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Distinct decalcification process of dentin by different cariogenic organic acids: Kinetics, ultrastructure and mechanical properties

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ABSTRACT

Objectives: We studied artificial dentin lesions in human teeth generated by lactate and acetate buffers (pH 5.0), the two most abundant acids in caries. The objective of this study was to determine differences in mechanical properties, mineral density profiles and ultrastructural variations of two different artificial lesions with the same approximate depth.
Methods: 0.05 M (pH 5.0) acetate or lactate buffer was used to create 1) 180 μm-deep lesions in non-carious human dentin blocks (acetate 130 h; lactate 14days); (2) demineralized, ~180 μm-thick non-carious dentin discs (3 weeks). We performed nanoindentation to determine mechanical properties across the hydrated lesions, and micro X-ray computed tomography (MicroXCT) to determine mineral profiles. Ultrastructure in lesions was analyzed by TEM/selected area electron diffraction (SAED). Demineralized dentin discs were analyzed by small angle X-ray scattering (SAXS).
Results: Diffusion-dominated demineralization was shown based on the linearity between lesion depths versus the square root of exposure time in either solution, with faster kinetics in acetate buffer. Nanoindentation revealed lactate induced a significantly sharper transition in reduced elastic modulus across the lesions. MicroXCT showed lactate demineralized lesions had swelling and more disorganized matrix structure, whereas acetate lesions had abrupt X-ray absorption near the margin. At the ultrastructural level, TEM showed lactate was more effective in removing minerals from the collagenous matrix, which was confirmed by SAXS analysis.
Conclusions: These findings indicated the different acids yielded lesions with different characteristics that could influence lesion formation resulting in their distinct predominance in different caries activities, and these differences may impact strategies for dentin caries remineralization.

1. Introduction

Demineralization of calcified tissues or biominerals occurs in organisms via either physiologic or pathologic processes. Physiologic demineralization such as bone resorption is an essential event in bone modeling and remodeling, tooth eruption and fracture healing, while diseases such as osteoporosis or dental caries are caused by excessive and pathologic demineralization followed by matrix degradation. Problems of human health have driven demineralization studies of calcified tissues with attention focused on the cause and characteristics of dental caries during the 19th and 20th centuries (Ehrlich, Koutsoukos, Demadis, & Pokrovsky, 2008). Knowledge of enamel caries and demineralization of enamel is substantial; in contrast, complex dentin caries and dentin demineralization need more meticulous investigations with the combination of current front-line microscopic and spectrometric techniques.

Dentin is made up of more organic components and water by volume (approximately 50%) than enamel (less than 3%). Thus, dentin caries is much more complex than enamel caries and involves at least two stages: the dissolution of biominerals by

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organic acids and the subsequent degradation of dentin matrix by proteases. In addition, natural dentin caries are geometrically complex with variations in width, depth, severity and color, making it difficult to find many similar lesions for *in vitro* studies. Artificial lesions offer significant advantages over natural lesions, since standard and reproducible lesions can be made routinely. Many attempts have been made to create artificial caries model systems using organic acids (Featherstone & Rodgers, 1981; Featherstone, Duncan, & Cutress, 1979; Featherstone, Cutress, Rodgers, & Dennison, 1982; ten Cate & Duijsters, 1982) and pH-cycling (Featherstone, 1994; Featherstone & Nelson, 1989; Featherstone, Glena, Shariati, & Shields, 1990; Featherstone, Stookey, Kaminski, & Faller, 2011; Stookey et al., 2011) to produce artificial lesions similar to natural caries. Such lesions of defined depth have also been used to evaluate various dentin remineralization procedures (Bertassoni, Habelitz, Pugach, Soares, & Marshall, 2010; Bertassoni, Habelitz, Marshall, & Marshall, 2011; Burwell et al., 2012). The changes in reduced elastic modulus (E_R) of hydrated artificial caries specimens were prepared and evaluated using an acetate buffer (pH 5) that produces a sloped mineral profile similar to natural lesions (Marshall et al., 2001; Pugach et al., 2009; Zheng, Hilton, Habelitz, Marshall, & Marshall, 2003). Noticeably deeper artificial lesions had a larger and highly demineralized outer flat zone of very low mechanical properties (Burwell et al., 2012) similar to the most severe active lesions (Burwell et al., 2012; Zheng et al., 2003). Both artificial root dentin lesions created with the same acetate buffer and natural root caries in extracted teeth have similar cross-sectional microhardness, and lesion profiles using qualitative polarized microscopy (McIntyre, Featherstone, & Fu, 2000).

