Published in final edited form as:

J Mech Behav Biomed Mater. 2015 April ; 44: 121–132. doi:10.1016/j.jmbbm.2015.01.006.

SYNERGISTIC DEGRADATION OF DENTIN BY CYCLIC STRESS AND BUFFER AGITATION

Santiago Orrego¹, Elaine Romberg², and Dwayne Arola^{3,4,♦}

¹Department of Mechanical Engineering, University of Maryland Baltimore County Baltimore, MD USA

²Department of Endodontics, Prosthodontics, and Operative Dentistry, Dental School, University of Maryland, Baltimore, MD USA

³Department of Materials Science and Engineering, University of Washington, Seattle, WA USA

⁴Department of Restorative Dentistry, School of Dentistry, University of Washington, Seattle, WA USA

Abstract

Secondary caries and non-carious lesions develop in regions of stress concentrations and oral fluid movement. The objective of this study was to evaluate the influence of cyclic stress and fluid movement on material loss and subsurface degradation of dentin within an acidic environment. Rectangular specimens of radicular dentin were prepared from caries-free unrestored 3rd molars. Two groups were subjected to cyclic cantilever loading within a lactic acid solution (pH=5) to achieve compressive stresses on the inner (pulpal) or outer sides of the specimens. Two additional groups were evaluated in the same solution, one subjected to movement only (no stress) and the second held stagnant (control: no stress or movement). Exterior material loss profiles and subsurface degradation were quantified on the two sides of the specimens. Results showed that under cyclic stress material loss was significantly greater ($p = 0.0005$) on the pulpal side than on the outer side and significantly greater ($p = 0.05$) under compression than tension. However, movement only caused significantly greater material loss ($p = 0.0005$) than cyclic stress. Subsurface degradation was greatest at the location of highest stress, but was not influenced by stress state or movement.

Keywords

dentin; demineralization; acid; fatigue; stress; non-carious cervical lesions; buffer agitation; microstructure; secondary caries

♦Corresponding Author Dwayne D. Arola, Ph.D., Associate Professor, Materials Science and Engineering Department, University of Washington, Roberts Hall, 333, Box 352120, Seattle, WA 98195-2120, +1 (206) 685-8158 Voice, +1 (206) 543-3100 Fax.

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1. Introduction

Resin composites have become the primary material used for tooth cavity restorations (Collins, 1998; Ferracane, 2011). Yet they have higher failure rates than the materials used in the past (Bernardo et al., 2007; Demarco et al., 2012). The primary mode of restoration failure is secondary caries (Dahl et al., 1978; Deligeorgi, 2001; Sarret, 2005), which most commonly develop at the margins of restorations (Mjör and Toffenetti, 2000; Mjör, 2005). Secondary caries are potentially facilitated by the localized high cyclic stresses at the interface and fatigue (Spencer et al., 2010; Pashley et al., 2011). Cyclic stress is detrimental to the durability of resin-dentin bonds (Mutluay et al., 2013a; 2013b). Resin composites also tend to accumulate more biofilm/plaque in the oral environment than other restorative materials (Zalkind et al., 1998; Beyth et al., 2007). The acid production of biofilms could act in synergy with the cyclic stresses to accelerate degradation of the margins and the formation of secondary caries.

Cyclic stresses contribute to degradation at different locations of the tooth structure. For example, Non-Carious Cervical Lesions (NCCLs) at the Cemento-Enamel Junction (CEJ) are considered multi-factorial, and attributed to a combination of erosion, abrasion, attrition and abfraction (Grippio, 2004; Bartlett and Shah, 2006; Ceruti et al., 2006; Michael et al., 2009; Pecie et al., 2011; Sena et al., 2012). Pioneering investigations by McCoy (1982) and Lee and Eakle (1984) identified that the CEJ experiences high stresses under non-axial loads. In fact, the term abfraction was adopted to distinguish stress-related type of lesions. Grippio (1991) suggested that stresses could foster microfracture of enamel and dentin under cyclic loading, thereby forming gaps or holes. However, the lack of direct experimental evidence of this process has led to some controversy (Litonjua, 2003; Michael et al., 2009; Sena et al., 2012; Grippio et al., 2012).

The effects of cyclic stresses to tooth structure are not only detrimental. Recent studies by Toledano et al (2014a, 2014b; 2015) report that remineralization may be achieved in demineralized regions of the hybrid layer by a period of cyclic stress. Thus, it is important to consider the contributions of cyclic stress and environment on mineralization changes in greater detail.

