RESEARCH REPORTS
Biomaterials & Bioengineering

M. Staninec[1]*, R.K. Nalla[2], J.F. Hilton[3], R.O. Ritchie[2], L.G. Watanabe[1], G. Nonomura[1], G.W. Marshall[1], and S.J. Marshall[1]

[1]UCSF School of Dentistry, Box 0758, San Francisco, CA 94143, USA; [2]Materials Sciences Division, Lawrence Berkeley National Laboratory, CA, USA; [3]Division of Biostatistics, UCSF School of Medicine, San Francisco, CA, USA; *corresponding author, stanin@itsa.ucsf.edu

J Dent Res 84(4):371-375, 2005

Dentin Erosion Simulation by Cantilever Beam Fatigue and pH Change

ABSTRACT

Exposed root surfaces frequently exhibit non-carious notches representing material loss by abrasion, erosion, and/or abfraction. Although a contribution from mechanical stress is often mentioned, no definitive proof exists of a cause-effect relationship. To address this, we examined dimensional changes in dentin subjected to cyclic fatigue in two different pH environments. Human dentin cantilever-beams were fatigued under load control in pH = 6 (n = 13) or pH = 7 (n = 13) buffer, with a load ratio (*R* = minimum load/maximum load) of 0.1 and frequency of 2 Hz, and stresses between 5.5 and 55 MPa. Material loss was measured at high- and low-stress locations before and after cycling. Of the 23 beams, 7 withstood 1,000,000 cycles; others cracked earlier. Mean material loss in high-stress areas was greater than in low-stress areas, and losses were greater at pH = 6 than at pH = 7, suggesting that mechanical stress and lower pH both accelerate erosion of dentin surfaces.

KEY WORDS: dentin, erosion, fatigue, pH, abfraction.

Received July 27, 2004; Last revision November 11, 2004; Accepted January 13, 2005

INTRODUCTION

Exposed root surfaces frequently exhibit non-carious notches representing loss of dentin by abrasion, erosion, and/or abfraction (Levitch *et al.*, 1994). Abrasion can be caused by brushing with a hard toothbrush and/or abrasive dentifrice (Hooper *et al.*, 2003). Erosion is caused by acid attack on exposed dentin (Maneenut and Tyas, 1995). Abfraction, in which crown contacts cause fatigue and flexure of the cervical hard tissue and eventual cracking or loss, is assumed to be caused by mechanical stress from bruxism and mastication (McCoy, 1982; Lee and Eakle, 1984), but no definite proof exists of a cause-effect relationship (Braem *et al.*, 1992). Cervical notches could be caused by any of these factors or some combination thereof (Bader *et al.*, 1996), but the etiology of these lesions is controversial (Litonjua *et al.*, 2003).

Teeth are subject to fractures through the crown or roots. In posterior teeth, fractures are frequently observed in the cusp, usually in teeth with large intracoronal restorations. In anterior teeth, fractures may occur at the gingiva, severing the crown of the tooth. This type of fracture often occurs at a cervical notch. Tooth fractures generally are assumed to be related to occlusal stresses, either as catastrophic events, or, more plausibly, to result from repeated subcritical loads, which cause fatigue-crack growth and eventual failure (Wiskott *et al.*, 1995). Surprisingly, there are limited relevant data on the fatigue behavior of human dentin (Dong and Ruse, 2003; Nalla *et al.*, 2003, 2004); furthermore, these studies vary with regard to the outcome variable and the testing environment. Nalla *et al.* (2003, 2004) characterized the stress-lifetime fatigue behavior and measured the rates of fatigue-crack fatigue behavior in Hanks' balanced salt solution (HBSS), while Dong and Ruse (2003) studied the role of the dentin-enamel junction in impeding fatigue-crack growth in water.

In the present study, to quantify the relative environmental effects of abfraction and erosion on dentin loss, we subjected cantilever beams of dentin to cyclic fatigue stresses under two different pH conditions.

