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Fundamentals of Dentine Demineralization and Remineralization with the Role of Bioactive Materials in Dentine Regeneration

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Abstract

Considering minimally invasive dentistry, recent restorative materials are expected to provide a therapeutic effect on the remaining tooth structure. Using inert restorations which lack interaction between restorative materials and tooth structure is not satisfactory nowadays. This has shifted the focus in biomaterial sciences towards innovative bioactive restorative materials that can remineralize remaining tooth structures and provide healing and protective properties. Understanding the remineralization process of demineralized dentine and the mechanism by which these bioactive materials potentially repair these tissues, will provide room for future advancements and clinical applications in restorative dentistry. This article provides a review of the basic dentine structure, the caries process, and different dentine remineralization models. Also, it details various bioactive materials with an understanding of their main classification in terms of remineralization potential.

Keywords: Dentine caries, Dentine remineralization, Remineralization pathway, Bioactive materials

Introduction

Today's dental practice places a strong emphasis on implementing minimal intervention dentistry (MID) in order to avoid tissue loss and patient discomfort. MID focuses on the least invasive therapeutic approaches by implementing caries control through preventive, and early intervention measures. Therefore, therapeutic tissue remineralization becomes the first fundamental principle of MID as it promotes biological and therapeutic methods rather than conventional surgical procedures for early tooth caries (Banerjee, 2018). Dentine tissues undergo degradation by the caries process and an opposing defense response by the dentine-pulp complex, which results in histologic alternation within the dentine structure into intermixed dentine carious layers (McConnell *et al*., 2007). Consequently, these layers are expected to respond differently to therapeutic remineralization procedures based on the availability and the condition of the remaining collagen fibers (Sajini *et al*., 2022a).

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In general, the remineralization process occurs naturally within the oral cavity in response to the cyclic pH change. It takes place under a neutral physiological pH level as calcium and phosphate mineral ions from saliva and plaque fluid are re-deposited within the carious lesion. This results in the formation of fresh hydroxyapatite crystals that are larger, and more resistant to acid breakdown (Hemagaran & Neelakantan, 2014; Kleszken *et al*., 2022). Enamel, dentin, and root cementum all undergo the demineralization-remineralization process on a chemically comparable basis. However, the various structural characteristics and varying proportions of mineral and organic tissue composition in each of these substrates result in significant variations in the nature and development of the carious lesion and the simplicity of the remineralization process (Almahdy *et al*., 2012). Remineralization occurs in different pathways based on the substrate and the material inducing it. The available bioactive restorative materials differ in their composition and therefore the remineralization pathway they promote differs as well (Sajini *et al*., 2022a). Regarding dental tissues; a demineralized enamel can be remineralized by simple calcium phosphate re-precipitation when these ions are present in high concentrations and the pH is high. On the other hand, dentine and cementum constitute of higher organic component that has a significant role in the initiation of mineral nucleation and regulating the form of the mineral phase (Martinez *et al*., 2009). This makes dentine remineralization in deep carious lesions a more complicated procedure. There are mainly two approaches for dentine remineralization; either utilizing existing seed crystallites by epitaxial growth of minerals or by providing minerals (such as Calcium and Phosphate) to continuously replace water spaces within the matrix in the form of apatite crystals. The growth and form of these crystals are controlled by the presence of matrix proteins (Liu *et al*., 2011b; Niu *et al*., 2014).

Regarding the management of deep carious lesions extending to dentin, the MID strategy encourages the removal of the diseased dentine solely and the replacement of these tissues with bioactive materials. Researches are still ongoing to offer novel biomaterials that will require less tooth preparation, reinforce the tooth's remaining structure with potentials of remineralization (bioactivity), and promote pulp healing (Mackenzie & Banerjee, 2017). Different studies have investigated the potential of the available materials to induce remineralization on different dentinal substrates (infected, affected dentine) (Sajini *et al*., 2022a; Sajini *et al*., 2022b). However, there are still critical gaps in understanding the process and the relation between the materials composition, the remineralization pathway, and the quality of the remineralized

tissues. Therefore, this article provides a review of the basic dentine structure, the caries process, and different dentine remineralization models. Also, it details various bioactive materials with an understanding of their main classification in terms of remineralization potential.

