Biomechanical Perspective on the Remineralization of Dentin

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Abstract

The objective of this article is to critically evaluate the methods that are used to assess outcomes of remineralization of dentin. Currently, the most used assessment methods fall either into quantitative analysis of the mineral content of the remineralized structures or dry measurements of their mechanical properties. Properties obtained from the dehydrated organic dentin matrix may not reflect the true mechanical behavior of the remineralized tissue under physiological and hydrated conditions. Here we seek to clarify the biomechanical aspects of remineralization of dentin, pointing out the effects of hydration and dehydration on the mechanical properties of treated tissues. We also emphasize that a more appropriate endpoint to evaluate the effectiveness of remineralization in dentin should be associated with the recovery of the mechanical properties of the hydrated tissue, which is presumed to correlate well with its overall functionality.

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Dentin is the most abundant mineralized tissue in the human tooth; therefore, its mechanical performance is of major significance to the overall function of teeth. Remineralization of carious dentin has, as its ultimate goal, the re-establishment of the functionality of the affected

tissue, which can be described as the competence of the dentin in accomplishing its role in the tooth. In brief, from a biological perspective, the dentin matrix can be described as a semipermeable barrier between enamel and pulp, or between the pulp and the outer surface of a cavi-
tated lesion with exposed carious dentin. From a microstructural perspective, the collagen fibrils in dentin serve as a scaffold for mineral crystallites that reinforce the matrix, supporting the surrounding enamel. From a biomechanical perspective, in brief, the mineralized dentin matrix preserves tooth function by helping to prevent propagation of cracks from the brittle enamel through the dentin-enamel junction into dentin [Imbeni et al., 2005], thus preventing fracturing of the enamel crown. Dentin properties are similar to the surrounding mineralized tissues, e.g. cementum and bone, thus reducing stress concentrations at the interfaces during deformation. There have been numerous investigations devoted to better understanding the biological and microstructural changes relating carious and remineralized dentin [Fusayama, 1997; ten Cate, 1997; Marshall et al., 1997; Paes Leme et al., 2006]. However, although previous investigations have suggested the importance of the mechanical recovery of dentin after remineralization [Kinney et al., 2003a; Suppa et al., 2006], there is a lack of information in the current literature with regard to the fundamentals of this phenomenon. Therefore, the rising minimally invasive approach in the control of dentin caries brings forth a need to assess models that can elucidate this aspect.

Dental caries results from the interactions of specific oral bacteria with constituents of an individual's diet

within the dental plaque. As a result of these interactions, acidic by-products, mainly lactic acid [Loesche, 1986; Paes Leme et al., 2006] diffuse initially within the enamel, or cementum, and subsequently through coronal or root dentin, dissolving mineral crystallites on their way [Featherstone, 1996]. Once the lesion reaches the dentin matrix, it progresses much more rapidly as compared to enamel, thus creating different zones that reflect differences of mineral content, mechanical properties and optical appearance as evidenced by a caries detector stain [Kuboki et al., 1977, 1983; Zheng et al., 2003; Pugach et al., 2009]. Previous work suggests that regrowth of mineral within these different zones may be possible [Shimizu et al., 1981; ten Cate, 2001, 2008; Maltz et al., 2002; Tay and Pashley, 2008]. Remineralization of carious dentin can occur either by a spontaneous incorporation of ions (calcium, phosphate and fluoride) from the oral fluid onto remnant crystallites in the demineralized tissue [Featherstone, 1990; ten Cate and Featherstone, 1991] or by treatments that incorporate the same ions from external sources.

The quality of dentin is dependent upon the sum total of characteristics of the tissue that influence its competence: microstructure, mineral density and especially the particular location of the mineral with respect to organic structures of the tissue. Therefore, the evaluation of remineralized tissues should reflect the integration of these features, accounting not only for mineral density, but also its intrinsic mechanical behavior under hydrated conditions, which reflects the interaction between the organic and inorganic components of the matrix. Here, in order to bring insight and stimulate discussion into this aspect, we critically examine some assessment methods currently available to evaluate the effectiveness of remineralization of dentin and emphasize the biomechanical perspective of this matter.

