Effects of Cyclic Fatigue Stress-Biocorrosion on Noncarious Cervical Lesions

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ABSTRACT

Problem: Although there is a high prevalence of noncarious cervical lesions (NCCLs), the etiology of these lesions remains contentious.

Purpose: To evaluate the combined effects of cyclic fatigue stress and biocorrosion activity on NCCLs. **Materials and Methods:** Extracted premolar teeth were allocated into four groups (N = 10). Two groups were cyclically fatigue loaded (100 N; 72 cycles per minute; 9,200 cycles) and placed in either hydrochloric acid gel (pH=0.1) or orange juice (pH=4). The other two groups were stored in identical chemical solutions without fatigue load. The buccal–lingual width of each tooth was measured before and after testing. The depth of biocorrosion, normalized by the percentage change in buccolingual width, normalized by time (hour) was calculated. The data were analyzed using a two-way analysis of variance and Tukey's HSD multiple comparison test ($\alpha = 0.05$).

Results: Mean (SD) of the depth of biocorrosion values were as follows: teeth receiving fatigue loading with hydrochloric acid gel exposure (1.003%/hour [0.063]) revealed a significantly higher depth of biocorrosion than the fatigue-loaded group with orange juice exposure (0.511%/hour [0.281]) (p < 0.01). For the groups without fatigue loading, those with hydrochloric acid gel (0.022%/hour [0.006]) had a significantly higher depth of biocorrosion than the group with orange juice (0.009%/hour [0.004]) (p < 0.01). The cyclically fatigue-loaded teeth with hydrochloric acid gel had a significantly greater depth of biocorrosion than either group without fatigue loading (p < 0.001). **Conclusions:** Cyclic fatigue stress—acidic biocorrosion had a significant effect on the depth of the NCCLs.

CLINICAL SIGNIFICANCE

In order to manage the destructive NCCLs lesions properly, it is essential to understand the etiology of these lesions. The present study indicated that the combined mechanisms of cyclic fatigue stress and biocorrosion could contribute to the formation of NCCLs.

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INTRODUCTION

Although there is a high prevalence of noncarious cervical lesions (NCCLs), the etiology of these lesions remains "contentious." NCCLs are characterized by a loss of tooth structure at the cement enamel junction where stress is commonly located. Frequently, these lesions result in esthetic problems, hypersensitivity, functional impairment, annoyance to the patient, and may result in tooth fracture. Previously, the etiology of NCCLs has been described as being caused by either toothbrush/dentifrice abrasion alone^{1–16} or by acids termed erosion.^{17–20} Following the introduction of the term abfraction by Grippo in 1991,²¹ further explained

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in 1995²² and amended in 2004^{23} to represent the microfracture of tooth substance in areas of stress concentration, the term abfraction remains misconstrued and misused.²³

In addition to the determinants of environmental factors, the structure and composition of teeth, the mechanisms of "biocorrosion"²⁴ (causing chemical, biochemical and electrochemical, and degradation), stress (manifested by abfraction), and friction (from toothbrush/dentifrice abrasion) should be considered in the etiology of NCCLs.^{13,22} If the combined effects of all potential etiologic factors are evaluated, clinicians may be able to alter the pattern of NCCLs formation and progression, thereby improving outcomes.

Biocorrosion can occur when the various chemical actions gradually etch away the tooth structure. The chemicals may be from biochemical intrinsic sources (e.g., gastroesophageal reflux disease, bulimia nervosa) or chemical extrinsic sources (e.g., carbonated beverages, acidic diet).^{22,23,25} Repeated or prolonged exposure of acids leads to selective dissolution of specific components of the tooth surface. It has been reported that the dissolution of tooth enamel can occur at a critical pH of 5.5.²⁶ A study done by Shellis and colleagues demonstrated that after an initial adjustment period, the dissolution rates of enamel and hydroxyapatite were constant, while that of dentin decreased with time. The dissolution rate increased as the pH decreased being most marked in the enamel.²⁷ This difference is very likely due to the highly inorganic content of enamel as was noted in a study by Grippo and Simring.22