MATERIALS & METHODS

Specimens

Human molars, recently extracted and sterilized by gamma radiation, were used. The use of human teeth for this experiment was reviewed and approved as exempt by the institutional IRB, so no patient consent was necessary. Sections ~ 1.5-2.0 mm thick were prepared from the central portion of the crown and the root in the bucco-lingual direction (Fig. 1a). From these sections, we prepared 23 beams (~ 0.88 x 0.88 x 10 mm) by grinding on wet-abrasive paper, finishing with 600-grit. Each dentin beam included some root dentin and some coronal dentin, with a small corner of enamel to indicate the orientation. The beams were marked with indelible ink, indicating positioning

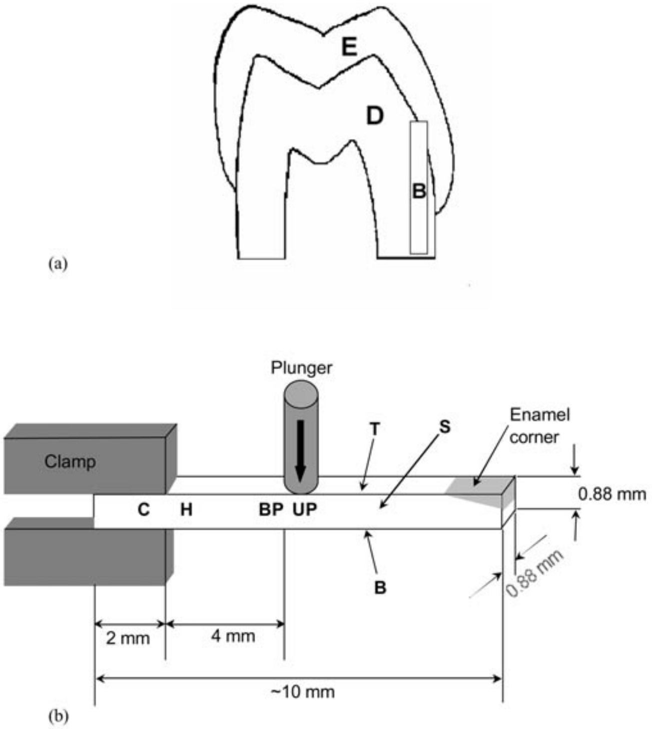

Figure 1. Schematic illustrations of **(a)** the location in the tooth from which the beam was prepared (E = enamel, D = dentin, B = beam), and **(b)** the cantilever beam geometry used for *in vitro* fatigue testing. Each dentin beam tested included some root dentin and some coronal dentin. Testing was conducted at ambient temperature. The locations where the measurements were made are indicated (C = clamp, H = high stress, BP = before plunger, UP = under plunger) for the top (T), side (S), and bottom (B) surfaces.

of the clamp and plunger, and were measured at 4 horizontal locations—inside the clamp (C), at the highest stress point (H), before the plunger (BP), and under the plunger (UP; clamp-plunger distance, 4 mm)—at each of 3 vertical locations—the top (T), side (S), and bottom (B) surfaces (Fig. 1b). Dimensional measurements (to a resolution of ± 0.1 µm) were made under a light microscope interfaced with a digital camera and a computer with digital image-processing software (ImagePro, Media Cybernetics, Inc., Silver Spring, MD, USA). The beams were measured in the same locations before and after fatigue cycling (repeated-measures accuracy SD 0.3%). After the fatigue cycling, specimen-specific changes in beam dimensions were estimated by the post-exposure, minus the pre-exposure, measurement at a given location and pH.

Simulation Design

The cantilever-beam specimens were loaded (Fig. 1b) with the use of a custom-made Delrin® rig. The apical end of the beam (location C) was clamped, and the beam was subjected to up to 1 x 10^6 fatigue cycles by being loaded at the coronal end (Fig. 1b) at a cyclic frequency of 2 Hz by a fatigue-testing machine (ELF 3200, EnduraTEC, Minnetonka, MN, USA) operating in load-control. In this way, the flexure that occurs at the cervix of the tooth as a result of applied coronal stress was simulated at location H, near the clamp. The maximum and minimum loads, L_{max} and L_{min}, were, respectively, 1.50 and 0.15 N, giving a load-ratio ($R = L_{min}/L_{max}$) of 0.1; the corresponding maximum and minimum stresses at the highest stress location H were, respectively, ~ 51-55 and ~ 5-5.5 MPa, calculated according to standard beam theory. The stresses decreased along the length of the beam, to negligible levels near the plunger (UP and BP).

