is known that optimal nutrition exerts an important role in this process. Temporary challenges are followingly managed by the dampening inflammation known to cause reversible state, while healing may occur. This process is called dentinitis [14, 15]. Excessive irritability leads to galloping inflammatory processes that are mainly irreversible in the tooth and recognized in the dentin by the name of caries.

2.3. A systemic approach and the impact of oxidation control

The systemic "angle" of caries may be construed as a link to diabetes mellitus. When enhanced blood glucose is registered in the hypothalamus, the production of free radicals like "reactive oxygen species (ROS)" is enhanced. These molecules serve as a warning signal for the hypothalamic gland to down-regulate the secretion of parotid hormone, while simultaneously upregulating the insulin secretion. Antioxidant loading has since long been known to manage the glucose-induced free radical storm sweeping the hypothalamic gland [16].

Antioxidants counteract the detrimental effects of the free radical damage, as shown in the so-called Asian Paradox, where heavy cigarette smoking is paralleled with reduced rates of coronary disease and cancer amongst consumers of green tea [17–19] that is famous for its antioxidant properties. However, along with the fact that systemically administered antioxidant effects of green tea reduce the incident of dental caries, vitamin K2 may prove to be an even more potent antioxidant [20–24].

2.4. A summary of the features of vitamin K2

Vitamin K2 is known as menaquinones, while vitamin K1 is phylloquinone. The quinones display oxygen-containing ring structures that render them suitable for the transport of electrons [25–27]. K2 was added to the vitamin K category of molecules, since it may be produced in the body from K1 [28]. K1 is deemed essential to blood clotting, and accordingly, our body has "invented" modes to recycle K1 for repeated use. Hence, it has rendered itself less dependent on a constant dietary intake of K1.

Vitamin K2 takes several forms that are linked to the structure of their side chains (e.g., MK4 and MK7). While MK4 is the form produced by our bodies produced from K1, supplemental MK4 is entirely synthetic. MK7 is a (more) biologically active form that entertains a longer half-life. Therefore, it is often the preferred or recommended supplement [29], especially in the treatment of osteoporosis and untoward soft tissue calcifications (ref).

Vitamin K2 is bounded to the transcription factor SXR/PXR (ref) and may serve as a cofactor of vitamin K dependent carboxylases. This enzyme, when associating with vitamin K2, will change the structure of proteins by the process of gamma-carboxylation, or the SXR/PXR-vitamin K2 complex may work to enhance the expression of a set of genes that are responsive

to the presence of vitamin K2 (ref). Some examples of these processes are osteocalcin, located in bones and teeth, and matrix GLA protein expressed in cardiovascular (i.e., soft) tissues. Both of these protein structures require the vitamins A and D, as well as vitamin K2 for their production [30, ref]. The carboxylation of osteocalcin by vitamin K2 allows it to attract and retain calcium that is good for bones [31, 32]. The "opposite" process is observed in cardiovascular (i.e., soft) tissues, since matrix GLA proteins allow for calcium to be deposited in arteries, when uncarboxylated, but shed or blocks the "entry" of calcium, when carboxylated with the assistance of sufficient vitamin K2 [33–35].

Dietary K2 is processed in the liver and released into the circulation via high and low density lipoproteins that make them readily available for uptake into extrahepatic tissues [36–38]. Fermented foods such as cheese have significantly higher levels of K2 than milk. The higher levels are obtained from bacterial sources. Natto (which is fermented soy) is, without any doubt the more potent source of vitamin K2 [30]. Most K2 supplements are cultured from natto; however, synthetic products with unsurpassed bioavailability and stability are now to be available in the market.

Menaquinones are taken up and stored in several tissues throughout the body. Some of the highest concentrations to be found are in the pancreas and the salivary glands [39]. Hence, it can be construed that there exists a close relationship between both of these exocrine/endocrine glands through the hypothalamus. High levels of vitamin K2 are also located in the brain, heart, and bone [40, 41] which definitely is of significance for many disease states, including dental caries, which has been shown to be associated with oxidative stress [6, 24].

2.5. How dental tissue is nourished

Saliva brings nutrients from the outside of the tooth directly to the inside [42]. The fluid holds active ingredients, like minerals and enzymes, as well as buffering agents. The free cytosolic calcium levels sustain the more important or critical role in the signaling potential of the salivary glands' contents [43–45]. Taken calcium's dependency on vitamin K2–assisted carboxylation related to osteocalcin and matrix GLA proteins for granted, findings to come may reveal that vitamin K2 exerts an impact on salivary signaling and composition and activation potency. Since long, one has known that insulin [46] and the exocrine secretions of the pancreas [47] are partially dependent on the presence of vitamin K2.

Furthermore, saliva serves as an important player in the maintenance of proper mineralization of the teeth's enamel. The saliva buffers demineralization seen with acid-induced mineral dissolution, and it delivers building blocks for remineralization "on request." The optimal pH for tooth health is variable [48], and is more related to saliva composition and flow. However, it is yet not known how saliva is connected to vitamin K2, but it has been associated with its contents of the pH-buffering inorganic phosphate, decreasing the counts of lactobacilli in the oral cavity [5].

Some types of cheese have been asserted to display anticariogenic properties [49], and this is due to its contents of fermented bacteria that produce higher amount of vitamin K2 [30, 50]. This favorable feature would serve as a source of systemically located vitamin K2, rather than

locally delivered. However, there is also a possibility that vitamin K2 is absorbed across the mucous membranes of the oral cavity. Quite successfully, one has applied ubiquinone topically to subdue periodontal inflammation induced by controlled oxidative stress [51].

Vitamin K2's effect on the tooth's outer surface can be seen via its impact on saliva component distribution. This secondary prevention, i.e., the "remineralization success" is mainly relying on whether the saliva composition is altered in order to produce a so-called facilitating "remineralization microenvironment."

2.6. Interpretation of "historical" data collected by Price

The famous set of data, collected by Price, when visiting groups of primitive cultures from different parts of the world, should be well known to vitamin K2 enthusiasts. Some groups visited were still primitive as to their alimentation culture and customs, while other groups and subgroups had adapted to modern civilized diets and ways of living.

Price subsequently analyzed food samples from these groups. And, with little deviation, he registered that the "primitive diets" were high in vitamins A and D, along with factor named "Activator X". This particular ingredient could be retrieved from butter by grass fed animals [5]. He launched the idea that it was a fat soluble nutrient that has now been identified as or linked to menaquinones, vitamin K2 [6].

3. Evaluation of "the" hypothesis

3.1. The support of a systemic theory of dental caries and K2 as a critical component

The "old" theory of dental health, called the 'acid theory,' has been linked to the oral environment as an isolated and unique process, involving the bacteria-acid axis, as it is the only cause-effect relationship.

The systemic version or theory of dental caries acknowledges the effect of refined carbohydrates on oral cavity through the impact of the hypothalamus and the endocrine system. Earlier, free radicals like ROS, typically having been construed as "exhaust energy" from mitochondria. *However, they are now thought of as "critical signals," conveyed to the hypothalamus in order to influence the secretion of hormones, like parotid hormone and insulin. The major task to be undertaken now is: find which nutrients optimally affect the hypothalamus and which would maintain the centrifugal fluid flow through the teeth*. Antioxidants such as EGCG of green tea have proven effective. Vitamin K2, however, may serve as a more potent nutrient.

3.2. What we hope to learn from Dr. Price's discovery

The exocrine functions of the salivary glands, i.e., the composition of the saliva, are nutritionally related. As for the prevention of dental caries, optimum nutrition with fat soluble vitamins like K2 may serve as much more significant factor, than the role of traditional dental recommendations which goes like this: *Eat less sugar to minimize the production of bacterial acids*

in the oral cavity. Dental disease will be construed and recognized as an inflammation related degenerative lifestyle disease in line with cardiovascular incidents, as well as with bone brittleness (osteoporosis) and diabetes mellitus.

4. Some recent articles "establishing" the vitamin K2 effect on teeth

It has been reported that vitamin D3 acts synergistically with vitamin K2 to prevent bone loss. A recent study evaluated the impact of vitamin K2 and vitamin D3, as an alimentary supplement in conjunction with scaling and root planning; SRP, as conventional periodontal, on gingival expression of the interleukins IL-1β and IL-10, serum bone-specific alkaline phosphatase (B-ALP), as well as tartrate-resistant acid phosphatase (TRAP, subtype 5b), and the steady state levels of alveolar bone calcium in rats subjected to experimentally provoked periodontitis. Alveolar bone mass in the periodontitis group was markedly larger than the ones in the other experimental groups. No significant differences were seen in the gingival contents of IL-1β and IL-10, blood B-ALP, TRAP-5b, and calcium, nor in alveolar bone mass between the groups receiving SRP and vitamins, and the experimental group receiving SRP alone. Furthermore, vitamin D3 and K2 alone, or combined, failed to affect gingival levels ofIL-1β and IL-10, as well as blood B-ALP and TRAP-5b, or the alveolar bone mass, as compared with traditional periodontal treatment *per se* [52].