While many acids or calcium chelating agents can solubilize the Ca–P crystals to create characteristic lesion models easily, acetic acid was commonly used in previous studies (Featherstone & Rodgers, 1981; Featherstone et al., 1979; Featherstone et al., 1982; Featherstone & Nelson, 1989; Featherstone et al., 1990; Featherstone et al., 2011; Stookey et al., 2011; ten Cate & Duijsters, 1982) to make artificial dentin or enamel lesions resembling natural caries. The acetate artificial caries system resulted in demineralization across the lesion similar to that observed in natural lesions of similar depths (Arends & ten Bosch, 1992). Organic acids produced by cariogenic lactobacilli are considered to play a major part in dentin demineralization (Byun et al., 2004). Distinct acids have been associated with caries activity. In active caries lactate appears to dominate, while acetate and propionate have been found in arrested carious lesions (Hojo, Komatsu, Okuda, Takahashi, & Yamada, 1994). We evaluated lesions produced by lactate and acetate buffers since the distinct association of acids with caries activities and prior work on enamel caries has shown that their demineralization characteristics may be different at the same pH (Featherstone & Rodgers, 1981).

The purpose of this study was to determine the kinetics of demineralization for two artificial dentin caries models based on lactate and acetate buffers at pH 5, a typical pH for caries demineralization, and to compare the mineral profiles and nanomechanical property profiles of large lesions of the same nominal depths produced to determine if the profiles are similar and to evaluate lesion characteristics as a foundation for their use in remineralization studies. We investigated both lesion types at the same depth (different demineralization periods) using all the analytical methods presented here for clinical relevance, as well as both lesion types demineralized at the same period of time for ultrastructural and SAXS analyses to confirm the distinct effects caused by two different acids. We sought to determine the properties of the hydrated tissue since dehydration of demineralized dentin results in shrinkage of the remaining matrix and its mechanical properties in this state do not reflect the state of the tissue *in vivo* (Bertassoni, Habelitz, Kinney, & Marshall, 2009). In addition, we investigated the ultrastructure of mineral/matrix of dentin lesions produced by these two acids, to correlate the modifications of structure to the changes of mechanical properties. The null hypothesis was that each buffer would produce similar demineralization profiles at the same pH and similar mechanical

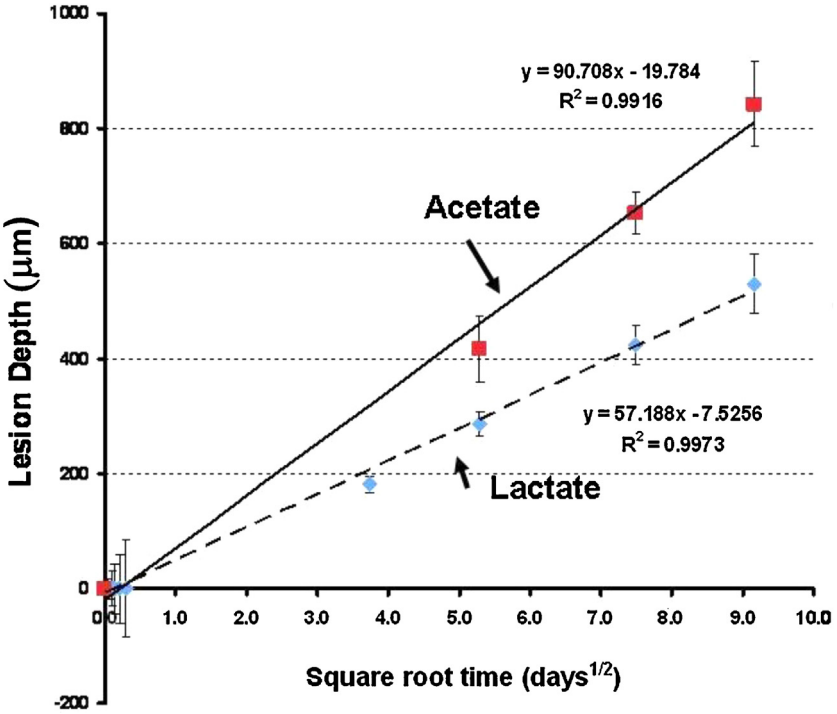

Fig. 1. Kinetic curves of dentin lesion formation (lesion depth versus square root of demineralization time) produced by acetate and lactate buffer at pH 5. Lesion depth was determined by polarized light microscope.