In addition to biocorrosion, stress concentration resulting from occlusal forces appears to be a cofactor in the etiology of NCCLs.^{22–25,27} Occlusal stress is dependent upon the magnitude, direction, frequency, and duration of force.²⁸ Stress can occur at various locations in teeth during interocclusal contact.²⁹ The principal axes of the teeth as well as the form, composition, and stability of the teeth can also affect the resultant stresses within the teeth.²⁷ Previous studies revealed that excessive cyclic and nonaxial loading resulted in cusp flexure and more stress concentration in the cervical region of the teeth.^{22,23}

The purpose of this investigation was to evaluate the combined physicochemical effects of cyclic fatigue stress and biocorrosion in the etiology of NCCL. The null hypothesis was that there is no difference in the depth of biocorrosion with or without cyclic fatigue stress under two different chemical sources. Orange juice was used as a surrogate for an extrinsic chemical acid source, whereas hydrochloric acid served as a surrogate for an intrinsic biochemical acid agent.

MATERIALS AND METHODS

Forty extracted intact human premolar teeth of typical morphology were selected. To be included, teeth had to be intact with no cracks or fractures in the crown, have no evidence of caries, and no prior restorations. The teeth were cleaned of surface debris, disinfected in 0.5% sodium hypochlorite, and kept in distilled water until the study began. The buccolingual and mesiodistal dimensions at the cervical of the each tooth were measured using a digimatic caliper accurate to within 0.01 mm (Mitutoyo series 551, Mitutoyo USA, Aurora, IL, USA). Five measurements were made at the greatest buccolingual width of the specimens, and the average width was determined for each tooth. The teeth were ranked according to the decreasing dimension. The ranked teeth were divided into four groups as follows: the first tooth was assigned to group 1, the second to group 2, the third to group 3, the fourth to group 4, the fifth to group 4, the sixth to group 3, the seventh to group 2, and the eighth to group 1. This procedure was repeated until each group had 10 teeth. Each group was assigned to one of four tested combination of the combined effects of cyclic fatigue stress and biocorrosion activity. The one-way analysis of variance (ANOVA) was tested and showed no significant difference between the dimensions of teeth in the four groups. The roots of the selected teeth were notched for retention, and a vertical planar enamel-bonding surface was prepared with a high-speed handpiece and diamond rotary cutting instrument (KS-1, Brasseler USA, Savannah, GA, USA).

The teeth were attached with sticky wax (Kerr sticky wax, Kerr, Orange, CA, USA) to a dental surveyor (J.M. Ney Co., Bloomfield, CT, USA) rod and placed in a vertical position so that the long axis of the tooth was parallel to the surveyor rod. The teeth were lowered into the copper cylinder positioning them in the center of the cylinder thus exposing 3 mm of dentin at the cementoenamel junction above the top edge of the cylinder. Premixed autopolymerizing resin (Pattern Resin, GC America, Scottsdale, AZ, USA) was injected into the cylinder until it was completely filled to the top rim. After the acrylic resin polymerized, the dental surveyor rod was detached and the each specimen was stored in distilled water at room temperature.

The mounted teeth were randomly allocated into four groups (N = 10). Two groups were cyclically loaded while the other two groups were without loading. All four groups were exposed to an acidic medium and tested for a period of 2 weeks. The four experimental groups were: (1) cyclically loaded with a hydrochloric acid gel application (L-HCL) (Tek Gel, surface gel, Mesa, AZ, USA) with a pH of 0.1 which was applied at the cervical areas of each tooth during loading, (2) nonloaded teeth with hydrochloricacid gel (NL-HCL) applied at the cervical area of each tooth twice a day for 2 weeks, (3) cyclically loaded teeth immersed in orange juice (L-OJ) (Tropicana, Tropicana Product Inc., Chicago, IL, USA) with a pH of 4, (4) nonloaded teeth (NL-OJ) were placed in orange juice for 2 weeks.