Structural Hierarchical Modeling of the Dentin Matrix

The microstructure of dentin, a composite mineralized tissue, suggests the necessity of a hierarchical approach to the understanding of its mechanical properties [Weiner and Traub, 1992; Kinney et al., 2003b]. The dentin matrix (fig. 1) is mainly composed of type I collagen fibrils with associated noncollagenous proteins, forming a three-dimensional matrix that is reinforced by mineral. The mineral is a carbonated nanocrystalline hydroxyapatite that is partitioned according to its location with respect to the collagen fibrils into: extrafibrillar mineral,

located in the spaces separating the collagen fibrils [Katz and Li, 1973; Katz et al., 1989; Landis, 1996], and intrafibrillar mineral that is generally believed to be mainly in the gap regions of the fibrils extending between tropocollagen molecules [Landis et al., 1993; Balooch et al., 2008; Jager and Fratzl, 2000]. There is uncertainty over the specific morphology of the mineral crystallites. Kinney et al. [2001b] used small-angle X-ray scattering to indirectly assess the micromorphology of the apatite crystallites in dentin and suggested that the mineral has a rod-like shape near the pulp and is more plate-like, with approximately 5 nm thickness, nearer the dentin-enamel junction. Similarly, Nalla et al. [2005], using transmission electron microscopy (TEM), also confirmed early observations from Boyde [1974] suggesting the presence of needle-like crystallites in the intertubular dentin region. On the other hand, Lowenstam and Weiner [1980], also using TEM, evaluated the ultrastructure of the crystallites in bone (which has a similar model of mineralization) after removal of its organic structures and suggested that the average length and width of the crystallites are 50 and 25 nm, with an approximate thickness of 2–3 nm, resembling plate-like structures.

Most micromechanical models of mineralized tissues assume that both the intrafibrillar and extrafibrillar mineral phases behave linearly elastically at small strains [Pidaparti et al., 1996]. However, more recent studies [Kinney et al., 2001a; Kinney et al., 2003a] provided substantial evidence that in the absence of the intrafibrillar mineral the expected linear relationship between mineral content and mechanical properties of dentin vanishes when the tissue is hydrated. These studies suggested that the reestablishment of functionality of affected dentin requires not only mineral formation extrafibrillarly, but also intrafibrillarly within the gap zones of the collagen fibrils. Therefore, mineral formation is a necessary, but may not be a sufficient, condition for reestablishment of dentin functionality after remineralization treatments. Hence, a more appropriate endpoint to evaluate attempts of remineralization in dentin should be the establishment of mechanical properties consistent with the normal tissue under hydrated conditions.

Assessment Methods of Remineralization in Dentin

Various methods have been suggested for evaluating the effectiveness of remineralization in dental tissues; however, to date they have been mostly based on the determination of changes in its mineral content [Arends et

Fig. 1. Structural hierarchical modeling of the dentin matrix. From left to right: SEM image of a fixed, demineralized dentin matrix showing the collagen fibrils surrounding dentinal tubules [Marshall et al., 1997]. In the schematic sequence, on the left, collagen fibrils show the extrafibrillar mineral between fibrils. In the middle, the arrangement of the collagen molecules yields 40-nm gap zones and 27-nm overlap zones resulting in the typical 67-nm periodicity of a collagen fibril. The length of the collagen protein triple helix is 300 nm. On the far right, the intrafibrillar mineral is represented sitting in the gap region between the collagen molecules. Non-collagenous proteins are not represented. Figure not drawn to scale. Modified from Rho et al. [1998].

al., 1997]. Recent studies have assessed the reincorporation of mineral into demineralized dentin using indirect qualitative analysis, such as polarized light microscopy [Arnold et al., 2007] and TEM [Tay and Pashley, 2008], and semiquantitative analysis such as transverse microradiography [ten Cate, 2001; Zaura et al., 2007]. Polarized light microscopy allows for the identification of dentin birefringence. In water or conventional mounting media, dentin birefringence is dominated by strong positive form birefringence (due to oriented submicroscopic pores) and weaker positive birefringence due to the collagen. As demineralization occurs, the strength of the birefringence increases by reduction of the weak negative birefringence due to the mineral, but the quantitative relationship between changes in mineral content and birefringence has not been fully established [R.P. Shellis, pers. commun.], which limits its usefulness in determining remineralization of dentin. TEM provides information on crystal shape and structure, but samples very small tissue volumes that may not represent the bulk material.