Mesenchymal stem cells have often been used for tissue engineering in regenerative medicine. The present application focused on the features of stem cells obtained from human exfoliated deciduous teeth (SHED) in comparison with dental pulp stem cells (DPSCs) and bone marrow-derived mesenchymal stem cells (BMMSCs). Cells retrieved from various sources displayed MSC characteristics (i.e., fibroblastic morphology and MSC markers). Their growth rate markedly elevated, as compared with that of DPSCs and BMMSCs. Furthermore, it was demonstrated that some 4400 genes altered their expression by a factor of 2.0 or more. A higher gene expression in SHED was witnessed for genes participating in reaction pathways such as "cell proliferation" and "extracellular matrix" [53].

Enhancement of intracellular Ca^{2+} concentrations is a feature commonly seen during the differentiation period of stem cells. The transient receptor potential melastatin 4 (TRPM4) serves as one of many ion channels controlling the Ca^{2+} signals in both excitable and nonexcitable cells. Nelson et al. [54] characterized TRPM4 in dental follicle stem cells (DFSCs) of the rat, and defined its impact on Ca^{2+} mediated signaling in the differentiation process. ShRNA-mediated suppression of TRPM4 decreased the channel activity, resulting in cell proliferation during osteogenesis, with a concomitantly augmented mineralization. Whole genome microarray analysis revealed that a plethora of genes, being associated with both vitamin K2 and SXR = PXR = NR1/2, were affected by TRPM4 during DFSC differentiation. These observations indicate that TRPM4 inhibit osteogenesis. The information provided suggests a link between the Ca^{2+} signaling pattern and gene expression during the differentiation process, including a recognizable influence of vitamin K2.

Wnt signaling pathways are now heavily linked to bone biology [55]. In the present review, recent advances in how Wnt/Lrp5-mediated signaling modulates osteoblast and osteocyte functioning, introduce new players in the Wnt signaling pathways, proving to play important

roles in bone development. Here, emerging areas of Wnt signaling in osteoclastogenesis are discussed, as well as the progress made in translating basic studies to clinical therapeutics and diagnostics centered around inhibiting Wnt pathway antagonists. These are sclerostin, Dkk1, and Sfrp1. In a recent study, (unpublished data) Osmundsen and coworkers have shown that vitamin K2 affects the Wnt system by modulating the expression of DKK1 (a Wnt inhibitor) during the development of teeth in developing molar teeth of the mouse.

Another report aims to reveal the biological and physicochemical features of MTA = mineral trioxide aggregate, related to its potency in eliciting reparative dentinogenesis. In comparison with calcium hydroxide-based materials, MTA is more efficient. It has been asserted that the action of MTA is associated with natural wound healing processes of exposed pulps, even though MTA may also stimulate matrix formation and its mineralization *in vitro*. Physicochemical analyses have shown that MTA may also interact with phosphate-containing fluids to precipitate apatite crystals. Furthermore, MTA shows better sealing ability and maintains structural stability, however [56].

Congenital diseases of tooth roots (e.g., developmental abnormalities of short and thin roots) may lead to tooth loss [57]. Recently, studies have shown that Osterix (Osx), serving as an important transcriptional factor, along with Runx1, Runx2, SP1, and SP3 [58], all participating in osteogenesis and odontogenesis, is thought to play a vital role underlying the mechanisms that determine the developmental differences between the root and the crown. During tooth development, Osx, particularly in odontoblasts and cementoblasts, promote and sustain their differentiation as well as and mineralization. Additionally, site-specific roles of Osx in the formation of tooth root have been established. Hence, Osx is construed as a promoter of odontoblast and cementoblast differentiation, as well as a factor determining root elongation. Research featuring mechanistic properties of teeth delineates a regulatory network involving Osx expression which is controllable via either BMP-signaling or Runx2-expression, pointing to a feasible way of promoting/sustaining Osx expression experimentally [59].

Calcium hydroxide $Ca(OH)_2$ has been extensively used in the dentistry; however, its mechanism of action remains unclear. Unraveling its modes of action will provide a broader understanding of the mechanisms associated with the induced dentinogenesis, as well as helping to optimize currently available treatment modalities to ensure specific regenerative processes of tooth preservation. A compilation of articles on "mechanisms of dentinogenesis involving calcium hydroxide" is featured in this paper, and recommendations related to dentinogenic mechanisms of $Ca(OH)_2$ range from direct irritating action by the material to induction of release of biologically active molecules, like fibronectin, BMPs (bone morphogenic proteins) like BMP-4 and BMP-7, TGFs (transforming growth factors) like TGFβ, IGFs (insulin-like growth factors), antiinflammatory interleukins, alkaline phosphatase (ALP), and others [60]. It is well known that a plethora of these factors are encoded by genes that are sensitive to the impact of vitamin K2 (via the transcription factor SXR = PXR = NR1/2), as well as the vitamin A (RXR) and vitamin D (VDR) receptors [61].

The extracellular matrix (ECM) provides physical support for various tissues. However, it also contributes to the development of same, their homeostasis, and prevention of disease. More than some 200–300 ECM molecules are listed as comprising the "core matrisome" in

mammals, based on analyses of whole genome sequences, and during the course of tooth development and growth, the structure-function relationship of the ECM is altered dynamically. In early phases, the basement membranes (BMs) separate into two cell layers of the dental epithelium and the mesenchyme. These matrix proteins are instrumental in cell functions like adhesion, polarity, as well as differentiation and mineralization of the enamel and dentin matrices [62, 63].

Interestingly, several of the genes known to be important in tooth development, referred to in the present paragraphs, can be retrieved as SXR and/or vitamin K2-sensitive, or as shown by Osmundsen and coworkers (personal communication), and tabulated underneath in: "Summary of information obtained from the microarray analyses."

5. Effects of mandibular injection of MK-7 on gene expression in the developing molar tooth

5.1. Methods

As an initial experiment, new born (at P1) Balb C mice were given 10 μl intra-mandibular injections of MK-7 (0.2, 2, and 10 mg/kg body-wt., dissolved in corn oil). The control mice were injected with vehicle only.

At 24 hours postinjection, the pups were killed and first right-hand side molar tooth germs were removed and transferred into RNA-Later solution.

Total RNAs were isolated from individual tooth germs and used for analysis of gene expression using deoxyoligonucleotides microarrays and real-time RT-PCR using the RNeasy Mini Kit. The quality of isolated RNA was monitored using the Agilent Bioanalyzer. RNA was isolated from three separate batches of tooth germs (three tooth germs per batch).

6. Results

Results from all dosages used suggested that numerous genes exhibited significantly altered expression. At this stage, results from pups given 2mg/kg have been more extensively analyzed.

These data results suggested that 281 genes were differentially expressed ($p < 0.05$), with changes in expression ranging from about 5- (Minpp1, Pdzd2) to about 0.05-fold (Zfp485, Slc2a5). Bioinformatics analysis (using Ingenuity Pathways Analysis) suggested that a major fraction of the observed changes in gene expression was associated with "cell death," the data suggesting a highly significant association to decreased apoptosis. This is likely mediated via altered expression of genes associated with regulation of metabolic substrates being converted to polyamine, retinoic acid-dependent regulation of apoptosis regulation (three genes), and altered osteoclast and osteoblast signaling (six genes). Several genes involved in synthesis of complex carbohydrates e.g., proteoglycans, heparin sulphate of chondroitin sulphate b, were upregulated.

Interestingly, also some genes related to enamel/dentine biosynthesis exhibited differential expression (e.g., Amelx, Ambn but not Enam). Microarray results were validated by real-time RT-PCR. Transcription factor analysis (using Ingenuity Pathways Analysis) suggested significant associations to the increased transcriptional activity of Myc (measured as changes in expression of Hspd1, Ctnnb1, Dkk1, and Psmb8 being in line with the predicted change). The downregulation of Dkk1 is interesting as denosumab treatment results in reduced Dkk1 level. Further, high Dkk1 levels have been associated with increased bone loss.

The data also suggested highly significant associations to decreased apoptosis. This is likely mediated via altered significant associations to polyamine regulation (three genes), altered retinoic, acid apoptotic regulation (three genes), and altered osteoclast and osteoblast signaling (six genes).