Specimens in the L-HCL group and the L-OJ were tested under load with a specialized loading machine (CoilCycler, Proto-tech, Portland, OR, USA) (Figure 1). To closely approximate in vivo loading conditions, each specimen was subjected to a fatigue load of 100 N (22.48 pound force) at a frequency of 72 cycles per minute. The mean of the physiological masticatory force has been reported to be in the range of 100 N to 400 N for the posterior dentition, and the frequency of the loading device, which conforms to the masticatory rate, has been reported to be in the range of 60 to 120 strokes per minute.³⁰ Therefore, the force applied falls within the limits of normal human bite forces and chewing cycles.³¹ According to the literature, 1.2 million cycles in the simulators corresponds to a clinical service



FIGURE I. Schematic photograph represent of specimen in group L-OJ, which tested under a cyclic fatigue load. The fatigue load was applied at 135 degrees to the long axis of the tooth to simulate a maximum bending motion of the tooth (maximum tensile stress) at the buccal cervical areas.

period of 5 years.³² In this present study, the number of cycle was selected at 9,200 cycles to represent 2 weeks of clinical services. The load was applied at 135 degrees to the long axis of the tooth to simulate a maximum bending motion of the tooth at the buccal cervical areas. After the fatigue loading was completed, the specimens were rinsed with water and gently dried prior to examination for any changes at cervical area under an optical microscope.

The level of cervical tooth substance loss in all teeth was determined according to a tooth wear index suggested by Smith and Knight.⁸ Any teeth where the cervical wear corresponded to an index level 2 or more (defect depth less than 1 mm deep) were considered to have NCCLs. Five measurements were made at the greatest buccolingual width of each specimen, and the average width for each specimen was calculated. The acid exposure time differed between the groups receiving cyclic loading and those that were not loaded. To adjust for this difference, the depth of biocorrosion normalized by percentage change of buccolingual width and, normalized by time (hour), were calculated to evaluate the difference among the testing groups.

Parametric statistical analyses were performed at a 95% confidence interval using statistical software (SAS V.9.1, SAS Institute, Cary, NC, USA). Groups were analyzed

Group	Depth of biocorrosion (% per hour)	Tooth wear index (%)					
		0	1	2	3	4	
L-HCL	1.003 (0.630)*	_	40	60	_	—	
NL-HCL	0.022 (0.006) [†]	_	80	20	_	_	
L-OJ	0.511 (0.281) [‡]	_	70	30	_	_	
NL-OJ	0.009 (0.004) [§]	10	80	10	_	_	

TABLE I. Mean (SD) values of depth of biocorrosion from the groups

Statistically significant difference (p < 0.05) is indicated by different superscripts. Tooth wear index: level 0 = no loss of contour;

level I = minimal loss of contour; level 2 = defect less than I mm deep; level 3 = defect I-2 mm deep; level 4 = defect more than 2 mm deep or pulp exposure or exposure of secondary dentin.⁸

using a two-way ANOVA, with fatigue load and acidity solutions as the variables, followed by Tukey's honestly significant difference multiple comparison test. In addition, specimens in each group were selected for a qualitative examination of the microstructure morphology on the enamel surface using a scanning electron microscope (FEI Sirion SEM, FEI Co., Hillsboro, OR, USA). The specimens were attached to scanning electron microscope (SEM) stubs with cyanoacrylate cement (UHU GmbH, Buehl, Germany) and sputter coated with gold palladium (Technics Hummer Sputter Coater, Anatech, Hayward, CA, USA) for 90 seconds.

RESULTS

The mean depth of biocorrosion values are presented in Table 1. Two-way ANOVA revealed a significant difference among the mean groups (fatigue stress and chemical activity) of depth of biocorrosion (p < 0.001). In addition, the interaction between stress and chemical (OJ) and surrogate biochemical (HCL) activity were significantly different (p < 0.05) (Table 2). The highest mean (SD) depth of biocorrosion was obtained from the L-HCL group (1.003%/hour [0.063]), followed by the L-OJ group (0.511%/hour [0.281]). This is approximately 50% to almost 100% more than the lowest mean depth of biocorrosion obtained from groups NL-HCL (0.022%/hour [0.006]) and group NL-OJ (0.009%/hour [0.004]). The HCL groups, either with or without load demonstrated a significantly higher depth of biocorrosion than group OJ (p < 0.01).

TABLE 2. Two-way ANOVA for the depth of biocorrosion

Source	Sum of squares	df	Mean square	F	Sig.		
Corrected model	6.705	3	2.235	20.727	0.001		
Intercept	5.965	I	5.965	55.320	0.001		
Fatigue stress (A)	5.492	I	5.492	50.939	0.001		
Biocorrosion activity (B)	0.637	I	0.637	5.911	0.020		
A×B	0.575	I	0.575	5.333	0.027		
Error	3.882	36	0.108				
Total	16.551	40					
Corrected total	10.586	39					
ANOVA = analysis of variance.							

