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Recovery after PILP remineralization of dentin lesions created with two cariogenic acids

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ABSTRACT

Objectives: Acetate and lactate are important cariogenic acids produced by oral bacteria. They produced different residual dentin structures in artificial lesions of similar depth. We evaluated if such lesions responded in the same way to a polymer-induced-liquid-precursor (PILP) remineralization.

Design: Dentin blocks obtained from human third molars, divided into 6 groups (n = 3). Blocks were demineralized with acetate (66 h) or lactate (168 h) buffer at pH 5.0 to create 140 μm target lesion depths. A-DEM and L-DEM groups received no remineralization. Other groups were remineralized for 14 days. 100 μg/mL polyaspartate was added into the remineralizing buffer for A-PIL and L-PIL, whereas A-CAP and L-CAP were treated with the same solution but without polyaspartate. Cross-sectioned blocks were examined for shrinkage and AFM-topography. Line profiles of reduced elastic modulus (E_r) were obtained by AFM-based nanoindentation across the lesion. Ultrastructures were examined with TEM.

Results: A-PIL and L-PIL recovered in shrinkage to the original height of the dentin and it appeared normal with tubules, with increases in E_r at both outer flat and inner sloped zones. At the sloped zone, acetate lesions lost more E_r but recovery rate after PILP was not statistically different from lactate lesions. A-CAP and L-CAP showed surface precipitates, significantly less recovery in shrinkage or E_r as compared to PILP groups. TEM-ultrastructure of PILP groups showed similar structural and mineral components in the sloped zone for lesions produced by either acid.

Conclusions: The PILP process provided significant recovery of both structure and mechanical properties for artificial lesions produced with acetate or lactate.

1. Introduction

Oral bacteria break down ingested carbohydrates and produce organic acids that induce demineralization of tooth tissues and lead to dental caries. Two of the most important of these acids are acetic and lactic acids (Distler & Kröncke, 1986). Standardized artificial caries lesions offer many advantages over efforts that utilize natural lesions because demineralization protocols can be reproducible and lesions of different size can be readily made based on demineralization kinetics of a given acid. Natural coronal dentin lesions have been characterized using nanoindentation, and have an outer relatively flat zone of low modulus in the most active lesions and an inner gradient zone as the modulus values increase with depth until normal dentin values are reached (Zheng, Hilton, Habelitz, Marshall, & Marshall, 2003). Mclntyre used both acetate and lactate to create artificial root dentin caries with similar zones and micro-hardness values to natural lesions (McIntyre, Featherstone, & Fu, 2000). The 2 acids used in McIntyre's study both demineralized dentin by diffusion-dominated mechanisms. In previous work we measured the kinetics of demineralization of coronal dentin using acetate and lactate buffers at pH = 5 and found that dentin demineralization is a diffusion-dominated process for both acids, but proceeded much more slowly using lactate as compared to acetate and the mineral content, mechanical property profiles and ultrastructures were significantly different in lesions of the same depth (Chien et al., 2016). These results suggest that lesions produced using the different acids may respond differently to remineralization treatments.

Remineralization of dentin lesions and demineralized dentin matrix using calcium and phosphate containing solutions has proven to be significantly more difficult than enamel lesions. Several related

Abbreviations: PASP, poly-aspartic acid; PILP, polymer-induced liquid-precursor; DEJ, dentin enamel junction

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processes have been introduced that have the capacity to remineralize collagen fibrils, bone and dentin matrices (Chen et al., 2015; Gower, 2008; Olszta, Douglas, & Gower, 2003; Tay & Pashley, 2008). The capacity to provide intrafibrillar mineralization of the collagen has been suggested to be critical to restoration of the mechanical properties of dentin (Bertassoni, Habelitz, Kinney, Marshall, & Marshall, 2009; Kinney, Habelitz, Marshall, & Marshall, 2003; Kinney et al., 2001). The system developed by Gower et al. (Gower & Odom, 2000; Gower, 2008; Olszta et al., 2003) has been shown to be particularly effective at remineralization of collagen matrices (Jee, Thula, & Gower, 2010), so we have applied this polymer-induced liquid precursor (PILP) mineralization process to artificial caries lesions in coronal dentin prepared from acetate buffer at pH = 5, and have shown that it provided complete recovery of mineral content throughout the lesion depth (as seen in micro-CT), while nanomechanical testing found the reduced elastic modulus (E_r) recovered to 50–60% of normal dentin in the severely demineralized outer zone and full recovery of the inner zone (Burwell et al., 2012).

However, the differences in residual dentin structure, such as more residual minerals left in acetate lesions, while lactate dissolved minerals much more slowly and along collagen fibers after their independent demineralizations (Chien et al., 2016), suggest that there may also be important differences in remineralization behavior, and therefore we undertook a comparative evaluation using lesions of similar depth produced from lactate and acetate buffers at pH = 5.

The hypothesis tested in this study was that artificial lesions created by lactate respond similarly to PILP remineralization treatment as acetate lesions of the same depth.

2. Materials and methods

2.1. Human teeth

Fresh human 3rd molars were obtained from subjects who required extraction according to protocols approved by the UCSF Committee on Human Research. After extraction the teeth were sterilized by gamma radiation for 24 h (Brauer, Saeki, Hilton, Marshall, & Marshall, 2008; White, Goodis, Marshall, & Marshall, 1994) then stored in DI water at 4 °C until used.