7. Conclusions

The results are based on the measurements from independent biological triplicates and therefore suggestive of effects of MK-7 on gene expression during the tooth development. A clear effect on gene expression was apparent also at a dosage of 0.2 mg/kg body weight. The results indicate increased transcription of genes involved in development of bone (increased biosynthesis of important carbohydrates) and of enamel/dentin.

Further investigations are, however, required to elucidate these findings. Such experiments will likely entail the establishment of a clear dose-response relationship as well as of a time-course of action. Also, effects of oral administration should be studied.

Summary of information obtained from the microarray analyses

Gene name	Description of function: *General* and bone-related	References
Lmcd1	*Transcriptional cofactor restricting GATA6 and GATA4 functioning by inhibiting DNA-binding*: Gata6 and Gata4 are transcription factors shown to be involved in TGFβ- and estrogen-mediated regulation of gene expression in osteoblasts, and thus bone mineralization to hamper the development of brittleness.	*Int J Biochem Cell Biol. 2013 Mar;45(3):696–705.* *J Bone Miner Res. 2014 Dec;29(12):2676-87.* *J Cell Physiol. 2013 Jul;228(7):1594–600.*
Dmxl2	*Protein involved in many functions including participation in signal transduction pathways, such as Notch signaling:* NOTCH signaling in BMSCs (bone marrow stromal/stem cells) is required for fracture repair performed by mature osteoblastic cells.	*J Biol Chem. 2010 Nov 5;285(45):34757–64.* *J Clin Invest. 2016 Mar 7. pii: 80672.*
Abcb4	*The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. The gene is heavily involved in a plethora of liver functions:* In experimental animals, i.e., Abcb4 (-/-) mice with hepatic osteodystrophy, serum RANKL and TGFβ-levels were augmented, resulting in an excess bone resorption rate, probably due to a dysregulation of genes like osteoprotegerin, osteocalcin, and osteopontin, as well as vitamin D metabolism.	*Bone. 2013 Aug;55(2):501–11. doi: 10.1016/j.bone.2013.03.012.*

Gene name	Description of function: *General* and bone-related	References
Slc12a6	*This gene is a member of the K-Cl cotransporter (KCC) family. K-Cl cotransporters are integral membrane proteins that lower intracellular chloride concentrations below the electrochemical equilibrium potential:* Human osteoblasts express functional K-Cl cotransporters in their cell membrane that seems to be able to induce activation of volume-sensitive channels by KCl, necessary for normal osteoblast membrane currents, and thus secretory functions.	*Am J Physiol Cell Physiol. 2003 Jul;285(1):C22–30.*
Mta1	*Transcriptional coregulator that can act as both a transcriptional corepressor and coactivator, and stimulates the expression of WNT1 by inhibiting the expression of its transcriptional corepressor, SIX3:* Hypoxia-induced MTA1 (via HIF-1α) stimulates growth (and inhibits differentiation) of osteoblastic (MC3T3) cells which is deemed important in the process of fracture healing.	*Eur J Med Res. 2015 Feb 3;20:10.*
Pou3f3	*This gene encodes a POU-domain containing protein that functions as a transcription factor. The encoded protein recognizes an octamer sequence in the DNA of target genes. This protein may play a role in development of the nervous system:* However, it was recently shown that this gene normally upregulates the genes of the Dlx-familiy (Dlx1, 2, 5, 6), as well as downstream genes like Gbx2, is involved in the patterning of the mammalian jaw.	*Development. 2008 Sep;135(17):2905–16.*
Dio2	*This gene belongs to the iodothyronine deiodinase family. It activates thyroid hormone by converting the prohormone thyroxine (T4) by deiodination to bioactive 3,3′,5-triiodothyronine (T3). It is highly expressed in the thyroid, but is known to be expressed many other peripheral tissues:* Cold exposure (in Misty mice) compensates for BAT (brown adipose tissue) dysfunction by increasing the expression of Acadl, Pgc1a, Dio2, and other thermogenic genes, by altering the expression of osseous Runx2 and Rankl. Genes upregulated by BMP-7 showed a strong enrichment for established osteogenic marker genes, and several others (MFI2, HAS3, ADAMTS9, HEY1, DIO2 and FGFR3) in osteoblasts. Furthermore, for DIO2 seems to impact osteoblastic differentiation. Outer ring deiodination (ORD) activity was seen in bone extracts of whole skeleton, bone marrow, and MC3T3-E1 osteoblasts. [1,25(OH)2VD]-treatment induced D2 activity, while estradiol, PTH, forskolin, leptin, TNFα, TGFβ, and dexamethasone did not.	*J Bone Miner Res. 2013 Sep;28(9):1885-97. doi: 10.1002/jbmr.1943.* *Bone. 2009 Jul;45(1):27–41. doi: 10.1016/j. bone.2009.03.656. Epub 2009 Mar 21.* *Endocrinology. 2005 Jan;146(1):195–200. Epub 2004 Oct 7*
Camk4	*Camk4 is a member of he serine/threonine protein kinase (PK) family, and the Ca^{2+}-calmodulin-dependent protein kinase subfamily. It serves as a multifunctional serine-threonine protein kinase, and has been implicated in transcriptional control of lymphocytes, neurons, as well as male germ cells:* Silencing of CaMK1β obliterates the proliferation ability of osteoblasts, as well as expression of c-Fos. However, this does not influence the skeleton markersRunx2, Osterix, and/or Osteocalcin. CaMKs activate pathways mediated by CREB and NFATc1. Inhibition of CaMKs obliterates CREB phosphorylation, lowering c-Fos, and NFATc1 expression, and thus osteoclastogenesis activated by NF-κB ligand (RANKL). Finally, BMP-receptor signaling in stem cells from human exfoliated deciduous (SHED) teeth enhances the expression of genes like BMP-4, Runx2, as well as DSPP.	*Bone. 2008 Oct;43(4):700–7.* *Nat Med. 2006 Dec;12(12):1410–6. Epub 2006 Nov 26* *J Endod. 2011 Dec;37(12):1647–52. doi: 10.1016/j. joen.2011.08.023. Epub 2011 Oct 6.*

Gene name	Description of function: *General* and bone-related	References
Ppp2r2b	*This gene encodes the family of phosphatase 2 regulatory B subunits. Protein phosphatase 2 functions as one of four main Ser/Thr phosphatases, and is involved in the inhibitory control system of cellular division:* Loss of estrogen during menopause causes changes in the female body. Hence, it is expected that HRT-associated gene expression is due to the changes in the DNA methylation profile (DMP). Of the DMP genes, ACBA1, CCL5, FASLG, PPP2R2B, and UHRF1 were differentially expressed, all of which are associated with HRT or estrogenic regulation. All genes were also associated with bone mineral content (BMC), while ABCA1, FASLG, and UHRF1 were also associated with body adiposity.	*Bahl A1, Pöllänen E, Ismail K, Sipilä S, Mikkola TM, Berglund E, Lindqvist CM, Syvänen AC, Rantanen T, Kaprio J, Kovanen V, Ollikainen M.*
Stard4	*Cholesterol homeostasis is regulated by sterol regulatory element (SRE)-binding proteins (SREBPs) and by liver X receptors (LXRAs). When sterols are depleted, LXRs are inactive and SREBPs bind promoter SREs and activate genes involved in cholesterol turnover. The protein STAR is involved in this process, and it is homologous to a family of proteins STARD4:* Estrogen-containing hormone replacement therapy (HRT) leads to a relief of typical menopausal symptoms, benefits bone and muscle health, and is associated with tissue-specific gene expression profiles. Hence, It is plausible that part of the HRT-associated gene expression is due to changes in the DNA methylation profile. The gene expression patterns of white blood cells (WBCs) and their associations with body composition, including muscle and bone measures of monozygotic (MZ) female twin pairs discordant for HRT were assessed. Of genes with differentially methylated regions (DMRs), five (ACBA1, CCL5, FASLG, PPP2R2B, and UHRF1) were also differentially expressed. These have been associated with HRT or estrogenic regulation, but were also associated with bone mineral content (BMC). Additionally, ABCA1, FASLG, and UHRF1 were also related to the body adiposity.	*Bahl A1, Pöllänen E2, Ismail K1, Sipilä S2, Mikkola TM2, Berglund E3, Lindqvist CM3, Syvänen AC3, Rantanen T2, Kaprio J1, Kovanen V2, Ollikainen M1.*
Tyk2	*This gene encodes the Janus protein kinases (JAKs). These proteins associate with and activate cytokine receptors with ensuing phosphorylation (activation) of receptor subunits. It is also a component of the interferon signaling pathways. It may therefore play a role in anti-viral immunity:* Interleukin-23 (IL-23) belonging to the IL-6/IL-12 family that plays a key role in autoimmune and inflammatory disorders. IL-23 binding to dendritic cells, macrophages and monocytes triggers the activation of Jak2 and Tyk2 which in turn phosphorylates STAT1, STAT3, STAT4, and STAT5 as well as induce formation of STAT3-STAT4 heterodimers. IL-23 is essential for the survival and/or expansion of inflammatory Th17 cells which, when activated by IL-23, sustain osteoclasto-genesis via the production of IL-17 (stimulator of the NF-kappa B) of mesenchymal cells. As a group, the IL-17 - IL-23 "axis" includes Th17 cells that play a major role in the development and maintenance of autoimmune arthritic inflammation.	*Scand J Immunol. 2010 Mar;71(3):134-45. doi: 10.1111/j.1365–3083.2009.02361.x.*
Pdcd5	*This gene encodes a protein that is upregulated during apoptosis. The encoded protein is a regulator of K(lysine) acetyltransferase-5, involved in transcription, DNA damage response and the cell cycle control, by blocking its degradation:* The programmed cell death gene (PDCD5) was overexpressed in an osteosarcoma (OS) cell line, MG-63. The results indicate that PDCD5 can induce apoptosis and G(2) phase arrest in MG-63 cells. Furthermore PDCD5 expression in established xenografted tumors was associated with a decrease in tumor size and weight. Furthermore, it was found that the Ras/Raf/MEK/ERK signaling pathway was hampered, leading to the inhibition of cyclin B and CDK1, and to the activation of caspases 3 and 9, respectively. These results are consistent with the G(2) phase arrest observed.	*Cell Signal. 2012 Aug;24(8):1713-21. doi: 10.1016/j.cellsig.2012.04.011. Epub 2012 Apr 25.*