The SEM images of the teeth at the cervical areas provided valuable information. From these two-dimensional images, a round-shaped lesion with an amorphous spongy-like microstructure on the surface was found in the group HCL (Figure 2A). In addition, numerous microporosities were detected in the substructure under high magnification in enamel (Figure 2B) and dentin (Figure 2C). In contrast, group OJ exhibited smooth surface structure with a minimal loss of surface structure contour (Figure 2D).

DISCUSSION

NCCLs' etiology has been considered as multifactorial (Figure 3). In the present study, two variables that affect



FIGURE 2. Scanning electron microscope (SEM) micrographs of teeth specimens at cervical areas. A, a round-shaped with an amorphous spongy-like microstructure was found in the group HCL; B, microporosities were detected in enamel in the group HCL (original magnification 1,000x); C, microporosities were detected in dentin in the group HCL (original magnification 1,000x); D, group OJ exhibited smooth surface structure with no significant change on the surface detected.

the depth of biocorrosion were evaluated: cyclic fatigue stress and the biocorrosion activity of two acids. The null hypothesis was rejected as the cyclic fatigue stress and the surrogate biochemical (HCL) and chemical (OJ) activity significantly affected the depth of biocorrosion on premolars.

In general, materials fail because of repeated loading from stresses that are too small to provoke spontaneous failures after only one application (fatigue). Numerous researchers have reported that the microfracture and loss of tooth substance in the cervical region was related to stress-induced flexure created by nonaxial occlusal forces.²³ In the present study, even though the fatigue stress application on the tooth was of short duration, a significant difference in mean values for depth of biocorrosion was observed. These results confirmed that the physicochemical effects of fatigue stress from occlusal force combined with the biocorrodents (OJ) and (HCL) contribute to NCCL formation. Therefore, where occlusal interferences are well established and diagnosed, occlusal adjustment might be considered. In vitro studies have demonstrated that both enamel and dentin degrade





when subjected to a stress combined with acidic agents acting as biocorrodents. $^{\rm 33,34}$

The present study confirmed that the depth of biocorrosion increased when the pH values decreased. There is substantial evidence from this study that exogenous (i.e., OJ fruit juice) and endogenous acids (i.e., HCL) are risk factors in the dissolution of enamel and dentin, and that the level of damage has been shown to increase during the frequency and duration of the acid exposure.

The exogenous agents from diet may be treated with dietary intervention with recommendations to remove or alter harmful habits. A recent study by Schlueter and colleagues has shown that the combined effects of trypsin from the pancreas and pepsin from the stomach act as proteolytic agents in causing biocorrosion of the highly organic dentin.³⁵

There are several limitations to this study. The results are applicable to only the premolar teeth selected and the two biocorrosive chemical and surrogate biochemical agents which were evaluated. The type of dental substrate could influence the results because tooth composition and surface hardness may vary between individuals or across age groups. The embedding method also did not simulate the clinical tooth mobility of the periodontal ligament, which may influence the results of the fatigue-loaded groups. Although the Smith and Knight tooth wear index is the most frequently used index in the dental literature and it records wear on all four surfaces (buccal, cervical, lingual, and incisal-occlusal), irrespective of the etiology of tooth wear, it has been reported that the data might not be quantitative. Future studies are required for the profilometry, impression-taking, surfometry, atomic force microscopy analysis to verify the depth of biocorrosion. In addition, the effect of

other risk factors on the depth of biocorrosion and stresses from loading forces, both individually and in disease models with other potential etiologic factors in the formation of NCCLs, are required for determination. An awareness of a multifactorial etiology may help the clinician to formulate an appropriate treatment plan for their patient.

CONCLUSIONS

Within the limitations of this in vitro study, physicochemical cyclic fatigue stress combined with the chemical (OJ) and surrogate biochemical (HCL) acidic agents were found to have a significant effect on the depth of biocorrosion in the formation of NCCLs. This study revealed that the teeth that had cyclic fatigue stress demonstrated more biocorrosion than the unstressed teeth.

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