The beams were randomly allotted to two erosion groups, then suspended in approximately 100 mL of either a mildly acidic pH = 6.0 (n = 13) or a neutral pH = 7.0 (n = 13) buffer, to simulate *in vivo* conditions. We prepared the buffers by adding concentrated hydrochloric acid to 0.1 M solutions of sodium tetraborate and Tris(hydroxymethyl)aminomethane, respectively. The buffer was not changed during the cycling of each specimen, and no significant changes in pH occurred during the experiment. It was anticipated that the clamp (at locations T and B) and the plunger (at location T, to a lesser extent) would shield the beam from the effects of erosion.

In addition to exposures to mechanical stress and buffer erosion, buffer agitation should be considered. As the plunger moved during the fatigue cycling, the beam flexed and moved correspondingly, effectively agitating the fluid. This agitation should enhance surface erosion along the length of the beam, from a negligible effect at the clamp to the highest effect at the plunger. Thus, erosive effects increased between location H and location UP, whereas mechanical stress effects decreased between them. Finally, change in the top (T) relative to the bottom (B) beam dimension contrasted the effect of tensile stress to that of compressive stress. To study these effects, we made measurements at the 12 locations indicated (Table 1).

Statistical Methods

The experiment was conducted according to a split-plot statistical design, studying material loss resulting from 12 exposure levels (Table 1) under 2 conditions (defined by pH). We modeled the effects of exposure levels, pH, and their interaction (1) on initial beam dimensions, to estimate uniformity of beam dimensions prior to intervention, and (2) on dimensional changes following intervention, to estimate means and 95% confidence intervals for effects on material loss at pH = 7, at pH = 6, and the difference. To quantify the relative effects of changes across the horizontal and vertical dimensions of the beams (*i.e.*, generate *P*-values), we also modeled changes as a function of the two dimensions and their interaction, stratified by pH. The models of changes also were adjusted for two specimen-level variables: initial maximum stress level, which is a function of the pre-exposure dimensions, and cycles completed prior to the beam breaking or the experiment ending. Since each of the 26 specimens contributed 12 pre-exposure dimension measurements and 12 change estimates, analyses were based on mixed-effects models, with specimens as random effects.

RESULTS

We examined the distribution of pre-exposure beam dimensions overall and as a function of the primary predictors, location and pH. In the neutral group, one specimen had an unusually high top dimension (31.0 µm higher than the average of its B and S dimensions), and another specimen had an unusually low bottom dimension (44.3 µm lower than the average of its S and T dimensions). When these locations were excluded, pre-exposure beam dimensions did not vary by location or pH ($P > 0.25$). Both specimens were retained in the sample, yielding an overall mean beam dimension of 878 µm (95% confidence interval, 874 to 882 µm). The median initial maximum stress level was 53.0 (min. 50.5; max. 60.1) MPa, and did not vary by pH level (2-sided Wilcoxon rank-sum test P = 0.28).

After the fatigue cycling, changes in beam dimensions were modeled as a function of location, pH, and initial maximum stress (Table 2) for assessment of the evidence of material loss at both pH levels and under different mechanical loading and agitation conditions. To the extent that, at pH = 7, there was no erosion, the combined effects of mechanical stress and agitation on material loss were isolated (Table 2a). In contrast, at pH = 6, the effects of stress and agitation were combined with the effect of erosion (Table 2b). Thus, the difference between the pH = 6 and pH = 7 conditions isolates the effect of erosion on material loss (*i.e.*, loss attributable to the difference between slightly acidic and neutral buffers; Table 2c).

Table 1. Anticipated Treatment Effects by Measurement Location

Vertical Beam Location	Under clamp (C)	Near clamp (H)	Near plunger (BP)	Under plunger (UP)
	Horizontal Beam Location			
Top (T) (tensile stresses)	s[*]: N/A[a] b: No a: N/A	s: High b: Yes a: No	s: Negligible b: Yes a: Yes	s: Negligible b: No/minimal[c] a: N/A
Side (S)	s: N/A[a] b: Yes a: No	s: Variable[b] b: Yes a: No	s: Negligible b: Yes a: Yes	s: Negligible b: Yes a: Yes
Bottom (B) (compressive stresses)	s: N/A[a] b: No a: N/A	s: High b: Yes a: No	s: Negligible b: Yes a: Yes	s: Negligible b: Yes a: Yes

[*] Legend: s, Mechanical stress level; b, Buffer action; a, Agitation; N/A, Not applicable/unknown.

