

Biomimetic Approaches of Dentin Regeneration

Vassileva R¹, Dyulgerova E¹, Gusiyska A², Mironova J², Gyulbenkiyan E².

¹Professor, Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University - Sofia, Bulgaria

²Assistant Professor, Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University - Sofia, Bulgaria

Abstract: *Dentin is a unique dental tissue in structure and composition that forms the main part of the teeth. Minimally invasive approaches today widely use enamel remineralization - a strategic concept in preventive treatment without restoration. The dentin remineralization is more complex but very promising for a variety of clinical applications. These applications are dentin and root caries, dentin hypersensitivity, acid-etched dentin that is incompletely infiltrated by adhesives and partially demineralized as a result of a carious process (i. e. affected dentin), especially in deep carious lesion. In order for remineralizing biomimetic materials to be implemented clinically and for practical recommendations to be offered, fundamental research studies on dentin remineralization and regeneration are still needed.*

Keywords: dentin, nanohydroxyapatite, nanoparticles, regeneration, remineralization.

1. General definitions and trends

Dentin, a dental tissue unique in structure and composition, forms the main part of the teeth. Its unique features are reflected the development of dental caries in dentin. For this, the standard treatment requires the removal of the carious part and subsequent restoration – the so-called (Drill and Fill technique). The most recent trends in conservative approaches aim to reduce the use of the usual restorative treatments and increase opportunities for the preservation of tooth structure.

Today, minimally invasive approaches generally apply enamel remineralization – a strategic concept in preventive treatment without restoration. Nanohydroxyapatite (HA) at a concentration of 10% and applied dynamic pH cyclical conditions are approved agents for remineralization. The deposition of minerals occurs primarily in the "external layer of the lesion" and is expressed at a pH of less than 7. The addition of specific substances, such as Gallachinensis extract (GCE), leads to large amounts of deposited crystals in "the body of the lesion". These crystals are evenly arranged, forming a dense, homogeneous structure in the outer enamel layer.

Unlike enamel remineralization, dentin remineralization is still in the early stages of development. The aim of the latter approach is to achieve significant recovery of the mechanical properties of the hydrated carious tissues – so-called functional remineralization. This requires further clarification of the mechanisms of biomimetic mineralization, particularly those for achieving optimal mineralization of collagen fibres both intra- and interfibrillar. This is hampered by the large amount of organic matter that grows in the carious dentin, as well as by the water content of the dentin. Of particular importance in operative dentistry are the characteristics of the so-called "affected" dentin [4], that is, the dentin subject to repair by remineralization. This dentin will have a reduced mineral content when compared to normal dentin. The amount of Mg is also reduced, which indicates a loss of peritubular dentinal matrix; the mineral crystals are dispersed, distributed randomly and varying in shape and size. Moreover, the apatite crystals are larger than those of normal dentin, and the intercrystalline spaces are

wider when compared to intact dentin. Furthermore, the collagen crosslinks are reduced in the organic phase. The dentin's mechanical properties are also reduced: it is softer, with lower tensile strength and lower Knopp hardness. These differences are commonly observed after the removal of carious dentin. Thus, it should be kept in mind that affected dentin is very different in terms of its morphological, mechanical and chemical characteristics when compared to normal dentin, and this, in turn, affects its bond strength with adhesive materials.

1.1. Mechanism of mineralization

It is assumed that secondary mineralization in carious dentin proceeds primarily through an increase in the amount of crystals retained in the lesion and not by a process of nucleation, precipitation or growth of mineral in the organic matrix, which in fact is mainly introduced by collagen type I [7, 8]. Early experiments involving remineralization with nanoscale bioactive glass particles and β -tricalcium phosphate show that the mechanical characteristics of healthy dentin cannot be reproduced. The modern approach involves the addition of polyacrylic acid (PAS) to a phosphate-containing liquid. As a result, a metastable amorphous calcium phosphate (ACP) nano-precursor is formed and deposited on the acid-demineralised collagen matrix. It is assumed that it undergoes a phase transformation associated with the formation of apatite crystallites arranged along the C-axis of the collagen fibres. With respect to the mechanical properties of the dentin, it is necessary to note that its mineral nanoparticles that are embedded in collagen protein fibres are pre-compressed, and this internal pressure helps prevent the spreading of cracks, thereby increasing the stability of the dentinal biostructure.

Another approach, proposed by F. Tay and D. Pashley [16], involves the use of white Portland cement as a source of Ca ions in the phosphate-containing liquid. This is used for guided remineralization of partially demineralized dentin, both when it is incompletely infiltrated with dentine adhesives and when the dentin is partially demineralized by a carious process. PAS and polyvinyl phosphoric acid are also included as nanoprecursors, and these transform into polyelectrolyte-stabilized apatite crystals that accumulate

along the surface of microfibrils. Some have suggested that the transition from nanoparticles to larger apatite structures is carried out by passing into the mesostructures. Other authors suggest that the so-called "smart" composites contain reactive Ca-silicate mineral dust originating from Portland cement [5].