Gene name	Description of function: *General* and bone-related	References
Psenen	*Presenilins are required for intramembranous processing of transmembrane proteins, such as the Notch proteins. Signaling by Notch receptors mediates a wide range of developmental cell fates.* *Titanium implant surfaces with modified topographies improve osteogenic properties in vivo. The activation of signaling stem cell pathways (such as TGFβ/BMP, Wnt, FGF, Hedgehog, and Notch) was characterized subsequent to incubations (24 and 72 h) with BCs to SLA and modSLA surfaces in the absence of osteogenic cell culture supplements.* *Key regulatory genes belonging to the TGFβ/BMP (TGFBRs, BMPRs, ACVRs, SMADs, Wnts, FZD1, FZDs, LRP5, NFATCs, PYGO2, LEF1) and Notch species (including PSENEN) pathways were upregulated on the modified surfaces. These data correlate with an increased expression of osteogenic markers (e.g. BSP and osteocalcin, as well as BMP2 and BMP6.* *These finding indicate that activation of proosteogenic cell signaling pathways by modSLA and SLA surfaces leads to enhanced osteogenic*	*Clin Oral Implants Res. 2014 Apr;25(4):475–86. doi: 10.1111/clr.12178. Epub 2013 Apr 21.*
Rhob	*RHOB (Ras Homolog Family Member B) is a Protein Coding gene. Diseases associated with RHOB include oculo auricular syndrome and sertoli cell-only syndrome. Among its related pathways are Signaling by GPCR and Developmental Biology. GO annotations related to this gene include GTP binding and GDP binding. An important paralog of this gene is RHOA.* *Defects (mild affection to complete destruction) in the sealing zone were observed in the OPG-deficient animals. Resorption lacunae were not detected, indicating the loss of osteoclast-mediated bone resorption activity. Treatment with OPG resulted in a significant decrease in the expression of a cluster of instrumental genes (like for instance) Rho guanine nucleotide exchange factors (RhoGEFs), RhoGTPases, ROCK1 and ROCK2. This resulted in damage to or destruction of the sealing zone, thus inhibiting osteoclast-mediated bone resorption.*	*Int J Mol Med. 2014 Sep;34(3):856–62. doi: 10.3892/ijmm.2014.1846. Epub 2014 Jul 10.*
Rs1	*The present gene encodes an extracellular protein serving an important organizational role in the retina. The protein encoded is assembled and secreted as a homooligomeric complex. Mutations in the present gene are lead to X-linked retinoschisis with ensuing severe loss in vision.* *G-protein-coupled receptors (GPCRs) are key regulators of skeletal homeostasis and important in fracture healing. It was earlier shown that blockade of G(i) signaling in maturing osteoblasts enhanced cortical and trabecular bone formation and prevented age-related bone loss in female mice. Furthermore, activation of G(s) signaling induced massive trabecular bone formation, but a concomitant cortical bone loss. Here, "labile" tibial fractures, where endogenous G(i) signaling are blocked by PTX, or G(s) signaling activated by Rs1, were achieved.* *Inhibition of endogenous G(i) activity gave a smaller callus, but enhanced net bone formation in both mice, irrespective of age.* **PTX treatment lowered the expression of Dkk1 and upregulated Lef1 mRNA upon fracture healing, indicating endogenous G(i) signaling in maintaining Dkk1 expression, while suppressing Wnt signaling. On the contrary, mice with activated Gs signaling demonstrated an increase in the initial callus size with enhanced callus bone production**. *These results indicate that G(i) blockade and G(s) activation are important for proper fracture healing.* *It was previously asserted that Rs1 constitutively activated Gs-coupled GPCR, under the control of the 2.3 kb Col I promoter, enhancing the steady state level mineral mass in trabecular bone of femurs. In this article, it was further concluded that Gs-signaling in OBs on enhanced intramembranous bone formation in calvariae of Col1(2.3)/Rs1 mice. Rs1 calvariae displayed a dramatic increase in bone volume with partial loss of cortical structure. Gene expression analysis of calvarial OBs showed that genes were affected by Rs1 signaling, featuring processes like: (a) differentiation, (b) synthesis of cytokine/growth factors, (c) angiogenesis, (d) coagulation, as well as (e) energy metabolism.*	*J Bone Miner Res. 2015 Oct;30(10):1896–904. doi: 10.1002/jbmr.2540. Epub 2015 May 14* *Exp Cell Res. 2015 May 1;333(2):289–302. doi: 10.1016/j.yexcr.2015.02.009. Epub 2015 Feb 20.*

Gene name	Description of function: *General* and bone-related	References
Trpm5	*This gene encodes a member of the transient receptor potential (TRP) protein family. This protein plays a role in taste transduction. It is activated by lower concentrations of intracellular Ca^{2+}, and inhibited by higher concentrations.* *Elevation of intracellular Ca^{2+} is commonly observed during stem cell differentiation (e.g., osteoblastogenesis), but cease after process completion. These findings suggest an inhibitory role for TRPM4 (Ca^{2+} ion channel) on osteogenesis while it appears to be required for adipogenesis. The data provide a link between the Ca^{2+} signaling pattern and gene expression during stem cell differentiation.*	*Stem Cells. 2013 Jan;31(1):167–77. doi: 10.1002/stem.1264.*
Amelx	*This gene encodes a member of the amelogenin family of extracellular matrix proteins. Amelogenins are involved in biomineralization during tooth enamel development.* *Research on enamel matrix proteins (EMPs) is centered on the understanding of their role in enamel biomineralization, as well as of their bioactivity for tissue engineering. It was shown that mRNA expression of AMELX and AMBN in mandibular alveolar and basal bones RNA-positive for AMELX. Furthermore, AMELX and AMBN mRNA levels varied according to: 1) ontogenic stage, and 2) tissue-type. In conclusion, it was asserted AMELX and AMBN may function as growth factor-like molecules in jaws, where they might play a role in bone physiology via autocrine/paracrine pathways, and especially during adaptation of stress-induced remodeling. Thymosin beta 4 is associated with RUNX2 expression through the Smad and Akt signaling pathways in mouse dental epithelial cells.* *Thymosin β4 (Tβ4) is associated with the initiation and development of the tooth germ, via enhancement of RUNX2. The transcription factor regulates the expression of genes involved in odontogenesis, like amelogenin, X-linked (Amelx), ameloblastin (Ambn), as well as enamelin (Enam).* *It appeared that the mDE6 mouse epithelial cell line expressed Runx2, Amelx, Ambn and Enam, and yielded calcified matrices upon the induction of calcification.*	*PLoS One. 2014 Jun 16;9(6):e99626. doi: 10.1371/journal.pone.0099626. eCollection 2014. Int J Mol Med. 2015 May;35(5):1169–78. doi: 10.3892/ijmm.2015.2118. Epub 2015 Mar 2.*
DKK1	*The present gene encodes a member of the dickkopf protein family. It is secreted, including two cysteine rich regions, and it parttakes in embrygenesis due to its inhibition of Wnt-ediated signaling. Enhanced DKK1 levels in bone marrow and blood correlates with bone osteolysis in patients suffering from multiple myeloma.* *In this article, the authors review advances and discrepancies in how Wnt/Lrp5 signaling regulates osteoblasts and osteocytes, and describe new players in Wnt signaling pathways exerting important roles in bone development, i.e., Wnt signaling in osteoclastogenesis, inhibition of Wnt pathway antagonists, such as sclerostin, Dkk 1, and Sfrp1.*	*Gene. 2012 Jan 15;492(1):1–18. doi: 10.1016/j.gene.2011.10.044. Epub 2011 Nov 3. Monroe DG1, McGee-Lawrence ME, Oursler MJ, Westendorf JJ.*