Both the CEJ and the margins of restored teeth experience a concentration of localized cyclic stresses and exposure to variable pH conditions. Rees (2000) suggested that the presence of acidic conditions combined with the stresses of mastication can cause more damage than either alone. But is there synergism between the cyclic stress and acidic condition that elevates the potential for degradation? Staninec et al. (2005) explored this question in an experimental study of material loss occurring to human dentin subjected to cyclic stress in an acidic environment. Results revealed that more material loss occurred when combined with mechanical stress, and that compressive stresses caused more loss than tensile stresses. Similarly, Mishra et al (2006a) found that more material loss occurred to bovine dentin in an acidic environment when combined with static loading and that greater material loss occurred by compressive stress. The investigators also found that subsurface demineralization increased when combined with static stress (Mishra et al., 2006b). While pioneering, the aforementioned studies did not consider the potential effects of fluid

movement and local dentin microstructure on the degree of material loss. Both factors could be relevant to degradation of the margins. Therefore, in the present investigation we explore the contribution of cyclic mechanical stresses and acidic buffer agitation to both surface and subsurface degradation, and assess the relative importance of dentin microstructure.

2. Materials and Methods

Caries-free third molars were obtained from dental clinics in Maryland according to an approved protocol issued by the Institutional Review Board of the University of Maryland (Approval Y04DA23151). All teeth were from young donors with 17 age 33 years. The teeth were kept in Hanks Balanced Salt Solution (HBSS) with 0.2% sodium azide as an antimicrobial agent at 4°C. Axial sections (~1 mm thick) were obtained from the central portion of the crown in the bucco-lingual plane, using a slicer/grinder (Chevalier Smart-H818II, Chevalier Machinery, Santa Fe Springs, CA, USA) with a diamond abrasive slicing wheel (#320 mesh abrasives) and water irrigation. Rectangular beams were obtained from the primary sections as depicted in Figure 1(a). All surfaces of the specimens were lightly polished using a very-fine silicon carbide paper (SiC Paper #2000, FEPA P-2000, Struers, Cleveland, OH, USA).

The dentin beams were subjected to four different loading conditions, with each conducted within a bath of approximately 100 mL of an acidic buffer (pH = 5) at 22°C. The solution was prepared by adding lactic acid to 0.1M solution of sodium hydroxide (NaOH), and the pH levels were verified with a calibrated pH meter (Model PH220-C, Extech, Waltham, MA, USA). Fresh buffer solution was used for each specimen and the pH was monitored over the period of evaluation to ensure consistency. The lactic acid environment was chosen according to a number of considerations. *Streptococcus mutans* (*S. mutans*), one of the principal bacterial species in the oral environment, metabolize fermentable carbohydrates to create lactic acid (Clarke, 1924; Kolenbrander, 2000). *S. mutans* carry out glycolysis and maintain adhesion to the tooth surface at pH levels as low as 4.5 (Padan et al., 1981; Padan and Schuldiner, 1986; Drobni et al., 2006). Experimental evaluations have considered pH values as low as 4.0 (Xu et al., 2011; Weir et al., 2012), but a pH below 5.0 causes instability and disruption of the bacterial community (Bradshaw and Marsh, 1998; Kolenbrander, 2000). Therefore a solution of lactic acid with pH=5 was considered to be clinically relevant and is consistent with previous evaluations (Do et al., 2013).

Of the four groups of specimens, Groups 1 and 2 were subjected to cyclic flexural loading (Figure 1(b)) using a universal testing system (BOSE Model ELF 3200, BOSE, Eden Prairie, MN, USA) operating in load control. A cantilever configuration was chosen after the methods applied in previous studies (Staninec et al., 2005; Mishra et al., 2006a; 2006b). In this arrangement the maximum stress occurs closest to the apical region of the specimen, akin to the stress concentration that develops at the CEJ. The apical end of the specimens was clamped and the coronal end (4.0 mm from the clamped edge) was loaded at a frequency of 5 Hz and a load ratio of 0.1 (min/max load) for a period of 1×10^6 cycles. The cyclic load was chosen to achieve a cyclic stress amplitude of ~40 MPa, which is just below the apparent endurance limit for this tubule orientation (44 MPa: Arola et al., 2006). The required maximum load was estimated from the specific specimen dimensions according to

standard beam theory (Popov, 1978). For the specimens in Group 1 the load was directed from the outer dentin inwards (i.e. toward the inner dentin or pulp), whereas for Group 2 the loading was applied in the opposite direction (Figure 1(a)). For Groups 3 and 4, the specimens were mounted in the same cantilever configuration and in the same environment. For Group 3 the specimens were maintained stagnant for a period of 56 hours without stress or movement, which is equivalent to the duration of cyclic loading for Groups 1 and 2. Specimens in Group 4 were subjected to cyclic movement at 5 Hz without stress for a period of 1×10^6 cycles. The maximum movement was 60 μm , which is equivalent to the average maximum displacement resulting from flexure loading of Groups 1 and 2. A total of 36 beams were evaluated with an equal distribution in the four groups ($n = 9$).