1. 2. The clinical areas of application

The applications of this type of remineralization and regeneration are diverse – dentinal caries, first, and also – root caries and dentinal hypersensitivity. A very important indication is the remineralization of acid-etched dentin that is incompletely infiltrated by adhesives and partially demineralized as a result of a carious dentinal process (i. e. affected dentin), especially in deep carious lesion.

2. Analysis of the dentin structure in terms of its regeneration treatment approaches

Dentin has a complex biological structure – fibrils of type I collagen form a 3D matrix that is reinforced with nanoapatite crystallites. This structure may be used as a basis (template) for further therapeutic strategies after the demineralization of dentin and the disclosure of collagen due to various reasons. At this point, however, it is important to remember that the disclosed collagen fibrils are subjected to proteolytic enzymes in the presence of biofilm. In advanced terms, the dentin represents a mineralized matrix of dentin tubules with intertubular dentin between them (Fig. 1) – the so-called tubular structure.

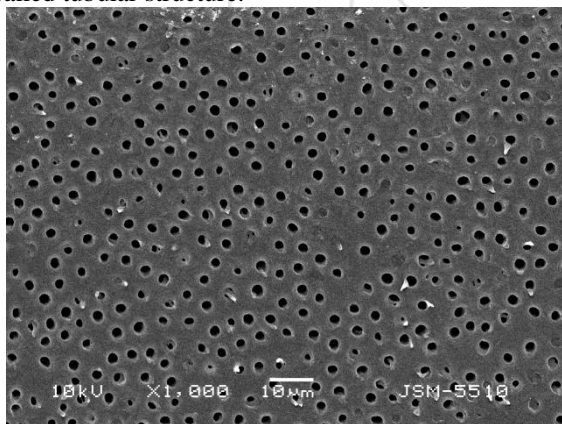


Figure 1: Dentin tubules with intertubular dentin between them - a SEM micrograph (magnification x 1 000).

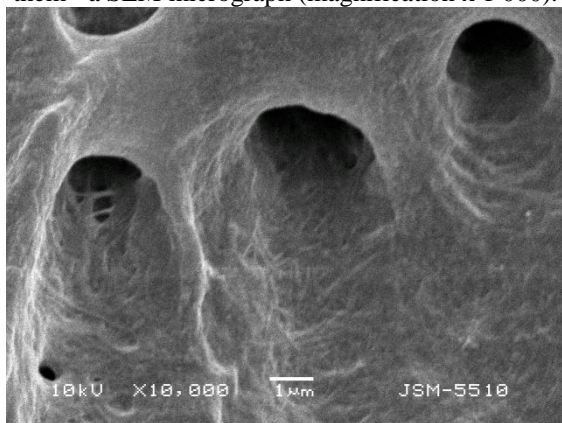


Figure 2: Dentin tubular structure - a SEM micrograph (magnification x 10000).

The complexity of dentinal structure leads to difficulties in assessing various therapeutic concepts applied to its preservation or remineralization (i. e. regeneration). In search of opportunities, there have been a number of experiments with different substances. For instance, colloidal HA and β -tricalcium phosphate are applied [15]. It was found that the latter regenerates the intermolecular collagen's cross-linking relations by combining them with adjacent interfibrillar areas, leading to better recovery of the dentin's micromechanical properties.

Other attempts include nanometrical bioactive glass particles applied in vitro [20]. The conclusions indicate an increase in mineral content, but without achieving the mechanical properties of natural dentin. However, it is emphasised in the study that the pastes that were subject to the in-vitro experiments contained HA nanoparticles and had a better effect in terms of mineralization than usual amino-fluoride toothpastes [19].

3. Indications of dentin regeneration (remineralization)

3. 1. Dentin hypersensitivity

Dentin hypersensitivity is a broad area for the clinical application of dentinal remineralization, as it affects mainly elderly patients and those with periodontal problems. Open dentinal tubules can be observed in these groups. Here, due to the presence and movement of dentinal fluid, the subodontoblastic plexus is severely irritated, especially when exposed to cold, hot or active osmotic stimuli. Different medical prescriptions can be applied. In one study, carbonate-apatite nanocrystals [14] are used for a 10-minute application, allowing time to sufficiently close the dentinal tubules (DT), and the results were evaluated by transmission electron microscopy (TEM). In a further double-blind study, zinc carbonate-apatite crystals in 70 patients [12] were used for a test. The findings show filling of the dentinal tubules, but the authors emphasise the need for repeated applications. The studies cited include commercial products. There are also *experiments* that include products synthesised specifically for the scientific experiment, such as nano-structured calcium phosphate and collagen [3], as well as nanometrical carbonate – apatite [10].