8. Emulation of the interaction between genes and microRNA species known to be instrumental in the development of the osteoblastic/odontoblastic phenotype by Mir@nt@n

The bioinformatics program Mir@nt@n, developed by Le Bechek et al. [64], was used to arrive at high stringency interactions between microRNA species known to be instrumental in the development and stability of osteoblastic and odontoblastic cells from stem cells. The dental = osteoblastic/dentinoblastic genes (being significantly regulated by Vitamin K2 in the present study) described in the present array were fed into the program along with other genes and microRNA-species known to be instrumental in the development and stability (both positively and negatively) of the osteoblastic and/or odontoblastic phenotype:

Lmcd1, Dmxl2, Abcb4, Slc12a6, Mta1, Pou3f3, Dio2, Camk4, Ppp2r2b, Stard4, Tyk2, Pdcd5, Psenen, Rhob, Rs1, Trpm5, Amelx, DKK1, SP1, SP3, SP7, Runx2, Runx1, NR1/2, ADRB3, Foxc2, PGC1α, PPARA, PPARG, Dio2, UCP1, Adipoq, LEP, BETA3AR/ADRB3R/B3AR, hsa-mir-155, and c/EBPB.

Hsa-mir-196a, hsa-mir-16, hsa-mir-455, hsa-mir-339, hsa-mir-125b, hsa-mir-328, hsa-mir-16, hsa-mir-149, hsa-mir-125b, hsa-mir-760, hsa-mir-133, hsa-mir-29, hsa-mir-27, hsa-mir-23, hsa-mir-320, hsa-mir-26b, hsa-mir-21, hsa-mir-302, hsa-mir-132, and hsa-mir-223.

9. Major findings

The genes, significantly modulated (directly or indirectly) by vitamin K2, are presented in **Table 1**.

Of major interest here, from a regulatory point of view, and as a minimal "cluster" of necessary and sufficient genes, are probably the following species: RUNX1, RUNX2, SP1, SP3, and DIO2, along with the microRNA-species 149, 328, 339, and 760 (see **Figure 1**). It is well known that the osteoblast and odontoblast phenotypes are "determined" and "stabilized" by the RUNX- and SP-families of transcription factors (upregulated), as well as the

Dental genes affected (directly or indirectly) by Vitamin K2

Lmcd1	=	LIM and cysteine rich domains 1
Dmxl2	=	Dmx2 like protein
Abcb4	=	ATP-binding cassette subfamily B member 4
Slc12a6	=	Solute carrier family 12 member 6
Mta1	=	Metastasis associated 1
Pou3f3	=	POU doman, calss 3 transcription factor
Dio2	=	Deiodinase 2
Camk4	=	Calcium/calmodulin-dependent protein kinase type I
Ppp2r2b	=	Serine/threonine-protein phosphatase 2A, subunit B
Stard4	=	StAR-related lipid transfer protein 4
Tyk2	=	Tyk2 tyrosine kinase 2
Pdcd5	=	Programmed celle death 5 (acetyltransferase 5)
Psenen	=	Presenilin - part of a secretase complex
Rhob	=	Ras homolog Family Member B
Rs1	=	Retinochisin 1
Trpm5	=	Transient receptor potential cation channel subfamily M member 5
Amelx	=	Amelogenin, important for tooth enamel development
DKK1	=	DKK1 dickkopf WNT signaling pathway inhibitor 1

For relations to tooth (enamel and dentine) development, see tabulation of specific gene/protein effect related to bone homeostasis

Table 1. "Dental" genes affected directly or indirectly by exposure to vitamin K2 (MK-7).

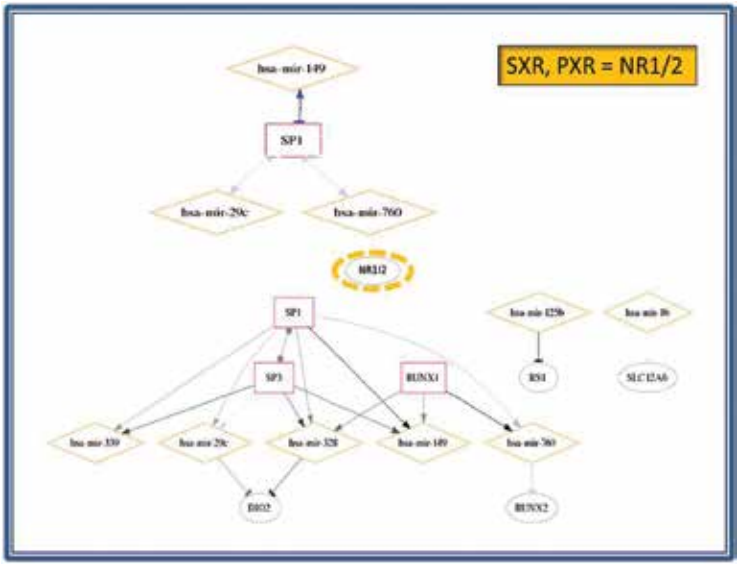


Figure 1. Interactions between transcription factors, "functional" genes, and microRNA species as emulated in the bioinformatics program Mir@nt@n.

microRNA-species 149, 328, and 339 (downregulated). Recently, it was shown [58] that mir-760 is involved in the effect of vitamin K2, since it associates with the transcription factor NR1/2 = SXR = PXR [65].

Using high/maximal stringency emulations rendered by the bioinformatics program Mir@nt@n [64], it was quite interesting to find that the gene DIO2 (deiodinase2) that encodes the enzyme transforming T4 to T3 in peripheral tissues, was associated with has-mir-760, also found to exert an impact on the levels of Runx2, as well as being involved in the steady state of SP1 and SP3, transcription factors upstream of the Runx species deemed to be markers of the osteoblast/odontoblast phenotype (see **Figure 2**). It therefore does not come as a surprise that bone tissue is heavily dependent on DIO2 activation to function properly, i.e., replenishing "lost" osteoblasts from precursor cell, as well as proper functioning of differentiated osteoblasts/dentinoblasts to maintain bone/dentine mass at a stable level [66]. It may though come as a surprise to many that, in fact, vitamin K2 serves a rather prominent role in this process.

Finally, when applying low-stringency criteria to the Mir@nt@n-emulation process, a larger and less rigid network of mutual interactions was obtained (see **Figure 3**). From the interactions predicted, one may hypothesize the following: It is not trivial to ingest a dose that is too small to see the broad spectrum of beneficial effects of vitamin K2 on osteoblasts/odontoblasts. Furthermore, the dose should be titrated to ensure proper levels and characteristics and amounts of bodily beige versus white adipocytes (confer the postulated

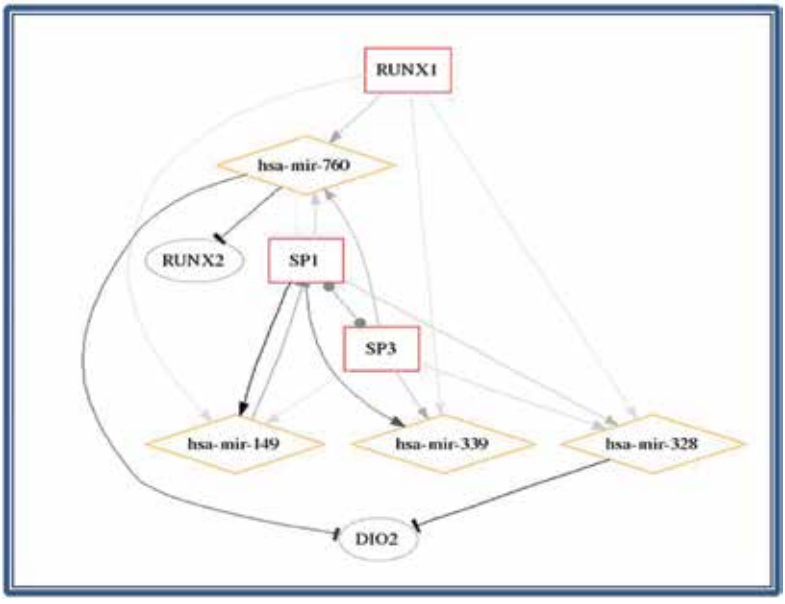


Figure 2. Interactions (high/maximal stringency emulation) of transcription factors, microRNA species, and differentiation / function-related genes in tooth germs from the rat.