Acid exposure caused exterior material loss realized as changes to the specimen geometry and surface quality. The two surfaces defined as the inner and outer faces (i.e. subjected to the maximum tensile and compressive stresses in flexure) were evaluated over the beam length (Figure 2(a)) using imaging processing techniques. Prior to cyclic loading/movement the specimens were mounted in a stainless steel fixture and small indelible marks were placed on one side of the beam along the x-axis for reference. In addition, the region of the beam extending outside of the 4 mm gage length (Figure 1(b)) was insulated with a thin coating of Super Glue. The glue minimized acid degradation and assisted in alignment of the acquired images. Microscope images were taken at 100X magnification before and after exposure of each specimen to the demineralizing environment using a light microscope (Olympus Model BX51, Olympus, Tokyo, Japan) and digital camera (Model DCM-300, Scopetek, Hangzhou, China). The images were recorded using Scopetek® software (ScopeTek, China, version 2009-11-02) with a resolution of 2048×1536 pixels. Multiple images were obtained along the beam length, and then processed and converted into binary format. A commercial software (Matlab®, Natick, MA, USA, ver. 2013b) was used to measure the distance from the central axis of the beam to the external edges (ε ; Figure 2b). The image acquisition and processing resulted in a resolution of 1.5 $\mu\text{m}/\text{pixel}$. A material loss profile was obtained along the beam length by subtracting the change in beam geometry before and after the treatment (Figure 3). Loss measurements were recorded $x=0.5$ mm (Location 1) and $x=3.5$ mm (Location 3) from the clamped edge, which correspond to the locations adjacent to the highest stress and beam deflection, respectively. The average normalized material loss was also estimated for each side of the beam by calculating the area under the material loss profile between Locations 1 and 3 and dividing that by the distance between these two locations.

The degree of acid penetration beneath the surfaces was evaluated using a Scanning Electron Microscope (SEM; JEOL Model JSM-5600, Peabody, MA, USA) in secondary electron imaging mode. Prior to evaluation, the specimens were dehydrated in a standard ascending ethanol series (70–100%) and then sputtered with gold palladium. Fast-fracture of the beams was performed in flexure at Locations 1 and 3. The fracture surfaces were then examined to measure the depth of acid penetration beneath the tensile and compressive surfaces.

Collected data was analyzed using commercial statistical software (IBM SPSS, Endicott, New York, USA, ver. 20.0). For the area loss measurements, a two-way ANOVA was used to compare the material loss between the four evaluated groups and between the treated

sides (i.e. inner and outer). An additional *t*-test was required to compare the demineralization occurring within the sides of each treated group due to the low *p*-values obtained in the two-way ANOVA. Same statistical methods were used to compare the average material loss specifically at Location 1. A three-way ANOVA was used to compare the material loss at Locations 1 and 3 of the two loaded groups (i.e. Groups 1 and 2). A one-way ANOVA was used to compare the material loss occurring exclusively at Location 1 for the loaded groups only to identify any potential effect of stress state (tension or compression). Finally two statistical analyses were performed to evaluate the sub-surface depth of degradation. A three-way ANOVA was used to compare the D values (Figure 7) between the evaluated groups, sides and locations. A one-way ANOVA was used to compare the degradation depth occurring exclusively at Location 1 for the two loaded groups (Groups 1 and 2) to identify potential effect of stress state. All the ANOVA analyses included a Tukey's post hoc analysis and were performed with $p = 0.05$ indicating a significant difference.

3. Results

A material loss profile from a representative specimen of Group 3 (control) is shown in Figure 3. As evident in this figure, there is considerable difference in the material loss on the two sides of the beam, with the largest loss on the inner side. Locations 1 and 3 are overlaid on this profile, which are the locations of the individual loss measurements.

A comparison of the area loss measurements that occurred over the period of cyclic loading (or equivalent term of exposure) is shown in Figure 4(a). The normalized area loss was determined from the measurements of the area under the profile divided by the length of the exposed beam. Specimens from Group 4 (movement only) experienced nearly three times greater loss (significant: $F=145.585$, $p=0.0005$) than that occurring in the other loading conditions. Surprisingly, the lowest degree of material loss did not result from the static group, but occurred for Group 1 in which loading was directed from the inner side of the beams outward. As evident from the statistical comparison, the material loss between the two groups subjected to cyclic stress was significantly different. Cyclic loading directed from outer to inner dentin (Group 2) causing significantly greatest loss and reversing the direction of loading decreased the magnitude of material loss by more than a factor of 2. Surprisingly, the material loss that resulted from the conditions of Group 2 was not significantly different from that of the static condition (Group 3). Figure 4(b) presents the average material loss for the four groups in terms of the two sides of the specimens (i.e. inner and outer). According to the statistical comparisons using multiple *t*-tests, the degree of material loss was significantly greater on the inner side of the beams than that on the outer side for all of the loading conditions evaluated.