Histopathology of Dentin Structure

Dentin is a specialized avascular connective tissue that constitutes the bulk of the tooth structure. The process of dentinogenesis (dentin formation), is carried out by cells called odontoblasts. Because the odontoblasts' cell bodies lie in the pulp cavity and their cell processes (Tomes fibers) extend further (100–200 m) into the tubules of the calcified dentin, they are considered a component of both pulp and dentin tissues. As a result, dentin is a living tissue that can serve a protective or reparative purpose in response to a pathologic challenge. Moreover, the developed dentine can bear stresses from masticatory forces and also protects the pulp as it is a highly mineralized, hard but elastic matrix (Pashley, 1991).

During dentin formation, the path of descending odontoblasts toward the pulp is represented by the dentinal tubules within the dentine structure. The dentinal tubule space is filled with cytoplasmic fluid and surrounded by the peritubular dentine matrix which is highly mineralized compared to the intertubular dentine matrix. Around 30% of intertubular dentine is made of mineralized collagen and is wrapped around the tubules perpendicular to their long axis. As a result, this three-dimensional microstructure that makes up dentine is crucial to the tissue's ability to function. The management of various dentine disorders, like as caries, and the understanding of the process of dentin demineralization and remineralization can be considerably improved by understanding its micro-architecture the composition of the organic matrix (Ritter & Walter, 2019; Sanari AA, *et al*., 2021).

Dentin organic content forms the extracellular matrix and it has two main components: collagen fibers and non-collagenous proteins. Collagen type I fibers make up the majority (90%) of the organic dentine material found in the extracellular matrix, with traces of collagen type III and V. It also has other non-collagenous proteins (NCPs), traces of blood serum proteins, enzymes (alkaline phosphatase and metalloproteinases), and proteins that bind calcium. The collagen structure in dentin consists of numerous polypeptide domains that are organized in a triple intertwining helical configuration creating a "coiled coil" structure. Under electron microscopy, these structures are presented in a staggering arrangement displaying a regular banding pattern with periodic overlaps and gap every 690 axial length. In collagen structure, these repeated units are referred to as D-periodic units. The majority of the organic matrix of dentine is made up of this distinctive interlocking collagen conformation, which offers regular gaps and acts as a scaffold for further crystallization and mineral deposition (Bertassoni *et al*., 2012). Additionally, non-collagenous protein components comprise approximately the residual 10% of the dentine organic matrix. Proteoglycans (PGs), proteins containing -carboxyglutamate, enzymes, and a family called small integrin-binding ligand N-linked glycoproteins (SIBLINGs) make up the majority of them. They cover the collagen fibrils to varying degrees and strongly regulate the process of the dentine matrix's mineralization (Qin *et al*., 2004). SIBLING's main role is to play a part in initiating the formation of apatite crystals, preventing spontaneous mineral deposition, and control apatite crystal growth to produce more appealing crystal forms. More specifically in dentin: dentine matrix protein 1 (DMP1), dentine sialophosphoprotein (DSPP) and its subdomains; dentine phosphoproteins or phosphophoryn (DPP) and dentine sialoproteins (DSP) are prominent proteins that are expressed during the mineralization phase dentine. Two opposing processes can be used to explain this regulatory role. These proteins exhibit a strong affinity for the CaP clusters found in the supersaturated solutions. Then, these amorphous mineral nuclei are stabilized on SIBLINGs proteins and exhibit a significant affinity for collagen molecules, which encourages regulated mineral nucleation on the collagenous template. However, when these proteins are present in large quantities, they also bind to the CaP nanoparticles and prevent the growing mineral nuclei from further growth and precipitation; this inhibitory action is crucial for preventing pathologic calcification (George & Veis, 2008).