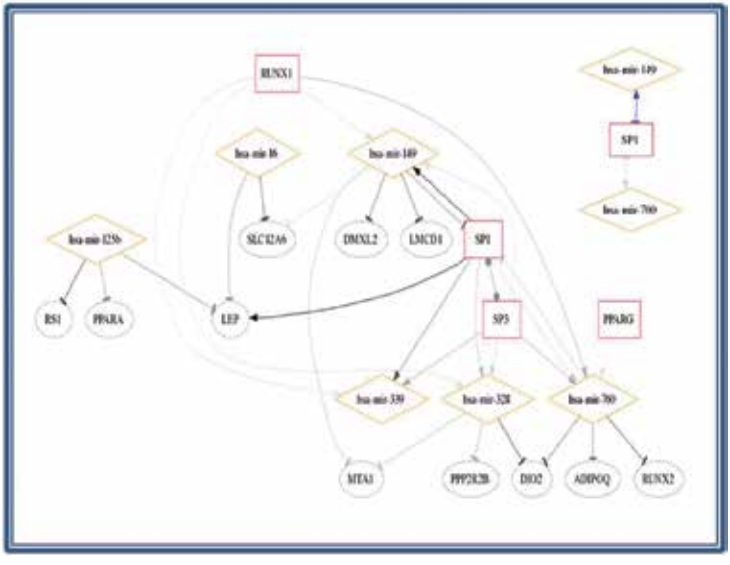


Figure 3. Extended interaction scheme (low stringency emulation) of transcription factors, microRNA species, and differentiation-related genes in tooth germs from the rat.

impact of vitamin K on mir-760 on SP1 and mir-149 with reciprocal regulatory loops), and the mir-760 "junction" between RUNX1, SP1, SP3, and PPARG versus DIO2, ADIPOQ, and RUNX2, which are all part of a mutually interacting network regulated by vitamin K2 odontoblasts/osteoblasts.

Finally, it should be emphasized that vitamin K2 (MK-7) upregulates Amelx and DKK1 in tooth germs, the former is instrumental in the building and maintenance of tooth enamel, and thus their resilience toward enamel erosion; the latter, DKK1 (dickopf1 WNT signaling pathway inhibitor 1), takes part in the modulation of osteoclast-related bone degradation, and in this context, the healthy transition between osteoclast-induced resorption and renewal of bone tissue with microcracks [67].

10. Pertinent question: is the dental filling material toxic to the living tooth? Contemplations on the making of live and artificial teeth

Monomers from methacrylate based dental materials both prior to and post polymerization have demonstrated adverse effects both *in vitro* and *in vivo* in terms of cytotoxicity [68], mutagenicity/genotoxicity [69–72], negative effects on fertility [73], xenoestrogenicity [74–76], and allergy induction [77]. The degree of cytoxicity will vary from the type assay used, materials tested, time intervals for testing, and cell types tested [78].

It is most pertinent to perform *in vitro* cytotoxicity testing on cells from cell types and tissues relevant to the area of *in vivo* placement of dental materials [79]. Recent studies on elution of monomers available in both dental composites and methacrylate- and epoxy-based root canal sealers looked at reactions in submandibular salivary gland acinar cells for the evaluation of cytotoxicity, cell proliferation, and apoptosis [69, 80]. The findings in such studies are of great interest and importance, but the authors of one of these studies [80] stated that their model would have been more realistic had they utilized human primary cells from direct target tissue. Tissues that often share the closest proximity to dental fillings are certainly not salivary glands but rather gingiva, mucosa, and, in particular, pulp tissues [79].

The pulp is a loose connective tissue within a nonresilient capsule of dentin and enamel. Pulpal inflammation is considered a protective mechanism and can either be of an acute or chronic nature. Acute and chronic responses are related to the "magnitude and duration of the insult [81]." Inflammation will inevitably cause vasodilation, increased vessel permeability which in turn will result in relatively large changes in tissue pressure [82]. Bacterial infection is the most common reason for pulpal inflammation, but any insult or stimuli will most probably result in a response. It is an established fact that many of the constituents in dental adhesive resin are cytotoxic [81], and the difference in cytoxicity varies among commercial materials commonly used by public dentists in Norway [unpublished results in a report to the Norwegian National Directorate of Health].

This project aims at elucidating the cellular effects of "leachables" (residual monomers) from dental filling materials exerted on dental pulp stem cells (DPSCs) *ex vivo*. It is important to

ensure that the cells used in the study are, indeed, stem cells. The International Society for Cellular Therapy has released a position statement wherein they list three criteria to define human stem cells: (1) adherence to plastic, (2) specific surface antigen expression, and (3) multipotent differentiation potential [83]. The cells to be used in this project fulfil all three criteria [84], and were isolated in accordance with a published procedure described by Sorrentino et al. [85].

11. Characterization of the DPSCs

Dental specimens were obtained from extraction after signed informed consent, and the pulp was exposed by cutting the tooth, while maintaining sterile conditions: The enamel of the tooth crown is partially cut, following the sagittal plane, applying a diamond bur. Thereafter, the cut is completed using a piezoelectric ultrasound scalpel to avoid overheating of the tissue. The pulp is then treated collagenase and dispase for 1 hour at 37°C, and then incubated in a bioSpherix chamber under normoxic conditions [85].

Phenotyping of the DPSCs yielded a CD-profile very much like the one seen for bone marrow mesenchymal stem cells (BM-MSCs) with an approximately identical percentage of cells expressing CD10 (CALLA), CD 13 (Aminopeptidase N), CD29 (β1-integrin), CD44 (H-CAM, Pgp-1), CD49acd (VLA-1,3,4 = α1,3,4-integrin), CD54 (I-CAM-1), CDw90 (THY-1), CD105 (Endoglin, TGFβ-R), CD140b (PDGF-Rb), CD146 (M-CAM), CD147 (Neurothelin/basigin), CD166 (Alcam, CD6-ligand), and also comparable amounts of GD2 (Neural ganglioside).

12. Tissue engineering using stem cells: can it be avoided?

It has been asserted that tissue engineering might be the future of endodontics [86]. It is stated in the abstract that pulpal regeneration after tooth injury is not easily accomplished, since the infected pulp is required for tooth extraction or root canal therapy. It is further asserted that an ideal form of therapy might consist of regenerative approaches where diseased or necrotic pulp tissues are removed and replaced with healthy pulp tissue to revitalize the affected tooth. The authors list different techniques, ranging from stem cell therapy, the use of growth factors, pulp implants, implant of 3D cell printed in hydrogels, injectable scaffolds, bioactive materials, the use of co-enzymes, and root canal revascularization. However, despite alleged advantages of the subject approaches, they also suffer major disadvantages like low cell survival, lack of de novo production of pulp, necrosis of reinfected pulp, and lack of vascularity, and requirement for precise root canal fitting.

By determining the cut point of toxicity (i.e., cell death/enhanced apoptosis and lack of proper differentiation induced by the leakage of monomers of endodontic filling materials), it is possible to develop new filling materials without an acute and long term detrimental effect on DPSCs. Hence, the development of a test battery to check the monomers that may diffuse into

the root canal, for cytotoxicity and ability to attain proper and functional cell phenotypes (i.e., odontoblasts, neural lattice, and endothelial cells constituting blood vessels) seems mandatory. The present project description aims to define such a test battery using highly sophisticated techniques like proteomics (including phosphoproteomics) and mass cytometry.

The advantage of using such techniques resides with the fact that extremely few cells may be used in complex arrays of incubation conditions, while still yielding reliable results. The technology described in the present project outline, may also enable the definition of a minimal and sufficient array of variables, which precisely describes a robust test battery to be implemented as a gold standard to be adopted in the development of endodontal biomaterial fillings in the future.

Author details

Jan Oxholm Gordeladze[1]*, Maria A. Landin[2], Gaute Floer Johnsen[3], Håvard Jostein Haugen[3] and Harald Osmundsen[2]

*Address all correspondence to: j.o.gordeladze@medisin.uio.no

1 Department of Molecular Medicine, Section for Biochemistry, Institute of Basic Medical Science, University of Oslo, Oslo, Norway

2 Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway

3 Institute of Clinical Odontology, Faculty of Odontology, University of Oslo, Norway

References

[1] Southward K. The systemic theory of dental caries. Gen Dent 2011:59(5):367–73.

