INVITED REVIEW

Biomineralization and Biomaterial Considerations in Dentin Remineralization

[1]Xu Zhang, [2]Zuohui Xiao, [3]Haorong Wang, [4]Anil Kishen

ABSTRACT

Dentin is a composite hard tissue, comprising of inorganic and organic matrices, and regulated by many proteins during development. The demineralization of dentin results from the loss of inorganic matrix [mainly hydroxyapatite (HAP)], but the organic matrix (mainly type I collagen) will sustain for a period of time after demineralization. Over the past decade, there has been a growing interest on the remineralization of demineralized dentin, primarily in connection with minimally invasive caries management. More and more biomaterials and methods are currently being evaluated to achieve newer approaches for the remineralization of demineralized dentin. These strategies are mostly based on biomimetic approaches and aim to achieve the characteristics of natural hard tissue. This article will present a complete review on the basic compositions and properties of dentin, which formed the basis for the biomimetic remineralization of demineralized dentin.

Keywords: Biomineralization, Collagen, Dentin, Non-collagenous proteins, Remineralization.

INTRODUCTION

Management of demineralized dentin is an important issue in dentistry. Clinicians routinely favor removing the softened dentin completely based on the hypothesis that softened dentin is typically infected dentin.[1] It is established that softening of dentin always precedes bacterial invasion and leaves a layer that is soft but not infected. This layer of demineralized dentin is thick in acute caries.[2] The term remineralization of dental tissue refers to the replacement of the mineral fraction lost

[1]Associate Professor, [2,3]Graduate Student, [4]Professor and Head

[1-3]Department of Endodontics, School and Hospital of Stomatology, Tianjin Medical University, Tianjin, PR China

[4]Discipline of Endodontics, Faculty of Dentistry, University of Toronto, Toronto, Canada

Corresponding Author: Anil Kishen, Professor and Head Discipline of Endodontics, Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, Ontario, Canada, e-mail: anil.kishen@utoronto.ca

during the demineralization process. From a clinical standpoint, optimal remineralization of a demineralized dentin layer will aid in preventing/treating dentin caries, root caries, and dentin hypersensitivity.[3] These strategies would serve as the foundation for minimally invasive approaches in the management of early dental caries.

Dentin remineralization is a complex process and most relevant research in dentin remineralization involves the understanding of the property of dentin matrix, the composition of remineralization solution and the interaction between the matrix and solution. In dentin matrix, besides inorganic fraction [mainly hydroxyapatite (HAP)], it also consists of significant levels of organic matrix that comprises of type I collagen and noncollagenous proteins (NCPs), such as dentin matrix protein (DMP1) and dentin phosphoprotein (DPP), also known as DMP2 or phosphophoryn, with highly phosphorylated serine and threonine residues.[4] The NCPs has an important role in the mineralization of collagen fibrils. According to the location within the collagen fibrils, the apatite crystallites are classified into extrafibrillar minerals and intrafibrillar minerals. Extrafibrillar minerals are located in spaces separating the collagen fibrils, while the intrafibrillar minerals are largely in the gap regions of the fibrils extending between tropocollagen molecules.[5-7]

It is suggested that remineralization mechanisms in enamel and dentin are similar.[8-11] Many studies have demonstrated that methods used in enamel remineralization would also work in case of dentin remineralization. A typical example for this is the application of fluoride. Currently, an accepted notion is that dentin remineralization occurs neither by spontaneous precipitation nor by nucleation of mineral on the organic matrix but by the growth of residual crystals in the lesions.[3] However, the role of organic matrix in association with the residual crystals (inorganic matrix) on dentin remineralization is debated. It has been indicated that if NCPs, as inhibitors of mineralization are removed, mineralization would occur on the completely demineralized dentin substrate.[3] On the other hand, Saito et al[12] reported that if completely demineralized dentin collagen (by EDTA treatment) contains a higher content of ester phosphate, it would induce remineralization. In addition, Kawasaki et al[3] have reported that the presence of fluoride could overcome the inhibitory effect of NCPs on mineralization.