[a] The exact stress states are somewhat indeterminate, owing to (unknown) clamping stresses.

[b] Stress varies from maximum tensile on the top surface, to maximum compressive on the bottom of the beam.

[c] The top surface of the beam was in contact with the plunger throughout fatigue-cycling, and hence, was considered to be mostly shielded from the buffer.

Table 2. Material Loss by Beam Dimension; Mean (95% confidence interval) in µm

Vertical Location	Under clamp (C)	Near clamp (H)	Near plunger (BP)	Under plunger (UP)
	Horizontal Location			
(a) pH = 7.0				
Top (T)	-11.1 (-22.0 to -0.2)	-33.7 (-44.6 to -22.8)	-29.0 (-39.9 to -18.1)	-16.9 (-27.8 to -6.1)
Side (S)	-10.4 (-21.3 to 0.5)	-31.7 (-42.6 to -20.8)	-31.0 (-41.9 to -20.1)	-22.1 (-33.0 to -11.2)
Bottom (B)	-11.8 (-22.7 to -0.9)	-40.8 (-51.7 to -29.9)	-34.3 (-45.2 to -23.5)	-31.2 (-42.1 to -20.3)
(b) pH = 6.0				
Top (T)	-22.6 (-33.1 to -12.1)	-70.1 (-80.6 to -59.7)	-63.0 (-73.5 to -52.5)	-34.3 (-44.7 to -23.8)
Side (S)	- 4.0 (-14.5 to 6.5)	-76.6 (-87.1 to -66.1)	-69.2 (-79.7 to -58.7)	-44.5 (-54.9 to -34.0)
Bottom (B)	-18.0 (-28.5 to -7.5)	-89.6 (-100.1 to -79.2)	-69.2 (-79.7 to -58.8)	-60.5 (-71.0 to -50.0)
(c) Difference, pH 6.0-pH 7.0				
Top (T)	-11.4 (-26.6 to 3.8)	-36.4 (-51.6 to -21.2)	-34.0 (-49.2 to -18.8)	-17.3 (-32.5 to -2.1)
Side (S)	+ 6.4 (-8.8 to +21.6)	-44.9 (-60.1 to -29.7)	-38.2 (-53.4 to -23.0)	-22.4 (-37.6 to -7.2)
Bottom (B)	-6.2 (-21.4 to +9.0)	-48.9 (-64.1 to -33.7)	-34.9 (-50.1 to -19.7)	-29.3 (-44.5 to -14.1)

At pH = 7, material loss was highest near the clamp (H) and decreased toward the plunger (H > BP > UP: Table 2a), implying a strong effect of mechanical stress [F-statistic (3, 132 df) = 95; $P < 0.001$], though it is not possible to separate out the effect of agitation (which comes into play for BP and UP). Material loss also increased along the beam from top (T) to bottom (B) at each horizontal location [F-statistic (2, 132 df) = 15; $P < 0.001$], demonstrating that the area under compressive stress (B) was more prone to material loss than the area under tensile stress (T). Similar patterns were observed at pH = 6.0; however, the variation across the horizontal dimension was stronger [F-statistic (3, 132 df) = 143; $P < 0.001$], and the variation across the vertical dimension (addressing the contrast between tensile and compressive stress) was weaker [F-statistic (2, 132 df) = 10; $P < 0.001$; Table 2b]. The effect of using a slightly acidic buffer rather than a neutral buffer was to approximately double the material loss at all locations, enhancing the effects of mechanical stress and agitation, except where the beam was shielded by the clamp, C ($P < 0.001$; Table 2c).