[2] Sorsa T, Tjäderhane L, Salo T. Matrix metalloproteinases (MMPs) in oral diseasesOral Dis 2004;10(6):311–8.

[3] Tjäderhane L, Larjava H, Sorsa T, et al. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions.J Dent Res 1998;77(8):1622–9.

[4] Listgarten MA. Serum IgA and IgG antibodies to Treponema vincentii and Treponema denticola in adult periodontitis, juvenile periodontitis and periodontally healthy subjects. J Clin Periodontol 1986;13(5):418–30.

[5] Nutrition and Physical Degeneration, 8th ed. Weston Price DDS. Price-Pottenger Nutrition Foundation Inc.; pp. 362–369, 398.

[6] On the Trail of the Elusive X-Factor: A Sixty-Two-Year-Old Mystery Finally Solved http://www.westonaprice.org/fat-soluble-activators/x-factor-is-vitamin-k2: 2013-09-14.

[7] The Stress of Life. Hans Selye MD. New York: McGraw-Hill Book Co.; 1978. pp.3–54, 57–168, 259–264.

[8] Leonora J, Tieche JM, Steinman RR. Further evidence for a hypothalamus-parotid gland endocrine axis in the rat. Arch Oral Biol 1993;38(10):911–6.

[9] Kesim S, Unalan D, Esen C, Ozturk A. The relationship between periodontal disease severity and state-trait anxiety level. J Pak Med Assoc 2012;62(12):1304–8.

[10] Leonora J, Tieche JM, Steinman RR. Stimulation of intradentinal dye penetration by feeding in the rat. Arch Oral Biol 1993;38(9):763–7.

[11] Zhang Q, Szalay AA, Tieche JM, et al. Cloning and functional study of porcine parotid hormone, a novel proline-rich protein. J Biol Chem 2005;280(23):22233–44.

[12] Leloup C, Tourrel-Cuzin C, Magnan C, et al. Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. Diabetes 2009;58(3):673–81. http://dx.doi.org/10.2337/db07-1056.

[13] Steinman RR, Leonora J. Effect of selected dietary additives on the incidence of dental caries in the rat. J Dent Res 1975;54(3):570–7.

[14] Liu Y, Chen M, Yao X, et al. Enhancement in dentin collagen's biological stability after pro-anthocyanidins treatment in clinically relevant time periods. Dent Mater 2013;29(4):485–92. http://dx.doi.org/10.1016/ j.dental.2013.01.013.

[15] Liu Y, Dusevich V, Wang Y. Proanthocyanidins rapidly stabilize the demineralized dentin layer. J Dent Res 2013;92(8):746–52. http://dx.doi.org/10.1177/0022034513492769.

[16] Leloup C, Casteilla L, Carrière A, et al. Balancing mitochondrial redox signaling: a key point in metabolic regulation. Antioxid Redox Signal 2011;14(3):519–30. http://dx.doi.org/10.1089/ars.2010.3.

[17] Barayuga SM, Pang X, et al. Methamphetamine decreases levels of glutathione peroxidases 1 and 4 in SH-SY5Y neuronal cells: protective effects of selenium. Neurotoxicology 2013;37:240–6. http://dx.doi.org/10.1016/j.neuro.2013.05.009.

[18] Ravenel MC, Salinas CF, et al. Methamphetamine abuse and oral health: a pilot study of "meth mouth". Quintessence Int 2012;43(3):229–37.

[19] Sumpio BE, Cordova AC, et al. Green tea, the "Asian paradox", and cardiovascular disease. J Am Coll Surg 2006;202(5):813–25.

[20] Nugala B, Namasi A, et al. Role of green tea as an antioxidant in periodontal disease: the Asian paradox. J Indian Soc Periodontol 2012;16(3):313–6.

[21] Yu H, Oho T, Tagomori S, Morioka T. Anticariogenic effects of green tea. Fukuoka Igaku Zasshi 1992;83(4):174–80.

[22] Ferland G. Vitamin K, an emerging nutrient in brain function. BioFactors 2012;38(2):151–7. http://dx.doi.org/10.1002/biof.1004. Epub 2012 Mar 15.

[23] Vervoort LM, Ronden JE, Thijssen HH. The potent antioxidant activity of the vitamin K cycle in microsomal lipid peroxidation. Biochem Pharmacol 1997;54(8):871–6.

[24] Oldenburg J, Marinova M, Müller-Reible C, Watzka M. The vitamin K cycle. Vitam Horm 2008;78:35–62. http://dx.doi.org/10.1016/S0083-6729(07)00003-9.

[25] Sharma C, Suhalka P, Sukhwal P, et al. Curcumin attenuates neurotoxicity induced by fluoride: an in vivo evidence. Pharmacogn Mag 2014;10(37):61–5. http://dx.doi.org/10.4103/0973-1296.126663.

[26] Atmaca N, Atmaca HT, Kanici A, Anteplioglu T. Protective effect of resveratrol on sodium fluoride-induced oxidative stress, hepatotoxicity and neurotoxicity in rats. Food Chem Toxicol 2014;70(5):191–7. http://dx.doi.org/10.1016/j.fct.2014.05.011. Epub 2014 May 22.

[27] Davidson VL. Electron transfer in quinoproteins. Arch Biochem Biophys 2004;428(1):32–40.

[28] Thijssen HH, Drittij MJ, Vermeer C, et al. Menaquinone-4 in breast milk is derived from dietary phylloquinone. Br J Nutr 2002;87(3):219–26.

[29] Sato T, Schurgers LJ, Uenishi K. Comparison of menaquinone-4 and menaquinone-7 bioavailability in healthy women. Nutr J 2012;12(11):93. http://dx.doi.org/10.1186/1475-2891-11-93.

[30] Kate R-B. Vitamin K2 and the Calcium Paradox: How a Little-Known Vitamin Could Save Your Life. Mississauga, Canada: Wiley; pp. 182–185.

[31] Knapen MHJ, Schurgers LJ, Vermeer C. Vitamin K2 supplementation improves hip bone geometry and bone strength indices in postmenopausal women. Osteoporos Int 2007;18(7):963–72.

[32] Hosoi T. Clinical implications of under carboxylated osteocalcin. Clin Calcium 2009;19(12):1815–21.

[33] Kyla Shea M, Holden Rachel M. Vitamin K status and vascular calcification: evidence from observational and clinical studies. Adv Nutr 2012;3(2):158–65.

[34] Kyla Shea M, O'Donnell Christopher J, et al. Vitamin K supplementation and progression of coronary artery calcium in older men and women. Am J Clin Nutr 2009;89(6):1799–807.

[35] Dalmeijer GW, van der Schouw YT, et al. The effect of menaquinone-7 supplementation on circulating species of matrix Gla protein. Atherosclerosis 2012;225(2):397–402.

[36] Patergnani S, Suski JM, Agnoletto C, et al. Calcium signaling around mitochondria associated membranes (MAMs). Cell Commun Signal 2011;22(9):19. http://dx.doi.org/10.1186/1478-811X-9-19.

[37] Ferland G. Vitamin K and the nervous system: an overview of its actions. Adv Nutr 2012;3(2):204–12. http://dx.doi.org/10.3945/an.111.001784.

[38] Schurgers LJ, Vermeer C. Determination of phylloquinone and menaquinones in food, effect of food matrix on circulating vitamin K concentrations. Department of Biochemistry and Cardiovascular Research Institute. Maastricht, The Netherlands: Maastricht University. Haemostasis 2000;30:298–307.

[39] Komai M, Shirakawa H. Vitamin K metabolism. Menaquinone-4 (MK-4) formation from ingested VK analogues and its potent relation to bone function. Clin Calcium 2007;17(11):1663–72.

[40] Li J, Lin JC, Wang H, et al. Novel role of vitamin K in preventing oxidative injury to developing oligodendrocytes and neurons. J Neurosci 2003;23(13):5816–26.

[41] Ferland G. Vitamin K and brain function. Semin Thromb Hemost 2013;39(8):849–55. http://dx.doi.org/10.1055/s-0033-1357481. Epub 2013 Oct 9.

[42] Dowd FJ. Saliva and dental caries. Dent Clin North Am 1999;43(4):579–97.

[43] Ambudkar IS. Regulation of calcium in salivary gland secretion. Crit Rev Oral Biol Med 2000;11(1):4–25.

[44] Ambudkar IS. Dissection of calcium signaling events in exocrine secretion. Neurochem Res 2011;36(7):1212–21.

[45] Ambudkar IS. Polarization of calcium signaling and fluid secretion in salivary gland cells. Curr Med Chem 2012;19(34):5774–81.