In dentin remineralization, the role of (a) inorganic matrix, (b) organic matrix, and (c) inorganic/organic additives in remineralization solution, needs to be further clarified. This knowledge would form the basis for newer methodologies for the remineralization of dentin or enamel. With improved understanding on the biomineralization of dentin and functions of NCPs, biomimetic strategies that are based on the interaction between organic macromolecules and inorganic/organic matrix could be developed for the remineralization of dental hard tissue. The advantage of biomimetic mineralization is that it simulates the natural process of formation of mineral crystals without using special equipment and strict conditions.[13] Some biomimetic remineralizing agents have been developed, such as nanocomplexes of stabilizer and amorphous calcium phosphate (ACP). These nanocomplexes can remineralize enamel lesions,[14] and when combined with a phosphorylated collagen, these nanocomplexes can remineralize dentin collagen as well[15] These strategies would provide the foundation for the nondestructive and more efficient methodologies for the minimally invasive management of dental caries. This review will focus on the biomineralization of dentin and the role of inorganic/organic matrix constituents on dentin remineralization.

BIOMINERALIZATION OF DENTIN

The process of biomineralization of dentin involves the interaction of NCPs with collagen fibrils and minerals.[16] The formation of dentin is a tissue-specific biomineralization process, which occurs at spatially independent sites throughout the organic extracellular matrices, mostly type I collagen fibrils and NCPs. The schematic drawing in Figure 1 depicts the highly polarized odontoblast with its process extending through the predentin into the dentin.[17] The mineralization front follows the cell movement at the same rate, maintaining an essentially constant width of the unmineralized predentin.[18] In predentin zone, the odontoblast actively secretes collagen to form collagen network and proteoglycan (PG). Several noncollagenous components, such as DPP, γ-carboxyglutamate-containing proteins of the osteocalcin type (Gla-protein) and a second pool of PG, are transported within the odontoblast process and secreted just in front of the advancing mineralized dentin. In the mineralization front, NCPs interacts with the collagen fibrils and inorganic ions to specifically trap calcium and phosphate ions to initialize mineralization.[19-21] Furthermore, a second pool of PGs appeared to be involved in the nucleation, orientation and growth of HAP crystal.[22-24] Dentin phosphoprotein is detected only in the dentin side of the mineralization front but not in predentin.[25-29] Therefore, a possible role of DPP in dentin mineralization may be to regulate the growth of crystals behind the mineralization front.
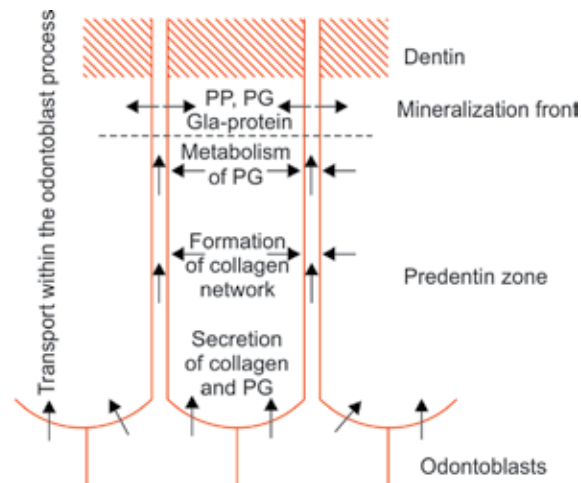

Fig. 1: Schematic drawing of the odontoblast-predentin region during dentinogenesis. Macromolecules are synthesized within the odontoblast cell bodies (below). The constituents of predentin, collagen, and proteoglycan (PG) are secreted close to the odontoblast cell bodies to form the extracellular, nonmineralized predentin matrix. Some of this proteoglycan fraction is metabolized in predentin. It should be noted that the 'mineralization front' is just an operative term, denoting that part of predentin where mineral formation is initiated[17]

Dentin collagen fibrils show a high degree of organization, a strong crystalline character and ability to interlock, which might be related to the specific type of NCPs that induced mineralization.[24,30,31] In the beginning of the mineralization, the HAP crystals start in gap regions or attach to the collagen fibrils that work as a scaffold. On the other hand, the body fluid composed of extracellular and intracellular fluid is known as a source of calcium and phosphate in biomineralization,[32] but the roles of other inorganic ions and organic macromolecules in body fluid and their synergism are not completely elucidated. It is considered that calcium ions are transported to the mineralization front by a transcellular route, where Ca^{2+}-activated ATPase, in concert with Na^+/Ca^{2+}-exchangers, calcium channels and intracellular calcium binding proteins, maintain a delicate calcium ion homeostasis in odontoblasts.[33-37] Phosphate ions are nonspecifically cleaved from compounds by alkaline phosphatase to support mineralization. Activity of alkaline phosphatase has been shown to increase at the mineralization front when crystal growth is occurring.[38]