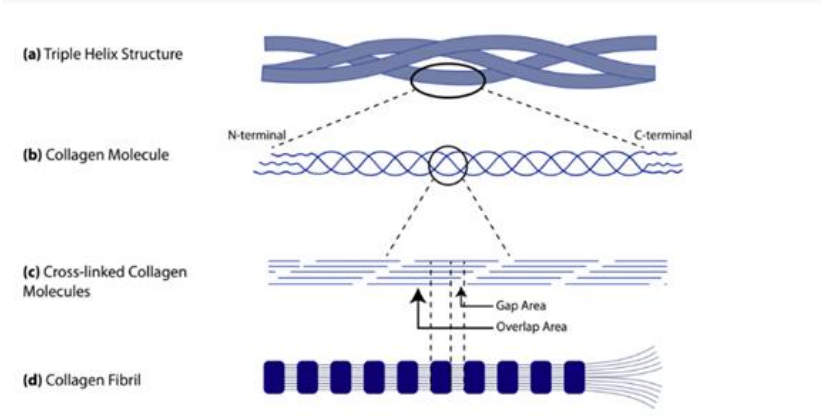

Figure 1. A diagram presenting (a) collagen triple helical structure. (b) Collagen molecule contains non triple helical areas at both ends. (c) Cross-linked staggering of collagen. (d) Collagen fibril showing the repeated periodic banding pattern and demonstrating the D-periodic unit with single overlap and single gap (modified from Cawston, 1998).

The mineral content in dentine is presented as apatite crystals which are low in calcium and high in carbonate Ca10 (PO4)6(OH)2. Dentine hydroxyapatite is called (non-Stoichiometric) and has a Ca/P ratio between 1.5 and 1.67, while bone hydroxyapatite is Stoichiometric apatite has a Ca/P ratio of 1.7. Due to its low calcium and high carbonate concentration, dentine apatite crystals are more acid soluble and therefore more susceptible to caries attacks than stoichiometric apatite. Mineral phase formation in dentine begins with these crystal nucleations within the matrix in the form of spheres structure that grow and unite with surrounding spheres to produce apatite crystals. The organic components have a significant influence on the crystals' growth and orientation during dentinogenesis. Collagen type I self-assembles in a way that creates enclosed spaces within its structure where mineralization by hydroxyapatite crystals while non-collagenous proteins regulate the crystallized apatite form, size, and direction. Further remineralization of dentine throughout its lifetime is controlled by the saturation of free calcium and phosphate ions. Amorphous calcium phosphate (ACP), octacalcium phosphate, and dicalcium phosphate dehydrate are examples of intermediate precursor polymorphs that are produced with high calcium and phosphate ion saturation and later develop into hydroxyapatites. At this point, type I collagen just offers the framework for crystal deposition. Further apatite development and nucleation are controlled by additional non-collagenous proteins (Niu *et al*., 2014).

Dentine Caries

The process of dental caries begins when the bacteria in the biofilm create acids and damage the enamel surface. Many elements are necessary for demineralization and caries attack to take place within the enamel, including time, microbial shift, adhesion of the biofilm, pH drop below the threshold value (pH 5.5), and oxygen tension (Dawes, 2003). At this time, dentine can still react to those acidic changes even though they were triggered at the enamel surface. This is because dentine has to infiltrate odontoblast extensions from the peripheral pulp and can respond to external stimuli. The initial dentine reaction is the development of reactive sclerotic dentine, which has intratubular mineral deposition and is found just below the demineralized enamel surface. Dentine caries is visible at this stage as a brownish discoloration near the enamel-dentine junction (EDJ), which may be arrested by regularly disrupting the biofilm by its mechanical removal, using salivary buffering agents, fluoridation, and diet modification (Banerjee & Watson, 2015). Afterward, for caries to progress into dentine, further cavitation, and demineralization should reach a considerable depth within the DEJ. DEJ is less resistant to caries attack than enamel or dentin which results in dentine caries spreading rapidly once it is cavitated and colonized by bacteria causing dentine demineralization and collagen degradation with a reactive opposing defense mechanism taking place at the same time (Ritter & Walter, 2019). On a histological level, this can be seen as intermingled structural changes without a definite biological boundary. These histological zones from DEJ towards the pulp vary in the amount of bacterial invasion, degree of mineral loss, and the extent of collagen destruction which can be broadly divided into the following layers; Soft-infected and Firm-affected carious dentin and hard dentin (Fusayama *et al*., 1979; Banerjee & Watson, 2015). Dentin collagen destruction which is more evident in soft-infected dentin was long believed to be caused by proteases from bacteria, but it has been found that once these enzymes have demineralized the dentin matrix, cariogenic bacteria do not further damage the collagen (Van Strijp *et al*., 1997). Inactive Endogenous matrix metalloproteinases (MMPs) are naturally present in saliva and sound dentin. Their activity is regulated by tissue inhibitors which restrict extracellular matrix breakdown but they are easily activated in an acidic environment produced by bacterial acids. As higher enzyme proteolytic activities in carious dentin have been detected, collagen destruction is believed to be caused by those activated MMPs from saliva and dentinal fluid (Vidal *et al*., 2014). Salivary MMP can readily access the outer carious layer, which may explain the destruction of collagen matrix in soft rather than firm carious dentin.