As evident in Figure 4, the material loss resulting from movement only (Group 4) saturated the results obtained for the other groups. Thus, results for Group 4 were omitted and a second comparison was conducted that concentrated on stress. The measures of average material loss that occurred at the location of highest cyclic stress (Location 1) for Groups 1 through 3 is shown in Figure 5(a). According to this comparison, cyclic loading from the outer dentin inwards (Group 2) resulted in significantly greater ($F=8.648$, $p=0.001$) material

loss. Figure 5(b) presents the average material loss at Location 1 for the two sides of the specimens when they are considered individually (i.e. inner and outer sides). Interestingly, the only group exhibiting a significant difference ($p=0.0005$) between the two sides was Group 2 according to the statistical comparisons using multiple t -tests. Therefore, the largest degree of material loss occurred when the inner dentin was subjected to cyclic compressive stress.

The relative influence of buffer agitation and the magnitude of cyclic stress on material loss was assessed from a comparison of results obtained for the two loaded groups at Locations 1 and 3. Results of this comparison are shown in Figure 6 and details of the corresponding three-way ANOVA are shown in Table 1. As evident from Figure 6(a), significantly greater ($F=68.857$, $p=0.0005$) material loss occurred when the loading conditions resulted in cyclic compression. When combining results for the two loaded groups, the results of Figure 6(b) shows that material loss was significantly greater ($F=60.015$, $p=0.0005$) on the inner side of the specimens (pulpal side) than that for the outer side (Figure 6(b)). These observations are in agreement with those obtained from the area loss measurements presented in Figure 4, which signify that the differences in microstructure on the two sides of the specimens is important. A comparison of the average material loss at Locations 1 and 3 is shown in Figure 6(c). Surprisingly, the dentin specimens underwent significantly greater ($F=42.524$, $p=0.0005$) loss at Location 3 (i.e. low stress and large movement) with respect to Location 1 (i.e. high stress and low movement). Thus, movement of the buffer caused significantly greater material loss than cyclic stress. It is important to note that there were additional significant interactions effects as well involving the stress state, microstructure and location as evident in Table 1.

The effects of lactic acid exposure and cyclic stress were also assessed in terms of the degree of subsurface demineralization. Characteristics of the microstructure after cyclic loading are shown in Figure 7. A micrograph of the degraded microstructure from a specimen of Group 1 (loading applied from inner to outer dentin) is shown in Figure 7(a). This micrograph was obtained from the inner side of the specimen at Location 1, which was subjected to cyclic tension. As evident in this micrograph, the depth of degradation was apparent from dissolution of the peritubular cuffs and an increase in the lumen diameters. Within the region of degradation, the intertubular dentin exhibited an increase in porosity as well, and exposed collagen fibrils were visible on the fracture surface in locations closest to the exposed surface (not shown). An additional micrograph obtained near Location 3 from this same group and from the tensile side is shown in Figure 7(b). Although the depth of degradation is lower than that evident in Figure 7(a), the changes to the microstructure are similar. In both micrographs presented in Figure 7 the lumens are distorted as apparent from the elliptical cross-section, particularly in the region closest to the exposed surface.

A summary of the degree of subsurface demineralization measurements is shown in Figure 8 and results from the corresponding three-way ANOVA conducted with these results is shown in Table 2. A comparison of the average depth of degradation for the two loaded groups is shown in Figure 8(a). Interestingly, the stress state did not have a significant influence on the depth of degradation ($F=2.796$, $p=0.107$). However, a comparison of the depth of degradation for the two evaluated sides (Figure 8(b)) showed that degradation of

the inner dentin was significantly greater ($F=8.829$, $p=0.007$). The average depth of degradation at Locations 1 and 3 for the specimens is shown in Figure 8(c). As expected, the degradation occurring at Location 1 is significantly greater ($F=10.183$, $p=0.004$) than that at Location 3. Thus, the degradation occurring in the region of highest stress was significantly greater than that in the region of largest movement. According to results of the three-way ANOVA (Table 2), the only significant interaction occurred between the stress state and location.

4. Discussion

Through the demands of daily oral functions, tooth tissues are regularly subjected to cyclic stress in the presence of simultaneous pH variations. Cyclic stresses have been implicated in the development of non-carious cervical lesions (e.g. Grippo et al., 2012; Sena et al., 2012) and in the development of secondary caries through localized fatigue degradation of the dentin (e.g. Spencer et al., 2010; Pashley et al., 2011). Previous studies have explored erosion of dentin (Staninec et al., 2005; Mishra et al., 2006a) and subsurface degradation (Mishra et al., 2006b) that occurs under simultaneous exposure to stress and acidic conditions. Their findings suggest that the degradation process is accelerated by stress. Results from the present investigation confirm these previous studies, but contribute new knowledge concerning the importance of dentin microstructure, stress state and relative influence of fluid movement to the nature of degradation. These topics warrant further discussion.

