Journal of the mechanical behavior of biomedical materials 66 (2017) 50–57

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

How mechanical stresses modulate enamel demineralization in non-carious cervical lesions?

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ARTICLE INFO

Keywords:
Non-carious cervical lesion
Tension
Demineralization
Fracture
Dental Enamel
Abfraction

ABSTRACT

Objective: To introduce an experimental non-carious cervical lesion (NCCL) model for studying the influence of presence and type of stress (tension or compression) on acid effects involved in NCCL formation on the enamel near the cement-enamel junction (CEJ).

Methods: 108 bovine incisors were cut into 18 × 3×3 mm^3 beams, with a notch in the cervical region to generate a standardized area of stress concentration. Half of the specimens were immersed in distilled water and the other half in acetic acid solution (pH 4.5) for 72 h. Each group was divided into three subgroups. Two subgroups underwent 800gf static loading, with the specimen positioned in a bending jig with the buccocervical region under either tension or compression. The load was applied simultaneously to immersion (in water or in acid). The third subgroup was not subjected to loading. Transversal and longitudinal 0.05 mm plates of the specimens were analyzed under a light microscope (40, 100 and 200×) to measure the enamel demineralization depth and to assess the presence of cracks, fractures and gaps at the enamel-dentin junction. The demineralization depth data were submitted to ANOVA and Tukey's test at a 5% significance level.

Results: Enamel demineralization depth (μm) was higher under tension (158 ± 19 in transversal sections and 229 ± 32 in longitudinal sections) than under compression (transversal: 129 ± 16 and longitudinal: 167 ± 10) or unstressed condition (transversal: 138 ± 21 and longitudinal: 187 ± 21). Specimens immersed in acid and subjected to tensile stress presented enamel micro fractures and wider gaps in the dentin-enamel junction.

Significance: Enamel demineralization was significantly higher in the presence of tensile stress, due to wider gaps between dentin and enamel, stress corrosion cracking and increased enamel permeability to acid.

1. Introduction

Non-carious cervical lesions (NCCL) are characterized by the loss of dental tissue near the cement-enamel junction (CEJ), unrelated with the presence of cariogenic biofilm. These multifactorial lesions have been associated with excessive abrasion (by toothbrushing) or acid corrosion (Litonjua et al., 2004; Nguyen et al., 2008; Sadaf and Ahmad, 2014). However, sub-gingival lesions cannot be explained by either mechanisms, since they do not occur subgingivally. In addition, these etiologic factors are not consistent with the fact that NCCL may be found on a single tooth, while the adjacent teeth remain unaffected.

Several studies explain the formation of single-tooth NCCL based on the abfraction theory (Braem et al., 1992; Dawid et al., 1991; Grippo, 1991; Lee and Eakle, 1984; Spranger, 1995; Xhonga, 1977). According to this theory (Lee and Eakle, 1984), enamel micro cracks are produced at the cervical area due to tensile stresses generated under non-axial occlusal loading. These micro cracks would predispose the tissue to erosion (corrosion) and abrasion (Bartlett and Shah, 2006). These lesions, called “abfraction lesions”, are typically wedge-shaped.

Although widespread among clinicians and researchers for years, this theory has been recently challenged (Michael et al., 2009). Some authors state that the association between NCCL and occlusal forces found in clinical studies does not represent a cause-and-effect relationship (Bartlett and Shah, 2006; Litonjua et al., 2003; Michael et al., 2009; Rees and Jagger, 2003; Senna et al., 2012). An additional

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http://dx.doi.org/10.1016/j.jmbbm.2016.11.003
Received 27 June 2016; Received in revised form 19 September 2016; Accepted 1 November 2016
Available online 04 November 2016
1751-6161/

criticism is that NCCL is predominantly observed in buccal faces, even when the occlusal contact suggests the presence of compressive stress in this area (Piotrowski et al., 2001).

The presence of micro fractures in the cervical region is also debated. Most of the studies that confirmed fractures in enamel and dentin due to non-axial loading were based on finite element analysis (Lee et al., 2002; Rees, 2002), while experimental or clinical evidences of these fractures are absent or weak. SEM and micro-CT images (Hur et al., 2011; Nguyen et al., 2008) of actual NCCL from extracted teeth found no evidence of enamel fracture as suggested by the theory of the abfraction mechanism.

Therefore, alternative theories have been postulated. Some authors suggested that the initial phase of "abfraction lesions" would involve loss of dentine at the enamel-dentin junction (Rees and Hammadeh, 2004), followed by fracture of unsupported enamel. Others (Grippo et al., 2013; Palamara et al., 2001) argued that mechanical stress contributes for NCCL formation by intensifying the demineralization effect of acids, but the specific mechanisms involved in this process are still unclear, as well as the stress type associated with it.

In the last decade, there was a breakthrough in understanding the mechanical properties of the enamel based on micro and nano-structure (An et al., 2015; Ma et al., 2016; Scheider et al., 2015). Several authors have emphasized that the enamel multi-level hierarchical organization is responsible for associating high mechanical strength, high fracture toughness and high damage tolerance in a highly mineralized material (Ma et al., 2016; Yilmaz et al., 2015). However, these studies focused on damage mechanisms due to isolated mechanical loading, without association with the corrosive environment, usually found in oral cavity.

The objective of this study was to introduce an experimental model for studying the possible interactions between stress (either tension or compression) and acidic environment on NCCL formation at the enamel near the CEJ. In addition, some considerations are made involving such mechanisms and the current knowledge of enamel multi-level hierarchical structure.