The outer zone of dentin caries is recognized clinically as a layer of soft, moist, dark brown tissue. Microorganisms have infected this zone significantly, completely demineralizing it and degrading the biological matrix to a point beyond repair (Fusayama *et al*., 1979). The mineral phase of dentine is targeted and completely lost at the ultrastructural level, exposing collagen. As a result, the exposed fibers experience denaturation and degradation, which disrupts the fibrillar structure, causes the loss of the distinctive banding pattern and significantly reduces cross-linkage (i.e. DHLNL and HLNL). These structural alterations to collagen molecules are irreversible and permanent (Nakornchai *et al*., 2004). An optical examination of these tissues revealed a noticeably greater fluorescent signal due to the exposed chromophore (collagen and organic component) which is linked to mineral reduction in this layer. Many studies found a correlation between the high fluorescence signal and the reduction in tissue hardness explained by both collagen destruction and mineral loss (Banerjee *et al*., 2010; Almahdy *et al*., 2012). Similar findings were found in the inner zone of firm (affected) dentin caries regarding fluorescence and tissue hardness but to a lesser degree which reflect more preserved collagen matrix and mineral content than the soft caries zone. The discolored firm dentine differs from the infected dentine in that there is less bacterial infiltration, partial mineral loss, and repairable collagen matrix degradation. Compared to sound dentine, this layer has some irregularly scattered crystals, the mineral phase is less crystalline and has a lower mineral concentration. Regarding the organic matrix in this layer, it has been shown that the general arrangement of amino acids inside collagen molecules is intact, however, the intermolecular crosslinks had changed to their precursor form, which could be seen as decreased cross-links (Kuboki *et al*., 1977). This organic transition is believed to be reversible with a capacity for remineralization and healing, therefore, the inner layer of dentine caries has recently attracted increased attention. Following the minimal invasive concept in dentistry that indicate conservative tissue removal and preservation of remineralize tissues, discrimination between soft and firm dentin is essential to determine the caries excavation endpoint. Clinically, this layer is characterized as being light brown, sticky, hard, and scratchy. However, there is still a lack of a clear clinical definition of the caries excavation limit that will guarantee a high-quality restoration while also protecting the tooth structure from

unnecessary removal. Various techniques have been used to distinguish between the two primary carious layers. Clinical techniques include the use of caries detection dyes as well as visual and tactile examination. Techniques based on auto-fluorescence (AF) detection have also been employed clinically and in laboratory studies. Besides, bacterial analysis and microhardness measurements (KHN) have been used in laboratory investigations (Sajini *et al*., 2022a).

In-vitro Dentine Demineralization Models

Several topics of dental research investigating the use of different preventive methods and treatment techniques, caries excavation techniques, and the effectiveness of dental biomaterials, have been carried out using in-vitro models representing natural carious lesions. The approach adopted for such studies should be carefully designed according to their distinct objectives. Therefore, several approaches have been put forth to create carious dentine models in an effort to address the standardization challenges when using natural caries samples. These approaches either use the biofilm caries model or chemical (acid) demineralization.