An interesting product, available from Guentsch et al. 2012 [6], is a glycerine gel enriched with gelatine and containing phosphate and fluoride ions. This product, which is seen as a biomimetic remineralization system, has a desensitizing effect that lasts for about 12 months, which is an undisputable success in terms of clinical outcome.

3. 1. 1. Using dentin remineralization in the case of root caries

For root caries, synthesized products with Ca-Na phosphosilicates in the form of bioactive glass are used. Upon the release of ions, new hydroxycarbonate apatite crystals (HCA) are formed, making the dentin more resistant to acid attacks and reducing the sensitivity of the tooth by filling the opened dentinal tubules with layers of HCA. These bioactive glasses are added to toothpastes that containing only high concentrations of fluorine. They can

also be incorporated into chewing gums to achieve a remineralization effect.

3. 2. Stabilization of the intermediate zone (interface) of dentin adhesives

There are several trends in this important area in terms of minimally invasive adhesive treatment.

3. 2. 1. Creation of antibacterial adhesives

In this technique, incorporated TiO₂ nanoparticles are used. These have a tendency to stabilize the structure of the dentin and to remineralize the dentin in the presence of tissue fluids [21]. They are expected to close the gap at the interface and consequently to reduce the occurrence of secondary caries, except through remineralisation, and to prevent bacterial infection. Other studies use nanoparticles of amorphous calcium phosphate (NACP) in concentrations from 10% to 40%. The nanoparticles are infiltrated into the dentinal tubules (not only those that are straight, but also those that are curved or irregularly shaped). This prevents the formation of biofilms and the development of secondary caries (11).

3. 2. 2. Improving adhesive polymerization through catalytic activity of nanoparticles

With this process, an adhesive layer is created, improving the dentin's mechanical properties and its resistance to degradation. For this purpose, colloidal platinum nanoparticles are used in the dentin treatment [22].

3. 2. 3. Antibacterial adhesives with nanosilver particles.

This system, used in orthodontics, is applicable in orthodontic treatment for enamel remineralization and for the inhibition of bacterial adhesion to orthodontic appliances and braces [1], and therefore will not be discussed.

3. 2. 4. Dentin adhesives visible on x-ray

This innovative approach uses flame-made Ta₂O₅ / SiO₂ nanoparticles in methacrylate binders.

3. 2. 5. Self-adhesive composites.

Here, the trend is to create flowable composites, such as the Vertise™ Flow (Kerr Dental) and Fusio™ Liquid Dentin (Pentron), into which adhesive systems are added. This simplifies the clinical application, and the implementation of the adhesive is skipped.

3. 2. 6. Self-healing adhesives

These adhesives are polyurethane nano-capsules that contain TEGDMA as a major component [13]. It is expected that this incorporated monomer will improve bond strength and durability.

One important method for the study of adhesion is high-speed atomic force microscopy (HS-AFM), which allows dynamic biological processes to be studied. Dimensional and temporal analysis are accurate within nanometers and milliseconds, respectively, and provide information about the biological processes at molecular level [2].

3. 4. Remineralization in deep dentinal caries – indirect pulp capping

Many attempts at remineralization with fluorides have been made in this regard [17], but it turns out that this method requires special conditions, and the process of remineralization is too long, making it unsuitable for application in clinical settings. Ca(OH)₂ has also been used for the same purpose [7], in both in-vitro and in-vivo experiments, and its remineralizing properties have been demonstrated. However, its effect on collagen fibres has not been fully investigated.

One group of researchers dealing with deep carious lesions used an innovative approach, applying clusters of amorphous calcium phosphate (ACP), which have outstanding remineralizing potential for collagen [23]. An analogue was made with PAC (polyacrylic acid) and PASC (polyaspartic acid), which stabilize ACP, because of their ability to chelate calcium ions (property due to their many carboxyl groups), and the composition of carboxymethyl chitosan was chosen. This derivative of chitosan also has many carboxyl groups, which may delay or inhibit the spontaneous precipitation of calcium phosphate and have chelating capabilities. The carboxymethyl chitosan is recommended as an indirect pulp agent in combination with ACP. Moreover, it can be converted into a matrix (scaffold) through lyophilization for further remineralization. Furthermore, the authors focus on its biological compatibilities and degradation, antibacterial properties and lack of toxicity.

These examples of the application of dentin remineralization prove that this relatively new approach can be quite promising in various areas of clinical application. This is so because biomimetic nanomaterials are used. They are completely new as a concept, and thanks to their nanostructure, they come close to natural tooth tissues. However, in order for them to be implemented clinically and for practical recommendations to be offered, fundamental research studies on dentin remineralization and regeneration are still needed.

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