Understanding the function of body fluid in biomineralization is significant for remineralization. Up to now, simulated body fluid has widely been used in the *in vitro* study of mimetic mineralization. What can be learned from biomineralization of dentin is that for remineralization of dentin or even for mimetic synthesis of other mineral crystals, the nucleation and regulation for morphology and structure in the crystal growth are crucial. Therefore, the study of biomimetic strategies should focus on these two following scenarios:

THE ROLE OF INORGANIC MATRIX OF DENTIN IN REMINERALIZATION

In remineralization process, the nucleation of mineral crystals on inorganic matrix is a rate-determining step, which is driven by local pH, supersaturation with respect to HAP, amount of seed crystals (nucleation sites) and surface area available for crystal growth.[39] The process of remineralization of more advanced subsurface lesions, where the diffusion of lattice ions take place through the surface pores is also considered to be a rate-determining step.[11] If the nucleation process occurs rapidly, the ions will be quickly depleted from the remineralization solution for nucleation and crystal growth. Consequently, a concentration gradient required for the diffusion will not occur and with progressive remineralization, the lesion pores may become blocked.[39] Thus, lower concentrations of calcium and phosphate are often preferable in remineralization solution to prevent immediate precipitation, which hindered ions diffusion into the deeper aspect of the lesion.

During the process of repair of early dental caries, the nucleation of mineral crystals on organic matrix should exceed above rate maintaining the balance. To achieve this goal, some additives are introduced to remineralization system to enhance the nucleation rate of mineral crystals. Fluoride is one the most successfully used agent. This is because the solubility product constant of FAP is 7.08×10^{-122} $mol^{18}.l^{-18}$,[40] which is less than that of HAP, 5.5×10^{-118} $mol^{18}.l^{-18}$ at 37°C.[41] The former agent is thus more favorable than the latter. Consequently, the presence of fluoride in saliva, biofilm fluid and remineralization solution can enhance remineralization.[42] In presence of fluoride, the nucleation and *de novo* formation of mineral crystals could occur, which could result in hyper-remineralization. For this point, a lower concentration of fluoride is recommended.

THE ROLE OF ORGANIC MATRIX OF DENTIN IN REMINERALIZATION

The organic matrix of dentin primarily consists of fibrous collagens and NCPs. Collagen constitutes 90% of the dentin matrix, and is principally type I.[43,44] Type I collagen itself in dentin does not seem to be able to induce significant remineralization of demineralized dentin[11,39,45] but may act as a template for mineral deposition.[46] NCPs constitute the remaining 10% of the dentin organic matrix. It is worth noting that some studies[4,11,20] indicated that NCPs in dentin could influence dentine remineralization by regulating the nucleation of mineral crystals. Hence, from the remineralization kinetic viewpoint, NCPs is also a rate-determining factor in nucleation of the mineral crystals during remineralization. Type I collagen in skin and tendon are not mineralized *in vivo*, where NCPs related to mineralization may be lacking or if present, may inhibit mineralization. Thus, it can be considered that collagen act as a foreign body/surface *in vitro* to induce nucleation based on the mechanism of heterogeneous nucleation. The carboxyl groups of collagen were considered to be the nucleation sites.[47] These findings emphasize that collagen substrate along with certain NCPs induce mineral growth.[48]

The NCPs in the matrix of completely demineralized dentin collagen are not equal to that of fully mineralized dentin. The nature and mount of NCPs in demineralized dentin would depend upon the method of demineralization. Thus, promoter or inhibitor of NCPs,[49,50,51] and the presence of NCPs in partially and completely demineralized dentin influences its remineralizing capacity *in vitro*. The major components of NCPs in dentin are attributed to the SIBLING (small integrin-binding ligand, N-linked glycoprotein) family,[52] and their gene is all clustered within an approximately 375 kb span of nucleotides on human chromosome 4q21 (mouse 5q21).[4] SIBLING family is composed of osteopontin (OPN), matrix extracellular phosphoglycoprotein (MEPE), bone sialoprotein (BSP), DMP1 and dentin sialophosphoprotein (DSSP). Osteopontin and MEPE acting as inhibitors of mineralization are found widely in mineralized and nonmineralized tissue, while DMP1 and the subdomains of DSSP [DSP (dentin sialoprotein), DGP (dentin glycoprotein) and DPP] are strongly engaged in bone and dentin mineralization.[4] Among NCPs in dentin, the properties and behaviors in mineralization of DPP (DMP2) and DMP1 are the most documented.[4]