Further, TEM imaging does not enable the distinction of mineral that is closely positioned to the organic matrix from mineral that is chemically bound to it. Transverse microradiography uses the degree of absorption of the X-ray intensity to quantify the amount of mineral incorporated into the tissue based on changes in gray levels in the through-thickness images when compared with standards. Other measures of mineral density have been obtained by thermogravimetric analysis, which is used to determine the weight of mineral gained compared to a control, and element-sensitive electron microscopy, which measures the ratio of calcium to carbon in treated specimens [Vollenweider et al., 2007]. Spectroscopic analyses, such as Raman and Fourier transform infrared spectroscopy, are also often used in dentin remineralization studies [Kawasaki et al., 2000; Rahiotis and Vougiouklakis, 2007]. These methods allow the determination of the nature of the mineral and also provide quantitative information on the changes in mineral and matrix composition as mineralization occurs [Boskey and Men-

delsohn, 2005]. Although these methods separate the responses of the mineral and the organic structures in the dentin matrix [Boskey and Mendelsohn, 2005], they do not differentiate the contributions of intra- and extrafibrillar mineral. Most importantly, they cannot evaluate the effectiveness of the remineralization procedure on the mechanical reinforcement and recovery of the mechanical properties of the damaged tissue. Therefore, evaluations of the effectiveness of restoring the functionality of the tissue cannot be made based on data provided by the above methods.

Biomechanical properties of dentin in particular and of dental tissues in general have been mostly evaluated by microhardness testing methods [Arends and ten Bosch, 1992; Featherstone, 1996], using either cross-sectional or surface assessments in dry conditions. In a microhardness test, a diamond indenter is brought into contact with the surface of the specimen at specific loads; the indentations are then measured (dry) and converted to a hardness value. However, the inability to measure the indentations in water is a significant drawback to the use of microhardness tests for the evaluation of the mechanical properties in composite mineralized tissues such as dentin, since the mechanical behavior of wet and dry dentin can be remarkably different [Kinney et al, 2003a, b; Angker et al., 2004].

The method of nanoindentation [Doerner and Nix, 1986; Oliver and Pharr, 1992], where force and indenter displacement are recorded continuously during the indentation and the elastic modulus and hardness are determined from the load displacement curve, has been applied to mineralized tissues only in the last decade. Balooch et al. [1998] applied an atomic force microscope with a nanoindenter to perform nanoscale indentations on fully hydrated specimens. In addition to allowing nanomechanical measurements to be done in water, it has also allowed precise positioning of the indenter and subsequent imaging of the indentation with submicrometer spatial resolution. Although nanoindentation probes only a thin surface layer (usually $<1\ \mu\text{m}$), the mechanical properties obtained compare well with bulk material measurements [Kinney et al., 2003b]. This permitted specific evaluations of the elastic mechanical properties of peritubular and intertubular regions in normal dentin [Kinney et al., 1996] and various zones of carious dentin [Marshall et al., 2001], the determination of the viscoelasticity of demineralized dentin [Balooch et al., 1998] and the isotropic elastic behavior of intertubular dentin [Kinney et al., 1999]. Although nanoindentation has now become a common technique for the determination of local mechanical

properties of structural features in biological hard tissues [Rho et al., 1999], many recent studies still rely on measurements of remineralization of dentin based on its mechanical recovery under dry conditions [Kielbassa et al., 2002; Turssi et al., 2006; Vollenweider et al., 2007; Shibata et al., 2008; Chu and Lo, 2008]. Such measurements made in dry conditions can produce nonphysiological results.

Dynamic Mechanical Behavior of Remineralized Dentin Matrix as Influenced by Hydration and Drying

The elastic properties of dentin should be related to the relative proportion of its constituent materials [Driesen and Verbeek, 1990]. In function, dentin is a hydrated tissue composed of approximately 50 vol% mineral, 30 vol% collagenous and noncollagenous proteins and the remainder of fluids [Marshall et al., 1997]. Therefore, its mechanical properties should be evaluated on the fully hydrated tissue.

It has been suggested that the extrafibrillar mineral in normal dentin can be considered as having a granular structure that is highly compliant due to moisture or attached proteins [Kinney et al., 2003a]. Recent studies have also suggested that the intrafibrillar mineral is rather resistant to demineralization and dominates the elastic behavior of collagen fibrils during loading [Balooch et al., 2008] so that the stiffness of the collagen fibrils is strongly dependent on the presence of the intrafibrillar mineral [Kinney et al., 2003a]. The higher resistance of the intrafibrillar mineral to demineralization may have important consequences for remineralization, as partially demineralized dentin may contain intact collagen fibrils with remnant mineral and associated noncollagenous proteins that could act as sites for regrowth of any lost mineral. This conjecture is further strengthened by observations by Clarkson et al. [1991] that demineralization also results in removal of noncollagenous proteins in two steps, a relatively easily removed soluble component and a more difficult-to-remove insoluble component that might remain bound within the collagen fibrils in association with the remaining mineral.