[46] Choi HJ, Yu J, et al. Vitamin K2 supplementation improves insulin sensitivity via osteocalcin metabolism: a placebo-controlled trial. Diabetes Care 2011;34(9):e147.

[47] Thomas DD, Krzykowski KJ, Engelke JA, et al. Exocrine pancreatic secretion ofphospholipid, menaquinone-4, and caveolin-1 in vivo. Biochem Biophys Res Commun 2004;319(3):974–9.

[48] Dawes C. What is the critical pH and why does a tooth dissolve in acid? J Can Dent Assoc 2003;69(11):722–4.

[49] Telgi RL, Yadav V, et al. In vivo dental plaque pH after consumption of dairy products. Gen Dent 2013;61(3):56–9.

[50] Elder SJ, Haytowitz DB, et al. Vitamin K contents of meat, dairy, and fast food in the U.S. Diet. J Agric Food Chem 2006;54(2):463–7.

[51] Yoneda T, Tomofuji T, et al. Anti-aging effects of co-enzyme Q10 on periodontal tissues. J Dent Res 2013;92(8):735–9. http://dx.doi.org/10.1177/ 0022034513490959.

[52] Aral K, Alkan BA et al. Therapeutic effects of systemic vitamin K2 and vitamin D3 on gingival inflammation and alveolar bone in rats with experimentally induced periodontitis. J Periodontol 2015;85(5):666–73. DOI: 10.1902/jop.2015.140467

[53] Nakamura S, Yamada, Y et al. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endodont 2009;35(11):1536–42. DOI: 10.1016/j.joen.2009.07.024

[54] Nelson P, Tran TDN et al. Transient receptor potential melastatin 4 channel controls calcium signals and dental follicle stem cell differentiation. Stem Cells 2013;31(1):167–77. DOI: 10.1002/stem.1264

[55] Monroe DG, McGee-Lawrence ME et al. Update on Wnt signaling in bone cell biology and bone disease. Gene 2012;492:1–18.

[56] Okiji K, Yoshiba K. Reparative dentinogenesis induced by mineral trioxide aggregate:a review from the biological and physicochemical points of view. Int J Dentist. DOI:10.1155/2009/464280 Volume 2009 (2009), Article ID 464280, pp 1-12.

[57] He YD, Sui BD et al. Site-specific function and regulation of Osterix in tooth root formation. Int Endodont J. DOI: 10.111/irj.12585. 49(12): 1124–1131.

[58] Gordeladze JO, Stakkestad Ø et al. Regulatory loops consisting of transcription factors and microRNA species determine the mineralizing characteristics of cell phenotypes –implications for bone engineering and prevention of soft tissue mineralization. In: "Cells and Biomaterial in Regenerative Medicine", 2014, ed. Daniel Eberli, http://dx.doi.org/10.5772/59149, pp 71–102.

[59] Sinha KM, Zhou X. Genetic and molecular control of Osterix in skeletal formation. J Cell Biochem 2013; 114(5):975–984.

[60] Sangwan P, Sangwan A et al. Tertiary dentinogenesis with calcium hydroxide: a review of proposed mechanisms. Int Endodont J 2013;46:3–19.

[61] Example of transcription factor (TF) regulation of the ALP gene: http://www.sabiosciences.com/chipqpcrsearch.php?gene=ALPL&factor=Over+200+TF&species_id=0&ninfo=n&ngene=n&nfactor=y

[62] Hynes RO, Naba A. Overview of the matrisome – an inventory of extracellular matrix constituents and functions. Cold Spring Harb Perspect Biol 2012;4:a004903.

[63] Yoshizaki K and Yamada Y. Gene evolution and functions of the extracellular matrix proteins in teeth. Orthod Waves 2013 March 1;72(1):1–10.

[64] Le Bechek A, Portales-Casmar E et al. Mir@nt@n: a framework integrating transcription factors, microRNAs and their targets to identify sub-network motifs in a meta-regulatory network model. Bioinformatics 2011;12:67.

[65] Horie-Inoue K, Inoue S. Steroid and xenobiotic receptor mediates a novel vitamin K2 signaling pathway in osteoblastic cells. J Bone Miner Metab 2008;26:9–12.

[66] Lecka-Czernik B, Stechschultea LA. High bone mass in adult mice with diet-induced obesity results from a combination of initial increase in bone mass followed by attenuation in bone formation; implications for high bone mass and decreased bone quality in obesity, http://dx.doi.org/10.1016/j.mce.2015.01.001, pp 35–41.

[67] Tai N, Inoue D. Anti-Dickkopf1 (Dkk1) antibody as a bone anabolic agent for the treatment of osteoporosis. Clin Calcium 2014 Jan;24(1):75–83. DOI: CliCa14017583.

[68] Huang FM, et al. Cytotoxicity of dentine bonding agents on human pulp cells is related to intracellular glutathione levels. Int Endodont J 2010;43:1091–1097.

[69] Samuelsen JT, et al. HEMA reduces cell proliferation and induces apoptosis in vitro. Dental Mater 2008;24:134–140.

[70] Schweikl H, et al. Cytotoxic and mutagenic effects of dental composite materials. Biomaterials 2005;26:1713–1719.

[71] Tadin A, et al. Genotoxicity in gingival cells of patients undergoing tooth restoration with two different dental composite materials. Clin Oral invest 2014;18:87–96.

[72] Walther UI, et al. Cytotoxicity of ingredients of various dental materials and related compounds in L2- and A549 cells. J Biomed Mater Res 2002;63:643–649.

[73] Al-Hiyasat AS, Darmani H, Elbetieha AM. Leached components from dental composites and their effects on fertility of female mice. Eur J Oral Sci 2004;112:267–272.

[74] Ruse ND. Xenoestrogenicity and dental materials. J Can Dent Assoc 1997;63:833–836.

[75] Tarumi H, et al. Estrogenicity of fissure sealants and adhesive resins determined by reporter gene assay. J Dent Res 2000;79:1838–1843.

[76] Wada H, et al. In vitro estrogenicity of resin composites. J Dent Res 2004;83:222–226.

[77] Raap U, Stiesch M, and Kapp A. Contact allergy to dental materials. J German Soc Dermatol 2012;10:391–396; quiz 397.

[78] Goldberg M. In vitro and in vivo studies on the toxicity of dental resin components: a review. Clin Oral Invest 2008;12:1–8.

[79] Huang FM, Chang YC. Cytotoxicity of dentine-bonding agents on human pulp cells in vitro. Int Endodont J 2002;35:905–909.

[80] Lodiene G, et al. Detection of leachables and cytotoxicity after exposure to methacrylate- and epoxy-based root canal sealers in vitro. Eur J Oral Sci 2013;121:488–496.

[81] Dahl JE, ØRstavik DAG. Responses of the pulp–dentin organ to dental restorative biomaterials. Endodont Top 2007;17:65–73.

[82] Tonder KJ. Vascular reactions in the dental pulp during inflammation. Acta Odontol Scand 1983;41:247–256.

[83] Dominici M, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.

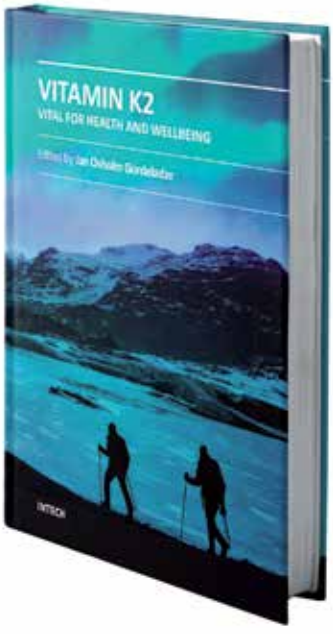
[84] Signore M, et al. Identity and ranking of colonic mesenchymal stromal cells. J Cell Physiol 2012;227:3291–3300.

[85] Sorrentino A, et al. Isolation and characterization of CD146+ multipotent mesenchymal cells. Exp Hem 2008;36:1035–1046.

[86] Deepak BS, et al. Tissue engineering: is it the future of endodontics? People's J Sci Res 2011; 4(1):76–82.

CHAPTER TITLE

VITAMIN K2 AND ITS IMPACT ON TOOTH EPIGENETICS



READ, SHARE
& DOWNLOAD FOR FREE

CHAPTER OFFPRINT FROM

VITAMIN K2 - VITAL FOR HEALTH AND WELLBEING

Edited by **Jan Oxholm Gordeladze**

ISBN 978-953-51-3020-8
Print ISBN 978-953-51-3019-2
Publisher: InTech
Published March 22, 2017

Book DOI: 10.5772/61430
Chapter DOI: 10.5772/66383

Edited Volume

OPEN ACCESS