Bacterial Biofilm Models

Biofilm models use cariogenic bacteria incubation with enamel or dentine. Several studies used the single-strain bacteria method where Streptococcus mutans was the main utilized species. It provides a simple and consistent biofilm model. However, bacterial interactions like competition, cross-feeding, or colony progression are not taken into account. Caries model using multi-species biofilm can be either a closed or open system. In the closed system, the culture medium is provided in a sealed environment where growth conditions vary based on the consumption of nutrients and the accumulation of bacterial by-products. This provides a simple, repeatable, and inexpensive model. On the other hand, there are open-system biofilm models such as the oral biofilm reactor or artificial mouth model. This system represents the in vivo environment more accurately compared to closed system models. It provides better control of the rate of biofilm formation by developing a steady state where waste metabolic products are regularly eliminated and nutritional medium is provided (Coenye & Nelis, 2010). However, due to the heterogeneity of the biofilm in the open environment, the experimental results are not easily repeatable. In addition, it is a complex model with a high risk of contamination and therefore, it is not commonly used.

Acid/ Chemical Model

Demineralized lesions (artificial caries) on enamel and/or dentin were created using chemical models used in the majority of the studies. This model mimics the caries process through the use of acid or an acid buffer to imitate the natural demineralization and remineralization cycles. Once the acidity (pH) falls below a specific point (critical pH), saliva and plaque fluid won't be saturated with calcium and phosphate, which leads to hydroxyapatite dissolution and demineralization. On the other hand, remineralization, or the re-deposition of minerals, happens as the pH rises opposing the demineralization process. Chemical models have several benefits, such as ease of research, low cost, effectiveness, reproducibility, and stability of the experiment. However, the fact that chemical models completely disregard the microbial component of tooth decay development is a major drawback. Moreover, collagen degradation which is a natural part of dentine caries cannot be reproduced using chemical demineralization.

Chemical models can utilize simple acid demineralization or pH cycling. Both processes aim to imitate the oral cavity environment at a chemical level instead of mimicking the biological changes (Skucha-Nowak *et al*., 2015). Acid etching is often used in dentistry to facilitate bonding to dentine and enamel after caries excavation. This aids in the removal of the smear layer, causes partial removal of minerals within the intertubular and peritubular dentine, and exposes the collagen fibers. In- vitro partially demineralized dentine samples often aim to resemble caries-affected (firm) dentine in terms of mineral content and physical properties and are often used to study the effect of different restorative materials on firm dentine (Farge *et al*., 2010; Cao *et al*., 2013). A simple acid demineralization model is created by using low pH agents such as mild organic acids and buffering agents to develop demineralized lesions. Many variables, including pH value, duration, temperature, mineral concentration, and inhibitors of mineral dissolution, can control the degree of demineralization. Changing these variables can influence the properties of lesions, like lesion depth and mineral loss ratio (Schwendicke *et al*., 2015). Since all variables can be managed, simple acid demineralization is thought to be an easy and efficient method for both enamel and dentine with repeatable and acceptable levels of demineralization. Using EDTA, phosphoric acid and acetate-containing solutions are the most common protocols (Cao *et al*., 2013; Schwendicke *et al*., 2015, Sajini *et al*., 2022a; Sajini *et al*., 2022b). The extent of dentine demineralization has been evaluated in a few studies to ensure that a considerable amount of minerals is removed while collagen fibers are partially intact to promote remineralization (Sajini *et al*., 2022a). This is a vital factor when evaluating the remineralization potentials of bioactive materials. Alternatively, the pH cycling method is based on subjecting the samples to alternating periods of demineralization and remineralization while the solution is continuously replaced. Depending on the different substrates and demineralized acid used, the pH of the demineralization solution ranges from 4.4 to 5.5 while the remineralization solution frequently has a pH of 7 (neutral acidity). This method aims to simulate the in vivo periodic pH alternation, which takes place in the mouth when sugars are processed resulting in caries development with the natural dynamic of t mineral loss and gain involved in the process. Using pH cycling to create enamel lesions has been found to result in higher demineralization than acid gelation but shallower lesions than those naturally produced, which has been attributed to the disregard of saliva and biofilm effect. Therefore, the pH cycling method is widely used in studies that involve dentine caries or used demineralized dentine models (Zhao *et al*., 2017).