The contribution of stress to erosion of dentin was first evaluated by Staninec et al (2005). In that study the extent of material loss at the location of highest stress extended to a depth of between roughly 60 and 100 μm , which is approximately 4 times higher than the average loss presented herein ($20 \pm 10 \mu\text{m}$). But Staninec et al. (2005) used a hydrochloric acid solution with $\text{pH}=6.0$ and an exposure period of 138 hours, which is more than twice the period in the present study (56 hours). Of equal relevance, Mishra et al. (2006a) evaluated the erosion of bovine dentin subjected to static flexure within a lactic acid solution at $\text{pH}=4.5$. The reported material loss within the region of highest stress ($44 \pm 13 \mu\text{m}$) is just over twice that reported in this investigation. Although Mishra et al. (2006a) also used a lactic acid solution, the period of exposure was more than 2 times greater (120 hrs). Exposure time and pH are the principal contributors to erosion of dentin (e.g. Buonocore, 1961; Breschi et al., 2002; Vanuspong et al., 2002). Vanuspong et al. (2002) showed that the degree of material loss is directly proportional to exposure time, which is one of the most likely contributors to the differences in results between the aforementioned studies. Linearly extrapolating the depth of erosion in this investigation to an exposure period of 120 hours results in an estimated loss of 40 μm , a value more consistent with that of these previous studies.

The stress state (i.e. tension vs. compression) was identified as an important factor to the extent of material loss. Significantly greater material loss occurred to the inner dentin under compressive stress than tension (Figure 5), which agrees with the previous investigations on erosion of dentin (Staninec et al., 2005; Mishra et al., 2006a). Interestingly, this behavior contrasts results for enamel, where tensile stresses were more conducive to material loss

than compression (Lee and Eakle, 1984). The difference in erosion behavior between these two tissues undoubtedly results from the larger brittleness of enamel (Park et al., 2008; Arola et al., 2010), and the potential for microcracking to accelerate the erosion process. One new finding in the present study is that the importance of stress state was not consistent for all conditions evaluated. Whereas cyclic compression caused significantly greater material loss to the inner dentin, reversing the direction of loading, i.e. with inner dentin subjected to cyclic tension, resulted in less than half the extent of material loss (Figure 5(b)). Mishra et al. (2006a) rationalized the importance of stress state through its contribution to diffusion. Elongation of the collagen fibrils in tension causes transverse compaction of the matrix on the surface through Poisson's effects. The compacted organic layer acts to resist diffusion of the acidic solution within the stressed surface layer. Previous studies subjected the inner dentin to compressive stresses only and did not consider a reversal (i.e. placing the inner dentin in tension). The lower dissolution rate of Group 1 (Figure 4) and diminished importance of stress state could be attributed to the lower mineral to collagen ratio expected of the outer dentin with respect to the inner dentin (Tesch et al., 2001; Ryou et al., 2011). More extensive demineralization is expected to the inner dentin due to the higher mineral/collagen ratio and the greater quantity of minerals available for ionic exchange (Hirayama, 1990). Stress state could also be more important to inner dentin due to the larger lumen diameters and extent of fluid movement within the lumens caused by mechanical pumping. The larger size and density of lumens towards the pulp results in greater permeability (Özok et al., 2002). As such, the outer dentin is expected to exhibit lower rates of material loss as well due to the smaller lumen diameter. Though plausible, the latter explanation is admittedly speculative and should be explored in more detail.

Interestingly, the stress state did not contribute significantly to the degree of subsurface degradation (Table 2). Mishra et al. (2006b) evaluated the contribution of stress to subsurface demineralization of dentin and observed that significantly greater demineralization resulted from compressive stress. The depth of degradation in the region of highest stress was $73 \pm 17 \mu\text{m}$, which is roughly twice that ($30 \pm 4 \mu\text{m}$) from the present study (Figure 8). Discrepancies in pH concentration and exposure time between these studies could contribute to the differences in extent of degradation. But what could cause the different interpretations regarding the importance of stress state? There are two additional factors that are necessary to consider. Interestingly, Mishra et al (2006b) found that there was no statistical difference between the demineralization that occurred on the fixed and free ends of the specimens, which correspond to the locations of maximum and minimum stress, respectively. That observation suggests that stress was not a significant factor. Furthermore, the study did not reverse the loading direction to explore the importance of stress state. Consequently, it is expected that the larger degree of demineralization in compression was also attributed to the differences in microstructure and chemical composition between the inner and outer sides of the specimens and not simply stress state. Regions closer to the pulp have higher mineral to collagen ratio (Tesch et al., 2001; Ryou et al., 2011) and therefore, higher rates of ionic exchange.

