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Indentation Damage and Crack Repair in Human Enamel*

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Abstract

Tooth enamel is the hardest and most highly mineralized tissue in the human body. While there have been a number of studies aimed at understanding the hardness and crack growth resistance behavior of this tissue, no study has evaluated if cracks in this tissue undergo repair. In this investigation the crack repair characteristics of young human enamel were evaluated as a function of patient gender and as a function of the distance from the Dentin Enamel Junction (DEJ). Cracks were introduced via microindentation along the prism direction and evaluated as a function of time after the indentation. Microscopic observations indicated that the repair of cracks began immediately after crack initiation and reaches saturation after approximately 48 hours. During this process the crack length decreased up to 10% of the initial length, and the largest degree of reduction occurred in the deep enamel, nearest the DEJ. In addition, it was found that the degree of repair was significantly greater in the enamel of female patients.

Keywords

Enamel; crack repair; microindentation; hardness; toughness; brittleness

1. Introduction

Human enamel is the hardest and most highly mineralized tissue in the human body. Enamel is composed of approximately 96% mineral, with the remainder consisting of water (3%) and organic proteins (1%) [Ten Cate, 2008]. The mineral portion consists of carbonated hydroxyapatite nano-scale crystals (~25 to 30 nm thick, ~60 to 70 nm width and considerably greater length) that combine to form long ‘keyhole’ shaped rods between 4 to 8 μm in diameter [Ten Cate, 2008; Robinson et al., 1995]. These micro-scale rods, also regarded as the enamel “prisms”, are arranged in a columnar fashion, extending from the Dentin Enamel Junction (DEJ) to the occlusal surface. The rods are partially surrounded by a sheath of non-collagenous organic matrix with thickness much less than 1 μm [Weber, 1973; Boyde, 1989; Ge et al., 2005].

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Due to its composition, the mechanical behavior of enamel is unique from that of other hard tissues [Arola, et al., 2010]. The hardness and elastic modulus of enamel ranges from 3 GPa to 6 GPa and 70 GPa to 120 GPa, respectively, both depending on the location of evaluation [Xu et al., 1998; Habelitz et al., 2001; Cuy et al., 2002] and patient age [Park et al., 2008a]. These two properties increase from the DEJ towards the occlusal surface. The large hardness and elastic modulus provide wear resistance and stiffness, properties that help enamel protect the underlying dentin and pulp from injury. When evaluated by the indentation technique, the apparent fracture toughness ranges from roughly 0.4 to 1.5 MPa m^{1/2} [e.g. Hassan et al. 1981; Xu et al., 1998; White et al., 2001; Park et al., 2008b; Padmanabhan et al., 2010; Hayashi-Sakai et al., 2012]. The resistance to crack growth has also been evaluated using a conventional fracture mechanics approach [e.g. Bajaj and Arola 2009a; 2009b; Bechtle et al., 2010], showing that human enamel exhibits a rise in toughness with crack extension and a fracture toughness exceeding 2 MPa m^{1/2}. Nevertheless, the fracture toughness of enamel is quite low overall, which raises an important question. Why has nature allowed dental enamel to possess such low resistance to fracture?

Though substantial effort has been aimed at understanding the introduction and growth of damage in enamel, there has been limited focus on its ability to repair damage. Cracks are frequently seen within enamel on the surface of teeth, but they seldom enable tooth fracture. Perhaps they undergo healing. Sognnaes [1949] studied the propensity of the so-called “tufts” to self-heal, which represent hypocalcified intrinsic defects near the DEJ [Osborn, 1969]. But there was no clear identification that healing occurs, or an evaluation of the underlying mechanisms. Hayashi [1994] identified mineral aggregates within existing cracks in occlusal enamel and reasoned that repair occurs by mineral deposition. More recently, Myoung et al. [2009] studied the effect of tufts on the crack growth. They argued that fluids enter the crack and promote development of an adhesive interlayer, which hardens with time and serves to heal cracks. However, no study has monitored healing over an extended period or quantified the process of crack repair.

The topic of self-healing has taken inspiration from biological systems. Currently applied to polymers, an advancing crack triggers the release of healing agents that fill and then heal the crack by chemical reaction [Wu et al., 2008]. If self-healing or crack repair occurs in enamel, an understanding of the process could find application to other material systems. In the present investigation we examine the indentation fracture resistance and crack repair characteristics of human enamel. Cracks are introduced within enamel via the indentation technique and the crack closure process is examined as a function of time.