Dentine Remineralization Pathways

Dentine structure consists of highly organized organic components where minerals are attached in a specific location and manner. This indicates that dentine remineralization is a complex process that

requires understanding at a molecular level. Various pathways have been described for dentine remineralization. The classic crystallization pathway, which involves producing large amounts of calcium phosphate crystals on collagen in a liquid environment containing minerals, was the main focus of early studies on dentine remineralization. These studies resulted in insufficient evidence, due to the fact that they were unable to accurately reproduce the proper arrangement of hydroxyapatite in the collagen fibers of natural teeth. Afterward, dentine biomimetic studies have become more prevalent as type I collagen and non-collagenous proteins research has evolved. Investigations on methods involving intrafibrillar remineralization, which more closely matches the development of natural teeth, have gained more interest in biomaterial research (Tay & Pashley, 2008). Based on the mechanism of how apatite crystallizes and grows, dentine remineralization pathways can be divided into classical and non-classical approaches.

The classical remineralization approach is often referred to as traditional top-down, ion-based remineralization (Liu *et al*., 2011b). The remineralization potential of fluoride-releasing materials is based on this approach. It depends on the epitaxial deposition of ions over pre-existing apatite crystals. These crystals serve as nucleation sites for the precipitation of primary ions from surrounding solutions until these ion clusters reach a size called "critical crystal nucleus. Later on, they grow using ion-by-ion attachment. Due to their relatively large size, these crystals are not able to penetrate the intra-fibrillar spaces within the collagen fibers, resulting in a collagen matrix with extra fibrillar remineralization only (Liu *et al*., 2011a). Current research on this form of classical-ion-based remineralization has labeled it as a non-functional or insufficient form of remineralization (Tay & Pashley, 2008; Liu *et al*., 2011a). Additionally, this approach cannot be used to remineralize caries-infected dentine or demineralized dentine, as these areas lack the presence of seed crystallites which are necessary for apatite formation.

On the other hand, the non-classical particle-based dentine remineralization mechanism is based on stabilizing ion-rich liquids using biomimetic analogs of non-collagenous matrix proteins rather than seed crystallites (NCP) (Liu *et al*., 2011b). An example of available bioactive restorative material that acts as a source of calcium ions and has been used to induce functional remineralization through this bottom-up remineralization technique is calcium silicate cement. In this approach the exposed collagen's mechanical properties are thereby restored, and brought closer to those of mineralized tissues, making this pathway a more functional remineralization strategy. Therefore, unlike the classical approach, it is thought to be highly beneficial when repairing completely demineralized dentine (Niu *et al*., 2014). One of the recommended analogs to be used in this pathway is a polyanionic molecule, such as polyacrylic acid. It has the role of creating flowable nano-aggregates (ACP) that act as nano-precursors for the further growth of the crystallites. As these molecules are small in size, they can penetrate collagen fibrils and subsequently precipitate as apatite nanocrystals in the water-filled gap zones. Later, a second analog is used, which acts as dentine matrix phosphoproteins in directing further precipitation and growth into apatite. This subsequently promotes crystal alignment and results in the desirable hierarchical dentine remineralization. Although some recent attempts to make these analogs available for clinical usage have shown promising outcomes, the use of these analogs remains difficult (Qi *et al*., 2012).