After partial demineralization, drying induces collapse and shrinkage of collagen fibrils that may exert compression on remaining extrafibrillar granular crystallites, inducing increased values of elastic modulus and hardness during indentation. When demineralized, the extrafibrillar mineral phase surrounding the collagen fibrils is preferentially removed [Balooch et al., 2008] and

Fig. 2. Schematic of the mechanical response of remineralized dentin. **a** Poor remineralization under hydration, suggesting the swelling phenomena of the organic matrix and poor interaction of mineral within collagen fibrils lead to low mechanical properties. **b** With drying, the collagen fibrils collapse, exerting compressive contacts constraining the unbound mineral, which results in a misleading interpretation of improved mechanical response. **c** When functionally remineralized, the collagen fibrils are reinforced by intrafibrillar mineral, and therefore the response under loading will be high (similar to normal dentin) even when hydrated.

the collagen fibrils and noncollagenous proteins remain exposed and swell in the presence of water. The water-infiltrated collagen becomes highly deformable under load, as the fluid is expressed by the indenter contact region and flows back as it recovers [Angker et al., 2004]. If the volume fractions of the dentin components are assumed to be fixed at a given stage of demineralization, any increase in the water content occurs mainly as a replacement for the lost mineral, especially from the extrafibrillar compartment. Thus the collagen fraction will remain nearly the same [Weiner and Wagner, 1998], but the fibrils will be suspended in a moisture-rich milieu that offers low resistance to loading. Consequently the demineralized dentin will theoretically contain more water than the 20 vol% found in sound dentin and, as a result, hydration yields greater effects in the demineralized dentin [Angker et al., 2004].

Remineralization of dentin can occur either by precipitation of mineral between collagen fibrils or functionally, bound to its structure. Therefore, simple precipitation of mineral into the loose demineralized dentin matrix, the so-called net remineralization, provides an increased mineral content, but may not necessarily provide an optimal interaction with the organic compo-

nents of the dentin matrix. In this case, with drying, the reduction in the contact compliance of the precipitated mineral combined with collapse of the partially demineralized collagen network could account for the linear dependence of the elastic modulus relative to the mineral content in the dry tissue [Kinney et al., 2003a]. Hydration, however, presents greater significance in the evaluation of the mechanical response of the tissue after remineralizing treatments. In the absence of the intrafibrillar mineral and an optimal interaction of the granular precipitate within the collagen fibrils, the dentin matrix can incorporate more water and tends to swell more than the sound tissue (fig. 2a). As a result, the compressive stresses that consolidated the mineral lying between the collagen fibrils no longer exist, and the elastic constants become largely determined by the highly deformable organic network and are therefore quite low (fig. 3). The net effect may be one of relatively high mineral content, but very low mechanical properties; thus, mineral content alone may yield misleading information on the actual effectiveness of the remineralization treatment. Hence, in agreement with our previous hypothesis [Kinney et al., 2003a], we suggest that changes in the mineral content alone do not necessarily result in

Fig. 3. Nanomechanical properties of dentin as influenced by hydration and drying. De- and remineralized dentin present striking differences of reduced elastic modulus (**a**) and nanohardness (**b**) between the dry and wet tissues. Dentin was demineralized with acetate buffer at pH 5.0 for 8 h and remineralized with a solution containing 1.5 mM calcium and 0.9 mM phosphate for 2 days. Mechanical properties were evaluated using atomic-force-microscopy-based nanoindentation as described by Marshall et al. [2001].

recovery of the mechanical properties of remineralized dentin.

Ideally, the regrowth of intrafibrillar and extrafibrillar mineral between the fibrils would lead to the full mechanical recovery of the demineralized dentin. This would yield properties comparable to normal dentin and indicate successful functional remineralization. In this situation, the collagen fibrils become reinforced by the reincorporated mineral, which facilitates the transfer of load onto the extrafibrillar mineral. A remineralized tissue that has restored its mechanical properties under hydration is an indication that mineral crystallites are in tight association or perhaps chemically bound to the collagenous matrix (fig. 2c).

