A New Perspective on the Biomineralization of Radicular Dentin

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Abstract

The biomineralization of radicular dentin involves complex molecular signaling. The exact mechanism of action remains elusive. However, the literature showed that protein binding sites for calcium ions and mineral precipitation are essential to promote the remineralization process. Dentin organic matrix possesses non-collagenous proteins, such as dentin matrix protein 1 (DMP1), that can initiate and regulate crystal nucleation. Recombinant DMP1 molecules have been thought to perform specific molecular recognition with the apatite surface, guiding calcium phosphate clusters during recruitment through the type I collagen matrix, which serves as a template for controlled biomineralization. The biological basis for the clinical benefits of this process has yet to be completely elucidated. From a clinical perspective, caries, acid erosion, or disinfection protocol treatments can cause dentin demineralization. Given this fact, dentin biomimetic remineralization strategies and treatment strategies aimed at preserving the dentin matrix have been proposed.

Keywords: Biomineralization; Carbonate apatite; Dentin remineralization; Non-collagenous proteins; Radicular dentin.

Introduction

Dentin is the mineralized tissue underlying the enamel and cementum constituting the main component of the tooth.\(^1\) Radicular dentin is a specialized type of dentin that is located below the enamel and cementum and provides structural support and protection for the tooth. Root dentin is characterized by its mineral content, microstructure, and mechanical properties, which are different from those of coronal dentin, which forms in the crown of the tooth.

Coronal and radicular dentin differ in their environment, structure, and in their organic and inorganic components. Coronal dentin exhibits significantly higher tubule density, whereas radicular dentin has larger diameter tubules and smaller peritubular cuff thickness, resulting in more peritubular dentin and a larger volume fraction of the matrix.\(^2\) Additionally, differences in the presence of highly phosphorylated proteins (SIBLING family) between coronal and radicular dentin were previously denoted.\(^3\) Among the most important findings, can be highlighted that DPP (DSPP fragment) levels in coronal dentin are approximately four times higher than those in radicular dentin, suggesting different substrates.\(^4\)

Due to its localization in the tooth, radicular dentin can be exposed to multiple factors that promote dentin demineralization, such as caries, erosion, pulp necrosis, infection of the root canal system, and diverse periodontal pathological conditions. Moreover, common chemicals used during endodontic procedures may cause collagen matrix degradation. Likewise, coronal dentin, when exposed, is surrounded by an environment with saliva and oral microbiota, whereas exposed radicular dentin is surrounded by cells in the periodontal ligament, bone, and tissular fluid with ions that provide an ideal environment to promote the biomineralization process.
Biomineralization is the process by which living organisms produce minerals, typically to harden or stiffen existing tissues. This can be seen in the formation of shells, bones, teeth, and various other structures in animals and plants. This biological process is mediated by extracellular matrix proteins, and regulated by a number of factors, including the presence of enzymes, growth factors, proteins, and other signaling molecules.

Biomineralization of enamel, dentin, and bone involves the deposition of apatite mineral crystals within an organic matrix. Moreover, the biomineralization process in radicular dentin involves the deposition of an organic matrix by odontoblasts, which acts as a template for the precipitation of mineral crystals, such as hydroxyapatite. During dentinogenesis, odontoblasts synthesize collagenous and non-collagenous proteins (NCPs) and form an unmineralized extracellular matrix (ECM), in which type I collagen is the major constituent. Collagen self-assembles into fibrils to form a passive scaffold that acts as a template for mineral deposition at specific sites in the biomineralization process.

Therefore, dentin biomineralization refers to the process by which is produced or reproduced dentin, through the precipitation of calcium phosphate minerals. The traditional view of dentin biomineralization focuses on the role of odontoblasts and the deposit of organic matrix. However, recent research has revealed a more complex and dynamic picture of the biomineralization process. The findings of our previous research suggested that the mineralized matrix is not a passive scaffold, it is actively regulated by a network of signaling pathways and molecular interactions.

Thus, it is essential to assemble previous data to understand the advancement of translational research regarding the biomineralization process in radicular dentin. Several studies have shown that using Mineral Trioxide Aggregate (MTA), as a bioactive material, promoted the precipitation of mineral crystals in the presence of phosphate-buffered saline (PBS). Reyes-Carmona et al. observed that the apatite formed by the cement-PBS system was deposited within collagen fibrils, promoting controlled mineral nucleation on dentin and triggering the formation of an interfacial layer with tag-like structures at the cement-dentin interface. Additionally, the biomineralization process positively influenced the resistance to dislodgement from dentin of all MTA-based-cements.