Applied Bioactive Restorative Materials

The minimally invasive dentistry approach should be applied whenever operative intervention is indicated. This approach aims to selectively remove the diseased tissue and replace it with a therapeutic-bioactive material that ensures extended longevity of the restoration. These materials are expected to remineralize affected-firm tissue that is left at the base of the cavity through the release of an adequate amount of minerals in a favorable environment. According to Larry Hench bioactivity means that materials should be able to "elicit a specific biological response at the interface that leads to bond formation between the tissues and the material" (Jones, 2015). This response is expected from the remaining tissues through ion release from the material that may lead to a chemical bond at the interface. Later on Kokubo and colleagues 1990, reached a consensus regarding a more universal definition of Bioactivity: The ability to form measurable surface apatite by 28 days in a specific SBF containing inorganic phosphate (Kokubo *et al*., 1990). This definition limits the restorative bioactive materials to those which induce functional remineralization only. Subsequently, ion-releasing materials such as Glass ionomer restorations are described as bio-interactive and not bioactive materials (Gandolfi *et al*., 2015; Minh, *et al*., 2021).

MI operative approach demands the use of bioactive restorative materials that can: 1) supply mineral ions (such as silica, calcium & phosphate); 2) form a bond with collagen (to form a template of calcium & phosphorus and in addition, act as a nucleation precursor for true apatite crystallization); 3) protect collagen from further deterioration; 4) provide a suitable pH to enable new mineral formation; and 5) prevent bacterial activity and growth (Toledano Pérez & Osorio Ruiz, 2016). These requirements are not present in all bioactive materials available in the market with the majority of them leading to incomplete remineralization, however, their important therapeutic role at the interface cannot be denied. Therefore, the classification of bioactive materials should be based on either functional or non-functional remineralization that enables a simple understanding of the materials' ability and the resultant properties of the remineralized tissues (McCabe *et al*., 2011; Abdulrahman, *et al*., 2022). Based on their behavior with the dental tissues, bioactive materials have been categorized earlier into three main categories: [1] materials that only remineralize hard tissues via minerals release such as fluoride, result in simple mineral deposition into the crystal voids to replace lost minerals in demineralized tissues. Examples of these materials are Glass ionomer cement; and its derivative, compomers, and giomers restorative materials where mainly fluoride supports the remineralization by carrying calcium and phosphate ions and participating them into the remineralized surface. However, in order to restore dentin functionality following remineralization therapies, such mineral formation is a requirement but not always sufficient. [2] The second category describes materials that form Apatite-like molecules at the interface especially when immersion in liquid that is similar to the normal physiological fluids. These

materials contain high amounts of calcium and phosphorus ions with the critical apatite pH that promote the formation, growth, and attachment of hydroxyapatite, e.g. Calcium aluminate materials. [3] The last category in this classification includes the materials that result in tissue regeneration besides ion remineralization and apatite formation. These materials can induce healing responses and develop dentine bridges, e.g. Calcium silicate cement. (Hamdy, 2018; Osipchuk, *et al*., 2023).

A proposed classification based on the remineralization pathway of the materials may offer a basic understanding of their potential and clinical use. Bioactive restorative materials can be classified into Classical and non-classical bioactive materials **(Figure 2)**.

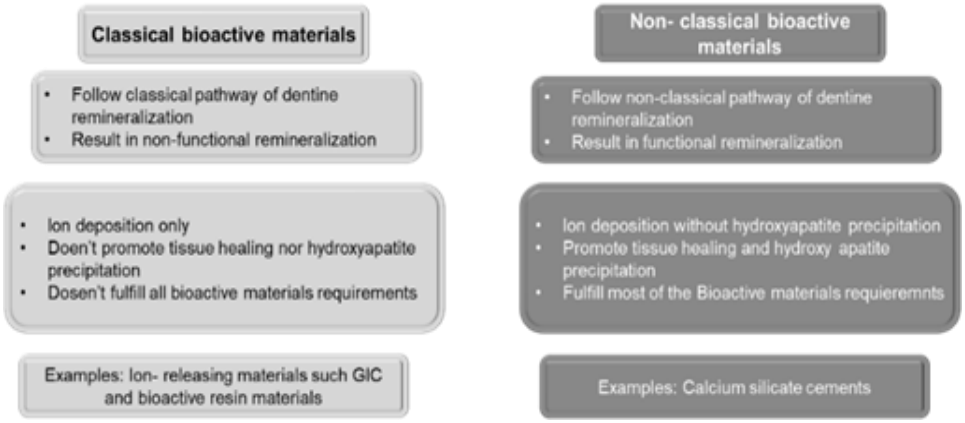

Figure 2. Classification of Bioactive materials based on dentine remineralization pathways