Although collagen fibrils in carious dentin may retain their structural identity as intact fibrillar entities [Yoshiyama et al., 2003], a recent study on the antigenicity of collagen and proteoglycans raised concern regarding the possibility of intrafibrillar (or functional) remineralization in sclerotic dentin [Suppa et al., 2006]. This hypothesis is testable, in principle, with the adjunctive use of atomic force microscopy and immunolabeling for TEM, as suggested by the authors.

Let us suppose that remineralization treatments actually restore the mineral content of the collagen fibrils. Will this necessarily lead to a recovery of the functionality of the dentin? From a biological perspective, dentin that has had its mineral content replenished but in which there is a poor association of mineral with the organic structures of the matrix may lack intrafibrillar mineral

and may be more susceptible to enzymic degradation than normal dentin, although it may be more resistant to demineralization, through increased size [ten Cate, 2008] and quality [Featherstone, 1996] of the mineral crystallites. From a microstructural perspective, the architecture of poorly remineralized tissue will present a decreased reinforcement of the collagen fibrils and an increased occupation of the extrafibrillar space by unbound mineral. Lastly, from a biomechanical perspective, the properties of dentin that has been remineralized without coupling between the organic and inorganic compounds should present significantly different properties from the normal tissue in water or under physiological conditions, as suggested in figure 3.

It is also valid to note that the observations described are mainly based on changes on mechanical properties obtained with quasistatic indentation techniques. More detailed determinations of biological, microstructural and other biomechanical changes of remineralized dentin will perhaps provide us with a broader understanding of the overall functionality of treated tissues. These developments should be encouraged so that better future strategies for remineralization of dentin can be designed.

Much work remains to be done to determine if successful remineralization of the intrafibrillar compartment in conjunction with the formation of additional mineral between the remineralized collagen fibrils will yield a regenerated structure that approximates the functionality of normal dentin.

Continuing Challenges

Although the reincorporation of mineral into the demineralized dentin matrix does not represent a full recovery of its functionality, it still plays a very important role, since the remineralized remnant crystallites in the subsurface of the tissue may be much more resistant to subsequent acid attacks [Featherstone, 1996]. Accordingly, the addition of fluoride to the lattice of the regrown mineral favors an improved resistance to acidic dissolution. Detailed electron-microscopic analysis of crystallites in various zones of caries lesions has also confirmed that remineralization occurs by growth of existing crystals [Silverstone et al., 1988], which may lead to the prevention of the recurrence of caries. Moreover, the mineral precipitated may work as a constant site for further nucleation of mineral ions present in the oral cavity, facilitating continuous remineralization over time. Therefore, even though the remineralized tissue may not exhibit the mechanical properties characteristic of normal dentin, it could have greater demineralization resistance, which stresses the necessity of studies using measurements of mineral reincorporation as well as mechanical properties.

Achieving remineralization of dentin remains one of the most difficult tasks in dentistry. In this article we have raised questions about the reliance on methods based on the traditional measurement of mineral content alone to evaluate the effectiveness of the remineralization of dentin. Assessment methods that would directly provide both quantitative and qualitative determinations of the exact positioning and mechanism of interaction of the mineral formed within the gap zones of collagen fi-

brils are still unavailable, and their development should provide improved understanding of remineralization strategies for carious dentin. Newer methods [Stock et al., 2008] using combined diffraction and small-angle X-ray scattering seem to be useful in this pursuit. Further evaluations of other biomechanical properties of remineralized dentin (e.g. flexural and compressive strength, fatigue) considering changes due to aging, which is known to induce dehydration of the dentin matrix [Bajaj et al., 2006], and differences in remineralization of demineralized to sclerotic dentin should broaden our knowledge of remineralization processes and are strongly encouraged. Remineralization approaches that guarantee an optimal incorporation of mineral within the gap zones of the collagen fibrils and the subsequent full mechanical recovery of the tissue must also be developed.

In summary, assuming that to date the concept of remineralization has been based upon 'reincorporation of mineral', we suggest that mineral concentration alone does not necessarily lead to improved functionality. Instead, the concept of functional remineralization stands upon the recovery of the mechanical properties of the affected dentin under hydration similar to the properties of normal dentin, which is presumed to correlate well with its overall functionality. Thus, we suggest that an important parameter to evaluate the effectiveness of remineralization in dentin should be associated with the recovery of the mechanical properties of the hydrated tissue.

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