2. Materials and methods

Bovine incisors (n=108) were cleaned and inspected using a magnifying glass in order to discard those with superficial structural defects. Teeth were stored in 1% thymol solution at 4 °C. Two days prior to specimen preparation, teeth were rinsed in running water and kept in distilled water at 4 °C. Two cross-sectional cuts were made on each tooth with double-sided diamond discs (Isomet) under cooling at low speed to remove the apical third of the root and the incisal third of the crown, leaving the tooth with a final length of 18 mm (8 mm crown and 10 mm root – Fig. 1, A). Afterwards, the teeth were cut axially to obtain 108 18 × 3 ×3 mm sticks.

In a first stick, constructed as a standard, a 0.8-mm radius groove was made in the pulpal wall of dentin in the cervical region (Fig. 1, C) using a cylindrical bur (3101 – KG Sorensen) at high speed. The groove was made in order to concentrate and standardize stress level around this area. In addition, as bovine enamel and dentine are usually covered by irregular formations of cementum, the buccal surface of the specimen was trimmed with a fine diamond cylindrical bur (1093 FF – KG Sorensen) at high speed, in order to flatten the surface and remove the cementum.

This standard stick was fixed on an adapted pantograph device (EDPC – University of Iowa 094-8B), and 108 replications of were prepared. The buccal surface of the replicated sticks was polished with a felt disk and diamond paste (Diamond gloss KG Sorensen), until gloss was achieved for removing any roughness left by the bur.

A small cavity was prepared at the incisal end of the sticks that would be submitted to a static bending load, using a round carbide bur at high speed. One of the ends of an L-shaped 0.5 orthodontic wire hook was cemented into this cavity, in order to establish a stable area for load application. The free end of the hook faced the stick lingual side for specimens submitted to compression, while for those submitted to tension, the free end faced the buccal surface (Fig. 1, E). The opposite 5 mm of the sticks were used for fixation in the testing device.

An adhesive tape (1.5 mm wide, 18 mm long) was applied to the central axial third of specimen buccal surface. Two layers of acid-resistant clear varnish were then applied to the entire surface of the stick. After 24 h, the tape was removed, leaving only a central band on the buccal surface to be exposed to acid (Fig. 1, D), while the varnished regions of the buccal surface served as references.

The sticks were divided in two groups (n=54), one group was tested with specimens immersed in distilled water and the other one in the acid solution. Each group was divided into three subgroups (n= 8). Two subgroups underwent 800 gf static loading in two bending modes: one producing tension and the other producing compression in the buccocervical region. The load was applied during 72 h, simultaneously to immersion (in water or in acid). The third subgroup was not subjected to load (Figs. 1-E and 2). The applied load (800gf) was established in a pilot study as 66% of the mean fracture load for 12 samples.

The specimens subjected to the acid solution were immersed in 900 ml of demineralizing solution of acetate buffer containing calcium chloride ($CaCl_2$) 2.2 mM, sodium phosphate (NaH_2PO_4) 2.2 mM and acetic acid 0.05 M. Potassium hydroxide (KOH) was used to adjust the pH (4.5). The solution contained 95 mg/l of thymol to prevent microorganisms from growing. Regarding the titratable acidity of this solution, it is necessary the addition of 4 ml of KOH 0.1 M for changing the pH of 200 ml from 4.5 to 5.0. This demineralization protocol was based on previous published studies (Pimenta et al., 1998; ten Cate and Duijsters, 1982).

After the end of the test, the sticks were immersed for 24 h in distilled water, which was changed four times. The varnish was removed from the surface of the sticks. For histological processing, the specimens were dehydrated with the following protocol: 70% alcohol for 24 h, 100% alcohol for 24 h, 100% alcohol renewed for 48 h and 100% alcohol renewed again for 56 h. Afterwards, in order to infiltrate the dental tissues and then embedding the sticks into polymer blocks, each one was immersed for three days in 10 ml of solution containing 15% dibutyl phthalate (activator of polymerization) and 85% dimethyl methacrylate monomer ("A solution"). Subsequently, each stick was removed from the first flask with the "A solution" and immersed for three days in a flask containing 10 ml of a new solution prepared with "A solution" plus 1% benzoyl peroxide (polymerization initiator). Then the specimens were removed and immersed in 10 ml of "A solution" with 2.5% benzoyl peroxide for three days, at room temperature. Finally, the specimens were placed in an oven at 37 °C until the final polymerization of the resin, which occurred after two days.

The blocks were sectioned using an Isomet cutting device, with a 0.3 mm thick double-sided diamond disc, at 400 rpm, under cooling. In each group of 18 specimens, 12 were transversally cut for obtaining a 0.5 mm plate with enamel of the cervical region. The other six specimens were cut in the axial direction, so that a 0.5 mm longitudinal plate in the central region of the stick (which had been exposed to acid) could be obtained.

After sectioning, one surface of each plate was sanded and polished using an automatic polisher with silicon carbide abrasive paper of 800, 1000, 1500, 2000, 2500, 4000 grain. The first polished surface of each plate was then bonded with cyanoacrylate adhesive on 76 × 26 × 1.5 mm acrylic slides for microscopy. After final curing of the adhesive, the other surface of the plate was also sanded and polished as described above. During the procedure of sanding and polishing, the thickness of the plates was periodically measured with a digital caliper until a final thickness of 0.05 mm.

The slices were photographed at 40, 100 and 200X magnification under a Olympus BX 60 light microscope equipped with an Olympus