Another variable which would be expected to affect the amount of material loss was the number of cycles to which the beams were subjected, since this influences the time of exposure to fatigue. Although we intended to cycle all the beams equally for 1 x 10^6 cycles, not all survived this number. Of the 26 beams cycled, only 7 withstood 1 x 10^6 cycles. This implies that the stress amplitudes used here, given by one-half of the difference between the maximum and minimum stresses (24.3 ± 1.0 and 23.6 ± 0.7 MPa for pH = 6 and 7, respectively), are above the corresponding stress-life fatigue limits (Nalla *et al.*, 2003). At pH = 7, 5 of 13 beams withstood 1 x 10^6 fatigue cycles without cracking, compared with 2 of 13 beams at pH = 6. The median cycle durations were 7.4 x 10^5 and 7.7 x 10^5, respectively, for pH = 7 and pH

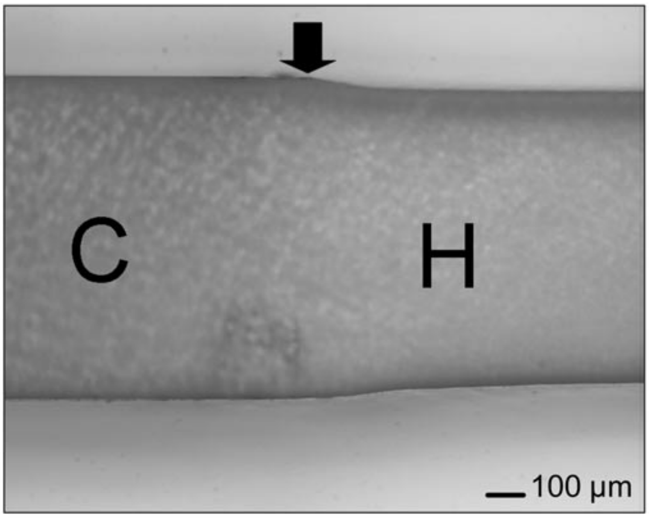

Figure 2. Optical micrograph showing the surface of a typical specimen near the high-stress area after fatigue-cycling at pH = 6. The surface loss is greatest near the clamp and decreases gradually toward the low-stress area, without any apparent localized notching. The arrow shows the location of the edge of the clamp (C = clamp, H = high stress).

= 6 (2-sided Wilcoxon rank-sum test $P = 0.52$). Material loss increased with longer cycle duration; however, after adjustment for the effects of mechanical stress, pH, and initial stress level, this effect was not statistically significant ($P = 0.23$) and was dropped from the models discussed above.

DISCUSSION

We have shown the effects of pH and mechanical stress on the material loss of dentin surfaces. These results are believed to be the first that have sought to evaluate erosion in the context of fatigue loading, in an *in vitro* model with standardized specimens. Both compressive mechanical stresses and lower pH were found to increase material loss. Assuming that no erosion occurred at pH = 7, our findings showed that the acidic buffer doubled the material loss at all locations of the beam, with these locations representing various levels of mechanical stress (horizontal axis; Table 2) and relative amounts of tensile and compressive stress (vertical axis; Table 2). This suggests that acidity increases the rate of material loss due to other factors. Our experiments support the hypothesis that mechanical stress, which causes flexure of the tooth at the cervix, contributes to surface erosion. Furthermore, compressive stress leads to significantly more material loss than tensile stress.

Since each cantilever beam had areas of various stress conditions and exposures to the buffer, each sample was its own control, if we simply compared the loss of surface material from the various areas with each other. The buffer solution caused some small changes in the non-stressed areas (BP and UP). In addition to pH = 7.0, we chose pH = 6.0, since this is the threshold for dissolution (Vanuspong *et al.*, 2002). Some material loss did occur at pH = 7.0, so it appears that, even at neutral pH, mechanical stresses cause some erosion of exposed dentin surfaces. We avoided calcium, phosphate and fluoride in the buffers, to maximize the possible material loss in this initial experiment. Further studies should address the inclusion of these ions.

We did not observe localized notching in the high-stress areas, but rather a uniform loss of material which decreased gradually toward the low-stress area (Fig. 2). The beam specimens are unlike a tooth in a clinical situation, because the tooth crown is covered by enamel, which adds stiffness and protection from the acidic challenge. This may account for some of the differences in the pattern of observed loss of material in our study as compared with clinical lesions. Clinically, dentin can be exposed due to loss of enamel or periodontal tissues (gingival recession) (Addy and Pearce, 1994). Furthermore, the tooth is often exposed to much stronger acidic environments physiologically (*e.g.*, from carbonated soft drinks, pH 2-3; gastric juices, pH 1-2); higher erosion levels should be expected under such conditions. Indeed, Vanuspong *et al.* (2002) reported increasing erosion depths with decreasing citric acid pH (2.5-6.0). Studies are currently under way to evaluate erosion under fatigue conditions in more aggressive environments, particularly with the aim of understanding the mechanisms behind the stress-induced acceleration of material loss. Another difference from the *in vivo* situation is the absence of a periodontal ligament to provide some cushioning. This could be incorporated into the model by cushioning the clamp; however, since the machine was run in force control, the resulting high and low stresses should be the same with or without cushioning.