Classical Bioactive Materials

Materials that use the classical, non-functional, top-down dentine remineralization pathway. They provide sufficient and essential ions by high initial influx that can attach to the existing crystals and start growing to fill the voids within the collagen. The development of these materials focuses on the continuous release of these ions that are regulated by the pH levels in the oral environment. They usually claim that those materials react to pH changes in the mouth. High amounts of calcium, phosphate, and fluoride ions are released during lower pH demineralization cycles. These ions supersaturate the saliva and are free to precipitate onto the tooth as hydroxyapatite or fluorapatite. Similar to this, teeth respond to pH cycles by releasing and recharging their ionic components (Pulpdent, 26-03-2023). These materials usually have an acid in their composition and use acidic etching to chemically bind to the tooth structure by forming an ion exchange layer. This process uses the Hydrogen ions which are released from acid to dissolve materials' particles and result in ion release. Simultaneously, they etch the underlying and lead to ion release from the tooth, the result is a thick ion exchange layer between the material and the cement (Atmeh *et al*., 2015; Sajini *et al*., 2022a). This process is known as 'Acidic etching of dentine". Glass ionomer cement, Giomers and compomers are examples of bioactive materials in this category.

Non-Classical Materials

In this category, the materials remineralization pathway is the non-classical, functional, bottom-up one. These materials are composed of calcium and phosphate ions that are solubilized and become available for remineralization. The development of these materials is based on providing amorphous ions to act as nano precursors for hydroxyapatite crystals and then regulating the position, and growth of these precursors into highly organized intrafibrillar apatite (Liu *et al*., 2011a). Usually, these materials are composed of calcium silicate, calcium aluminate, or calcium aluminoferrite and propose an alkaline nature (high pH). This alkalinity has an important role in the effective intrafibrillar remineralization of dentine. It is found that the materials' high pH results in caustic dentine degradation occurs in conjunction with a mineral-rich tag-like structure that infiltrates into the underlying dentinal tubules as well as an interfacial mineral infiltration zone that is usually found with classical bioactive materials (Atmeh *et al*., 2015; Sajini *et al*., 2022a). This etching process has been termed the "caustic etching of dentine" (Watson *et al*., 2014; Kopzhassarova, *et al*., 2021; Sajini *et al*., 2022a). Moreover, this alkaline nature has an important role in the reduce MMP activity in addition to beneficial anti-bacterial effects (Tawil *et al*., 2015). Examples of materials in this category are calcium silicate cement such as Biodentine TM and Theracal LC and calcium aluminate restorative materials.

Conclusion

There are multiple pathways of dentine remineralization that bioactive materials follow. It is crucial to know the potentials and limitations of commercial bioactive materials to match the clinical situation with the best material to use. In combination with good diagnosis, practitioners can select the best restorative materials to apply on the substrate left behind after caries excavation. Caries affected dentine can be remineralized with any category of bioactive restoration. Conversely, caries-infected dentine has no remaining crystals therefore, only non-classical bioactive materials may be able to remineralize the dentine (Sajini *et al*., 2022a). For effective remineralization of both substrates, the pH and MMP activity in the remaining dentine must be controlled. This can be achieved by developing a therapeutic bonding system that depends on an understanding of the ion-releasing mechanism of materials. Combining the use of such contemporary adhesive systems with ion-releasing restorative materials will result in decreasing the

degradation of the tooth-restoration interface and long-lasting performance.

The development of bioactive restorative materials is challenged by balancing between bioactive properties and essential physical and mechanical ones. Intensive research evaluating the performance of these materials is available but still lacking (Sajini *et al*., 2022c).

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