One of the most important contributions to the degree of surface and subsurface degradation was the evaluated side of the specimen. The inner side of the specimens underwent significantly greater material loss than the outer side for all conditions (Figure 4(b)), even

for those specimens not subjected to cyclic stress (Groups 3 and 4). For those groups that underwent cyclic stress, the side of the specimen was equally important as contributions from the stress state and location (Table 1). During exposure of dentin to the lactic acid solution, hydrogen ions interact and exchange ions with the hydroxyapatite crystals (Featherstone and Lussi, 2006), thereby causing progressive dissolution. Evaluations of this process at the micro- and nano-scales have revealed that peritubular dentin is dissolved before the intertubular dentin when exposed to acidic environments (Meurman et al., 1991; Kinney et al., 1995; Marshall et al., 1993; Marshall et al., 1997). The dissolution rate of intertubular demineralization reaches a plateau, while for peritubular dentin the rate is continuous and linear. Hence, there are two potential causes for the more extensive material loss occurring to the inner dentin. Based on studies of dentin tubule dimensions and their distribution (Garberoglio and Brännström, 1976; Pashley, 1989; Marshall, 1993; Marshall et al., 1997), the inner side of the specimens possesses a larger number of tubules and tubule diameter. That results in a greater area fraction of peritubular dentin within the inner dentin and facilitates more extensive material loss via dissolution. Subsurface degradation was significantly greater on the inner side of the specimens as well (Figure 8(b)), which can also be attributed to the mineral to collagen ratio and preferential cuff dissolution. Clearly the larger tubule diameter within the inner dentin increases dentin permeability and penetration of the acid solution (Pashley, 1989).

The largest degree of material loss overall occurred to the specimens of Group 4 subjected to movement only (Figure 4(a)). Wiegand et al (2007) examined the erosive effects of acids flowing over dentin specimens with different velocities and duration. They found that dentin loss increased with increasing acid flow rate. The contribution of external fluid movement to demineralization can be explained by the so-called “Nernst diffusion layer” (Tobias et al., 1952). A semi-static protective layer is created at the solid-acid interface when the liquid reaches its saturation point during ionic exchange. The Nernst layer acts to protect the surface, thereby preventing further ion exchange between the mineral and the acidic environment. Agitation of the fluid rapidly removes the layer and accelerates the demineralization process (Wiegand et al., 2007; Ehrlich et al., 2008; Lussi et al., 2011; Attin et al., 2012). In the cantilever configuration, cyclic loading of the specimens caused buffer agitation. As evident in Figures 6(c), the location of greatest deflection (Location 3) underwent more than twice the material loss experienced at Location 1. Thus, the degree of material loss by external fluid movement was greater than that caused by cyclic stress. Higher flow rates increases the ion exchange and therefore the rate of dissolution. Prior investigations on enamel corroborate this concept (Maupome et al., 1999; Eisenburger and Addy, 2003; Shellis et al., 2005, Attin et al., 2012). The mineral of dentin is more soluble than that of enamel (Shellis et al., 2005), which is relevant to the growth of non-cariou lesions under the movement of saliva. Hence, cyclic stress may contribute to the development and growth of non-cariou lesions, but it appears that the movement of saliva about the ensuing lesion plays an equally important role.

It is important to consider the present results in light of recent investigations by Toledano et al., (e.g. 2014a, 2014b; 2015), which suggest that cyclic and sustained loads promote remineralization of dentin in previously acid treated regions, i.e. at the hybrid layer of resin-dentin bonded interfaces. Interestingly, the authors show that remineralization occurs in the

regions that experienced stress via increases in nanohardness and mineral to collagen ratio. Apparently, the newly formed mineral was seeded from the remaining crystallites in the partially demineralized dentin. Those novel findings are fundamentally different from the present investigation as the environmental conditions for cyclic loading were not acidic. Cyclic loading herein was performed with constant acid exposure, thereby preventing any possible localized mineralization, which could be considered a limitation of this investigation. Alternatively, the findings could be considered to emphasize the severity of degradation posed by the acid secretion of biofilm at the bonded interface. Preventing biofilm attack is essential.

Dentin is considered a porous material and it undergoes permeation when exposed to fluidic environments. The importance of the dentin tubules to fluid transport and the spatial variations in permeability related to the tubule architecture have received considerable attention (e.g. Brannstrom and Astrom, 1967; Pashley, 1989; Prati, 1994). Temperature variations, physical loading and/or osmotic changes can activate fluid movement within the tubules. Mechanical loading of dentin causes both axial and transverse deformation of the tubules, which acts as a pump and stimulates fluid flow (Ratih et al., 2007; Su et al., 2013). Paphangkorakit and Osborn (2000) demonstrated that normal chewing forces caused displacement of fluid within the dentinal tubules. This suggests that there is a fluid-structure interaction, which could potentially be studied using poroelasticity theory (Cowin, 1999). It is important to highlight the elliptical cross-section of the effected lumens after dissolution of the peritubular cuff in Figure 7. Clearly the change in shape of the lumens is a product of the cyclic stress, and the increased distortion towards the surface results from the reduction of stiffness with mineral loss.