2.2. Artificial caries lesions

2.2.1. Demineralization

Dentin blocks measuring 6 (length) × 3(width) × 2.5(height) (mm) were prepared from the coronal regions of the teeth. The occlusal surface of each dentin block was cut and ground so that the surface was just below the dentin enamel junction (DEJ) to simulate progression of the natural caries in coronal dentin. The occlusal surface was polished with SiC abrasive papers and a series of aqueous diamond solutions on polishing cloths to 0.25 μm (Buehler, Lake Bluff, IL). Two coats of nail varnish (Revlon #270, New York, NY) were painted on the polished surface except for a window 3 × 3 mm that was left to create an artificial lesion upon acid exposure. The nail varnish protected area served as a reference after the treatments rendered (as shown in Fig. 1). Demineralization buffers containing 2.2 mM calcium phosphate and either 0.05 M acetic acid or lactic acid, pH adjusted to 5.0, were used to create artificial caries lesions similar to those reported previously (McIntyre et al., 2000). Each nail varnish coated dentin block was placed in a 50 ml tube with 40 ml of the selected buffer. Demineralization time was calculated from the kinetics curve obtained from a previous study (Chien et al., 2016), 66 h for acetate buffer, 168 h for lactate buffer to create lesions with a target depth of approximately 140 μm. After demineralization, samples were harvested, rinsed thoroughly with DI water and stored in 100% humidity to maintain hydration until studied.

2.2.2. Remineralization

Demineralized dentin blocks were randomly assigned to 6 groups (n = 3/group) as shown in Table 1. For groups 3–6, each block was placed in 40 ml of remineralizing buffer containing Tris, 4.5 mM calcium and 2.1 mM phosphate, at pH 7.4 for 14 days in an incubator at 37 °C, with agitation provided by a rocker platform for remineralizing treatment. 100 μg/ ml of Poly-L-aspartic acid (PASP) sodium salt, with molecular weight of 27 kDa (Alamanda polymers, Huntsville, AL) was added to A-PIL and L-PIL groups (Burwell et al., 2012; Jee, Thula et al., 2010). Negative control groups (A-CAP and L-CAP) were treated in the remineralizing buffer prepared in the same way except without PASP. The "no" treatment groups (A-DEM and L-DEM) did not receive any remineralizing treatment and served to show the characteristics of demineralized lesions.

2.3. AFM and mechanical property

2.3.1. Embedding and cross sectioning

The degree of remineralization can be assessed using different methods such as by recovery of mechanical properties (hardness, modulus), mineral content evaluated by x-ray attenuation, and changes in microstructure, often using TEM. Remineralization solutions are supersaturated and if lesions are shallow, surface precipitation may alter properties and mineral content to give the misleading suggestion that remineralization has occurred. Such spontaneous precipitation of calcium-phosphate tends to occur, which can increase mineral density at near surface depths of 10 to 20 μm. However, portions of deeper lesions may not be remineralized. Thus it is important to use deeper lesion depths. In addition, evaluating only from the surface can mask the challenge of delivering the mineral into the main body of the lesions.

Evaluating the remineralization treatment efficacy according to an increase of mineral content alone may not allow differentiation of extra- and intra-fibrillar mineral deposits because mechanical properties of the dentin are sensitive to the restoration of intrafibrillar mineral of collagen (Bertassoni et al., 2009; Kinney et al., 2003). As noted above, the lesion depths evaluated by mineral density and mechanical properties do not always match (Burwell et al., 2012; Chien et al., 2016). Thus we take the approach of doing a series of nanoindentations along the cross sectioned lesions which cover all the regions, starting with the embedding material, fully demineralized outer zone, to the transition zone and finishing on the non-affected dentin, and while in hydrated conditions. This is in addition to the structure and mineral density analysis to fully understand the reconstruction of dentin.

Excess moisture was removed by blotting the dentin blocks containing the treated lesions prior to embedding them in room temperature curing epoxy (Epoxycure, Buehler, Lake Bluff, IL). The embedded blocks were cut with a slow speed saw under water (Buehler, Lake Bluff, IL), perpendicular to the treated occlusal surface to reveal the lesion profile. A thin slice obtained from the center of the specimen (~1200 μm) was glued onto the AFM specimen discs (Ted Pella, Redding, CA) with a small amount of cyanoacrylate (QX-4, MDS Products, Laguna Hills, CA), then polished to 0.25 μm with diamond as described in the dentin block preparation section above.

2.3.2. AFM based nanoindentation

An atomic force microscope (Nanoscope IIIA, Digital Instrument, Santa Barbara, CA) equipped with a Triboscope load-displacement transducer (Hysitron, Minneapolis, MN) and nanopositioner (nPoint, Middleton, WI) was used for nanomechanical property testing. A diamond Berkovich tip (fluid cell, ~100 nm tip radius) was used and the system was calibrated with a fused silica standard in wet and dry conditions. Site specific reduced elastic modulus (E_r) was obtained with loads of 200 μN to 500 μN with a 3 s trapezoidal loading curve as described previously (Burwell et al., 2012). Each indentation yielded a load-displacement curve, from which E_r was determined as previously