We chose to ignore the possible contribution of abrasion to the loss of material. Although abrasion with a toothbrush and dentifrice could be incorporated into the model, it would increase its complexity. However, abrasion may play an important role in the clinical situation and should be explored in future studies. It should be noted that, although the stresses were high enough to cause failure of the beams, the amount of surface loss was relatively small, particularly at pH = 7. Clinically, it is common to observe cervical notches 1 mm or deeper (Piotrowski *et al.*, 2001), whereas we observed mean losses of 41 µm at pH = 7 and 90 µm at pH = 6 in the highest stress areas.

In summary, based on a series of *in vitro* fatigue-cycling experiments on human dentin cantilever beams in two different environments, both mechanical stress and lower pH accelerated material loss of dentin surfaces. Compressive stresses generally led to more loss than tensile stresses, and a change in pH from 7 to 6 nearly doubled the loss observed.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health, National Institute of Dental and Craniofacial Research, under Grant No. P01DE09859.

REFERENCES

Addy M, Pearce N (1994). Aetiological, predisposing and environmental factors in dentine hypersensitivity. *Arch Oral Biol* 39:33S-38S.

Bader JD, McClure F, Scurria MS, Shugars DA, Heymann HO (1996). Case-control study of non-carious cervical lesions. *Community Dent Oral Epidemiol* 24:286-291.

Braem M, Lambrechts P, Vanherle G (1992). Stress-induced cervical lesions. *J Prosthet Dent* 67:718-722.

Dong XD, Ruse ND (2003). Fatigue crack propagation path across the dentinoenamel junction complex in human teeth. *J Biomed Mater Res* 66(A):103-109.

Hooper S, West NX, Pickles MJ, Joiner A, Newcombe RG, Addy M (2003). Investigation of erosion and abrasion on enamel and dentine: a model in situ using toothpastes of different abrasivity. *J Clin Periodontol* 30:802-808.

Lee WC, Eakle WS (1984). Possible role of tensile stress in the etiology of cervical erosive lesions of teeth. *J Prosthet Dent* 52:374-380.

Levitch LC, Bader JD, Shugars DA, Heymann HO (1994). Non-carious cervical lesions. *J Dent Res* 22:195-207.

Litonjua LA, Andreana S, Bush PJ, Tobias TS, Cohen RE (2003). Noncarious cervical lesions and abfractions: a re-evaluation. *J Am Dent Assoc* 134:845-850.

Maneenut C, Tyas MJ (1995). Clinical evaluation of resin-modified glass-ionomer restorative cements in cervical 'abrasion' lesions: one-year results. *Quintessence Int* 26:739-743.

McCoy G (1982). The etiology of gingival erosion. *J Oral Implantol* 10:361-362.

Nalla RK, Imbeni V, Kinney JH, Staninec M, Marshall SJ, Ritchie RO (2003). *In vitro* fatigue behavior of human dentin with implications for life prediction. *J Biomed Mater Res* 66(A1):10-20.

Nalla RK, Kinney JH, Marshall SJ, Ritchie RO (2004). On the *in vitro* fatigue behavior of human dentin: effect of mean stress. *J Dent Res* 83:211-215.

Piotrowski BT, Gillette WB, Hancock EB (2001). Examining the prevalence and characteristics of abfractionlike cervical lesions in a population of US veterans. *J Am Dent Assoc* 132:1694-1701.

Vanuspong W, Eisenburger M, Addy M (2002). Cervical tooth wear and sensitivity: erosion, softening and rehardening of dentine; effects of pH, time and ultrasonication. *J Clin Periodontol* 29:351-357.

Wiskott HW, Nicholls JI, Belser JC (1995). Stress fatigue: basic principles and prosthodontic implications. *Int J Prosthodont* 8:105-116.