The evaluation of subsurface degradation via microscopic inspection showed that the region under highest flexural stress (Location 1) exhibited significantly greater degradation than the low stress region (Figure 8(c)). Subsurface demineralization of the dentin specimens was assisted by the subsurface transport of the acid solution within the tubules. A significantly greater depth of degradation occurred to the inner dentin (Figure 8(b)), which now appears to result from the increased permeability rendered by the larger lumen diameters. At least in part, the greater mineral/collagen ratio of the inner dentin and larger peritubular cuffs contributed as well. Indeed, the importance of tubule size and density is evident from comparing Figures 7(a) and 7(b). Due to the decrease in fatigue strength of dentin with demineralization (Do et al., 2013), deep dentin would be the most vulnerable to fatigue failure and the development of secondary caries under cyclic stress.

Results of the present investigation have provided new understanding regarding the importance of microstructure to surface and subsurface degradation of dentin when subjected to cyclic stress and fluid movement. Nevertheless, there are some recognized limitations to the investigation and concerns that are essential to highlight. One important concern is the loading protocol. Although cyclic loading was conducted at a frequency of 5 Hz, mastication occurs at an average frequency of between 1.5–2.5 Hz (Ostry DJ and Flanagan JR, 1989). The fatigue strength of dentin is frequency dependent, with higher frequencies resulting in greater fatigue strength (Nalla et al., 2003). If fatigue contributes fundamentally to the dissolution of mineral, the increase in loading frequency could have

diminished the relative importance of cyclic stress to the degree of surface and subsurface degradation. In addition, the frequency of loading is relevant to the rate of fluid movement and the consequent degree of material loss caused by buffer agitation. Thus, the prominent contribution of movement to the erosion of dentin could at least partly be attributed to the higher loading rate that was used. While unlikely, further work addressing this topic may be worthwhile. As previously mentioned, the loading environment consisted of lactic acid solution maintained at constant pH and temperature (22°C). The pH level of the oral environment is constantly changing (Shellis et al., 2010) and is undoubtedly important to the nature of ion exchange and material loss. Overall, results of the investigation have shown that the degradation of dentin within an acidic environment is not only a function of stress and stress state, but is also a complex function of fluid movement and the local microstructure. These factors should be considered in future studies aimed at understanding the synergistic relationship between stress and degradation of dentin.

6. Conclusion

An experimental investigation was conducted to evaluate the relative importance of cyclic stress and fluid movement on the degradation of dentin in an acidic environment. Specimens of radicular dentin were obtained from human teeth and subjected to cyclic-flexure, free movement (no stress) or maintained stagnant (control) within a lactic acid solution (pH=5.0). Degradation of the tissue caused by the experimental conditions was evaluated in terms of material loss from the surfaces (i.e. erosion) and subsurface degradation (i.e. demineralization). Results showed that those regions located closer to the pulp (inner dentin) underwent a significantly greater ($p < 0.05$) degree of erosion than the outer dentin for all groups (i.e. loaded and unloaded) evaluated. Although the erosion was significantly greater under compressive stress than tension, the material loss occurring as a result of fluid movement was substantially (up to three times) greater than that from cyclic stress. The degree of demineralization was significantly greater within the inner dentin and regions of high stress as well, but there were no significant effects of stress state or fluid movement on subsurface degradation. Synergism in the degradation (surface and subsurface) of dentin will occur in conditions of combined fluid movement and cyclic compressive stress, with the propensity for degradation increasing with depth from the dentin enamel junction to the pulp.

Acknowledgments

Support for the following investigation was provided by the National Institutes of Dental and Craniofacial Research (R01 DE016904) and a seed grant from the University of Maryland Baltimore County.

This work was supported in part by grant R01 DE016904 from the National Institute of Dental and Craniofacial Research (PI D. Arola) and a seed grant from the University of Maryland Baltimore County.

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Figure 1.

Preparation of the specimens and loading configuration. (a) view of a sectioned tooth and outline of a specimen to be sectioned, indicating the two sides of interest (i.e. inner and outer); b) beam dimensions in millimeters and loading arrangement. The direction of loading was either from the inner to outer dentin or in the reverse direction.

Figure 2.

Demineralization of the dentin specimens and evaluation of material loss. (a) measurement of material loss and locations of interest. The measured loss (δ) was determined by superposing microscopic images of the beams before and after the period of exposure. The material loss (δ) was measured along the two sides of the beam (inner and outer). Location 1 is at $x=0.5$ mm and Location 3 is at $x=3.5$ mm from the cantilever support. (b) optical micrograph showing the demineralization of a typical specimen after cyclic loading in the lactic acid solution (pH=5).

Figure 3.