The role of DPP in mineralization process of calcium phosphate *in vitro* was the most exhaustively studied in the past three decades. Due to the application of different extraction methods and dentin source (human or animals), the DPP used in early studies were altered in molecular weight, type and content of amino acid. Despite these differences in DPP used in different studies, the enrichment of aspartic and serine, high phosphorylation of serines and (Asp-Ser-Ser) repeats are common characteristics. A typical human DPP is a 140 KDa protein containing approx 38% aspartic acid and 42% serine.[50,53] Phosphorylation of serine residues results in a very high negative charge molecule that can be considered a virtual sink for the binding of calcium ions.[4] *In vitro* studies of DPP were shown to inhibit the formation of HAP *de novo* in solution,[54] and gel systems[55,56] but to promote HAP formation when it was covalently immobilized on agarose beads and collagen.[57-59] These findings suggest that the formation of HAP is inhibited by soluble DPP but promoted by immobilized DPP. In addition, in the

gel system the formation of HAP is independent on the concentration of DPP (decreasing by 100 μg/ml of DPP and increasing by 0.01–1 μg/ml).[56] The inhibition or promotion of mineralization by free DPP in solution depends on its concentration, while immobilized DPP on a certain surface could induce mineralization. Furthermore, studies have shown that EDTA-soluble DPP, which are inhibitor of mineralization, when removed from dentin collagen, would facilitate remineralization of completely demineralized collagen.[12,60,61,62,63]

Dentin matrix protein was identified as a new serine-rich acidic protein of the dentin matrix by cDNA cloning, containing 489 amino acid residues.[49] Compared with DDP, DMP1 has a high content of Glu residues and does not contain $(DSS)_n$ repeats. Dentine matrix protein is present in phosphorylated form at low levels in the dentine matrix as shown in immunochemical and organ culture biosynthetic studies.[49] Its effect on remineralization of demineralized dentin has not been investigated, but its role in mineralization of collagen *in vitro* has been studied, which could suggest remineralization of dentin. He et al reported that two peptide clusters at C-terminal of DMP1, 349DSESSEEDR357 and 424EENRDSDSQDSSR437, were identified as collagen-interactive peptides.[64] The binding between DMP1 and collagen is driven by electrostatic interaction as indicated by the acid residues in these two peptides. On the other hand, the N-terminal domain of DMP1 can stabilize ACP due to the high negative charge density created by aspartic acid residues that can favor and stabilize the amorphous nuclei.[65] Nanocomplexes of DMP1/ACP as a precursor to apatite penetrates the gap zone of collagen and finally transform into HAP crystals to mineralize collagen. It was shown that DMP1 could serve dual roles during dentin formation: inhibiting spontaneous mineral precipitation and promoting controlled mineral nucleation on a collagenous template.[65]

Based on the stabilization effect of ACP by DMP1, analog materials that mimic DMP1 have been designed to simulate the remineralization function in dentin. These materials are equipped with high negative charge due to rich carboxyl groups, such as polyacrylic acid (PAA) and polyaspartic acid (PASA).[15,66-68] While some other materials, such as phosphorylated chitosan (Pchi) and carboxymethyl chitosan (CMC) could not only stabilize ACP due to their charge characteristic, but also could be processed into scaffolds by lyophilization for certain specific clinical application.[69-71] In summary, DPP and DMP1 play a dual role in mineralization. They can act as an inhibitor of crystal nucleation/growth in solution or as a template for crystal nucleation when immobilized on a solid surface. The mineralization of collagen based on the immobilization of DPP on collagen and the interaction of DMP1 with ACP and collagen would suggest a possibility for remineralizing the demineralized dentin.

SUMMARY

Improved understanding on the role of organic matrix in the biomineralizaton of dentin, highlighted that collagen could play a key role in the remineralization of dentin. Thus, remineralization could be achieved by modifying collagen, while dismissing the conventional concept of depending only on the inorganic matrix to induce remineralization of dentin. Therefore, remineralizing nanomaterials, especially biomimetic nanomaterials and their remineralizing rationale, would broaden the horizon in minimally invasive or microdentistry.

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