Moreover, there are few published data confirming the occurrence of the biomineralization process in vivo. Previously, the process of biomineralization was evaluated after implantation of dentin tubes filled with MTA in the subcutaneous tissue of mice. In the period of 12 hours after implantation, numerous apatite-like clusters were observed on collagen fibrils all over the surface of dentin tubes. The formation of these deposits became more extensive over time, resulting in the formation of a compact layer on the surface of dentin tubes within 7 days.

The ultrastructural examination of the precipitates allowed verification of the presence of different types of minerals. Generally, higher amounts of spherical precipitates with acicular crystallites were observed along the periphery, being characteristic of the pH-dependent autocatalytic transformation of the metastable amorphous calcium phosphate phase into an apatite phase. The occurrence of petal-like precipitates was observed, and related to octacalcium phosphate (OCP) formation, being that OCP structure is composed of apatite layers separated by hydrated layers. Furthermore, OCP has been proposed as a mandatory precursor phase for the formation of non-stoichiometric, biologic apatites.

At physiologic pH, the formation of carbonated apatite can be associated with a two-stage process. Initially, the Ca/P ratio of the precipitates is lower than 1.5 and is associated with the alkalinization of the solution. During the second stage, there is an increase in the Ca/P ratio and a drop in pH, which can be attributed to the incorporation of OH− ions released from the bioactive material into the OH− sites of the apatite. On the basis of this information, it is possible to deduce that initially an amorphous calcium phosphate phase is formed, which acts as a precursor to the secondary phase during which carbonated apatite is formed.

Bioactivity is an expression that describes the beneficial effect of a material implanted in living tissue. The capacity of the material to interact with the living matter allows the integration of the biomaterial into the environment. When a bioactive material is implanted, a series of biochemical and biophysical reactions occur at the implant-tissue interface, resulting in the formation of a carbonated apatite layer.

However, the role of the collagen matrix during the nucleation of amorphous calcium phosphate and its transformation into oriented crystals of apatite remains controversial. Thus, our group designed a biomimetic remineralization system of radicular dentin to evaluate the functional relationship of MMPs (MMP-2 and MMP-9) and NCPs (DSPP and DMP1-CT) with the initiation and maturation of apatite.
Figure 1. Biomimetic remineralization system of radicular dentin. SEM microphotographs showed the presence of spherule precipitates with acicular crystals, and mainly petal-like precipitates, which can be observed predominantly in the demineralized dentin (red arrow).
Indeed, our study developed an experimental model in which radicular dentin, the tissue to remineralize, was not in direct contact with the source of calcium ions. Our novelty results demonstrated the migration of the mineral precipitates from their ion source (bioactive material) to dentin, indicating the presence of an attraction flux and specific sites of nucleation. As observed in figure 1, spherule precipitates with acicular crystals, petal-like, and compact lath-like precipitates were identified, mainly in the demineralized dentin. We hypothesized that this phenomenon occurs because the dentinal collagen matrix is a NCPs reservoir, which functions as a template for remineralization. The demineralization procedure exposed specific phosphoric acid-containing signaling molecules such as DMP1-CT and DSPP within the underlying dentin layer, initiating the specific binding of mineral precipitates to specific sites of dentin and triggering the remineralization process. Thus, these findings suggest that the remineralization of radicular dentin can be achieved with the aid of bioactive materials and a standardized demineralization procedure to expose NCPs in the dentin matrix, allowing MMPs to convert structural matrix proteins into signaling molecules and generating peptides, which allows a specific attraction flux of apatites through the PBS solution, to guide mineral nucleation and maturation.

Indeed, from a clinical perspective, it is important to state that the dentinal matrix possesses its own biological resources to regenerate, and clinicians must perform clinical protocols aimed to preserve NCPs when dentin remineralization and/or tissue regeneration are to be achieved. However, recently we demonstrated that common final irrigation protocols promoted a significant alteration of radicular dentin composition, as well as an important proteolytic effect on DMP1-CT, concerning the lack of potential of this remaining radicular dentin for regeneration and/or remineralization.

Understanding the properties and formation of radicular dentin is important for developing treatments aimed to regenerate. Biomimetic remineralization strategies should reproduce the dimension and structural hierarchy of apatite deposits within a demineralized collagen matrix. Further research in this area has the potential to lead to a new understanding of the underlying mechanisms of biomineralization and inform the development of new treatments for dental diseases.

References


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