A typical material loss profile along the beam length for a specimen of Group 3. No loading or movement was applied to this profile. The area loss was measured between Location 1 and Location 3 as represented by the hatched areas under each profile. The material loss was also evaluated at the two locations and the two sides of the beams.

Figure 4.

Measure of the normalized area loss for the four evaluated conditions (i.e. area loss divided by the length of the exposed beam). (a) Average material loss (both sides of the beams) for each group. A two-way ANOVA-Tukey HSD revealed significant differences of the material loss between the groups ($F=145.585$, $p=0.0005$). The greatest demineralization occurred in Group 4. (b) A comparison of the average material loss for each of the two sides. The four t -tests revealed significant differences for all groups ($p<0.05$). The inner side exhibited the greatest loss in all groups. Group 1: load applied from inner to outer dentin; Group 2: load applied from outer to inner dentin; Group 3: static; Group 4: movement only.

Figure 5.

Material loss at Location 1 (the region of highest stress) for the loaded groups. (a) Average material loss (both sides of the beams) for each group. A two-way ANOVA with Tukey HSD revealed significant differences between Group 1 and Group 2 ($F=8.648$ and $p=0.001$). (b) A comparison of the material loss for Groups 1 through 3 in terms of the two sides. The t -test revealed a significant difference within the evaluated sides of Group 2 ($p=0.0005$). No significant differences were found for Groups 1 and 3 ($p>0.05$ for the two cases). Group 1: load applied from inner to outer dentin (inner side under tension stress and outer side under compression stress); Group 2: load applied from outer to inner dentin (inner side under compression stress and outer side under tension stress); Group 3: static (no stresses applied).

Figure 6.

A comparison of the material loss for the loaded groups at Locations 1 and 3. (a) A comparison of the overall material loss for Groups 1 and 2. Significantly greater loss ($F=68.857$ and $p=0.0005$) occurred when the load was applied from outer to inner dentin. (b) A comparison of the overall material loss for the two evaluated sides. Significantly greater loss occurred at the inner side ($F=60.015$ and $p=0.0005$). (c) A comparison of the overall material loss at Locations 1 and 3. Significantly greater loss occurred at Location 3 ($F=42.524$ and $p=0.0005$). Group 1: load applied from inner to outer dentin; Group 2: load applied from outer to inner dentin.

Figure 7.

Micrographs showing the depth of degradation (D) beneath the exposed surfaces of inner dentin (Group 2) after cyclic loading. a) Microstructure beneath the surface at Location 1; b) Microstructure beneath the surface at Location 3. The fracture surfaces are perpendicular to the x -axis. Note the increase in porosity, preferential removal of the peritubular cuffs and distortion of the lumens in both micrographs.

Figure 8.

A comparison of the depth of degradation for the loaded groups at Locations 1 and 3. (a) A comparison of the average D for Groups 1 and 2. No significant difference ($F=2.796$ and $p=0.107$) was found. (b) A comparison of the average D for the two evaluated sides. Significantly greater degradation ($F=8.829$ and $p=0.007$) occurred on the inner side. (c) A comparison of the average D at Locations 1 and 3. Significantly greater degradation ($F=10.183$ and $p=0.004$) occurred at Location 1. Group 1: load applied from inner to outer dentin; Group 2: load applied from outer to inner dentin. Columns with different letters are significantly different.

Table 1

Results for the three way-ANOVA conducted with the material loss measurements at Location 1 and Location 3. The comparison includes the loaded groups only (Groups 1 and 2).

Source	df (num)	df (den)	Group	Mean	SD	F	p
Stress State	1	35	Group 1	5.14	3.60	68.857	0.0005
			Group 2	12.14	7.72		
Microstructure	1	35	Outer Side	5.37	4.06	60.015	0.0005
			Inner Side	11.91	7.70		
Location	1	35	Location 1	5.89	4.98	42.524	0.0005
			Location 3	11.39	7.57		
Stress*Mic	1	35				19.878	0.0005
Stress*Loc	1	35				9.131	0.004
SIDE*Loc	1	35				1.987	0.164
Stress*Mic*Loc	1	35				0.121	0.729

Table 2

Results for the three way-ANOVA conducted with the depth of degradation measurements at Location 1 and Location 3. The comparison includes the loaded groups only (Groups 1 and 2).

Source	df (den)	df (num)	Group	Mean	SD	F	p
Stress State	1	15	Group 1	27.71	2.76	2.796	0.107
			Group 2	26.27	3.88		
Microstructure	1	15	Outer Side	25.72	3.03	8.829	0.007
			Inner Side	28.27	3.33		
Location	1	15	Location 1	28.36	3.70	10.183	0.004
			Location 3	25.62	2.47		
Stress*Mic	1	15				0.493	0.489
Stress*Loc	1	15				7.962	0.011
Mic*Loc	1	15				3.225	0.085
Stress*Mic*Loc	1	15				3.122	0.09