2. Materials and Methods

Human third molars (N=59) were obtained from selected patients with written consent and following all the protocols required by both the Cooperative University of Colombia (UCC) Dental Clinic and Eafit University. The teeth were obtained from patients of Medellin, Colombia between 18–25 years of age with nearly equal number of males and females. Third molars were selected in order to reduce the effect of cuspal wear on the enamel thickness and potential variances in chemical composition. All of the molars were caries-free and had not been previously restored. Immediately after extraction, all the specimens were kept in Hank's Balanced Salt Solution (HBSS) at 2°C to avoid dehydration and loss of mineral [Habelitz et al., 2002]. In addition, the specimens were tested within two weeks of extraction to limit the potential for loss of mineral or organic materials.

Each molar was sectioned along the longitudinal axis using diamond abrasive slicing equipment with continuous water coolant. Secondary sections were made to obtain the desired portion of the crown. The specimens were then mounted for indentation analysis

with cold-cured epoxy resin (Figure 1). The exposed occlusal enamel in the resin mount was polished using silicon carbide abrasive paper with successive smaller particle sizes until reaching #600 mesh. Further polishing by means of standard polishing cloth wheel was then performed using diamond particle suspensions until reaching 0.05 μm particle size. The polished specimens were kept within an HBSS bath solution prior to testing.

Ten specimens were used to study the Vickers hardness load dependence of human enamel and to identify the indentation load corresponding to the development of cracks. A previous study [Park et al., 2008] reported an indentation size effect in the hardness of enamel, and a reduction in hardness with increasing indentation load. Microindentation was performed using a micro-hardness tester (Wilson Instruments, Model 402 MVD, Norward, MA, USA) with a Vickers diamond indenter. At least 10 indentations were made at six different loads (0.1, 0.25, 1.0, 2.0, 3.0 and 5.0 N) in each specimen. Indents were carefully placed with a distance of at least 10 diagonals in length from each other, or from the DEJ. The Vickers hardness number (HV) was estimated following ASTM C1327 [2008] according to

$$HV = \frac{0.1891F}{L^2}, \quad (1)$$

where F is the indentation load and L is the indentation diagonal.

The apparent fracture toughness ($K_{c(app)}$) was measured on 28 specimens using the indentation approach. Based on results from the indentation size effects analysis, an indentation load of 3 N was used, which exceeded the critical load corresponding to the transition point hardness. Differences in properties were compared using an Analysis of Variance ANOVA with the critical value (alpha) set at 0.05. The indentation diagonal lengths and average crack lengths extending from the indentation corners were measured for each indentation (Figure 2a). Ten indentations were made on each specimen starting from the occlusal surface. Polishing was then performed to remove approximately 400 μm of material, after which another 10 indents were performed. This procedure continued until reaching the DEJ, and enabled characterization of $K_{c(app)}$ as a function of distance from the occlusal surface. Indentation of enamel results in the development of Palmqvist cracks [Park et al., 2008b]. Thus, the apparent fracture toughness for each indentation was calculated as [Niihara et al., 1982]

$$K_{c(app)} = 0.0084 \left(\frac{E}{HV} \right)^{2/5} \left(\frac{2F}{L} \right) \frac{1}{c^{1/2}} \quad (2)$$

where HV, F, L and c are the Vickers hardness, indentation load, average diagonal length and crack length, respectively. The elastic modulus (E) distribution for enamel as a function of normalized depth was obtained from the results of Park et al., [2008b].

In addition to estimating the apparent fracture toughness, the indentation brittleness (B) of enamel and its variation from the occlusal surface down to the DEJ was estimated according to [Quinn et al., 1997]

$$B = \frac{HV \cdot E}{K_{c(app)}^2}. \quad (3)$$

Repair of the indentation cracks was studied using an additional 21 specimens following a procedure similar to that described for evaluating the $K_{c(app)}$. Briefly, indentations were

made using a load of 3 N and crack length measurements were performed afterwards. The specimens were placed in deionized water at room temperature and then removed to measure the crack lengths after periods of 6, 24, 48, 96 and 168 hours. The original crack lengths (c_o) and that after specific crack lengths (c_t) were used in estimating the repaired crack length (c_h) as shown in Figure 2(b). The repair efficiency (η) was then defined as a function of time according to

$$\eta = \frac{c_o - c_t}{c_o} = \frac{c_h}{c_o}. \quad (4)$$

3. Results and Discussion

The influence of indentation load on the hardness of enamel is shown in Figure 3, which shows substantial indentation size effects. At the lowest load ($F=0.1$ N) the hardness reached values exceeding 7 GPa, whereas for loads of 3 N and greater the hardness decreased to a plateau nearly 50% lower than the maximum. These results are consistent with those of Park et al. [2008], who attributed the size effect to microcracking of enamel at larger loads. Indeed, this is the case for indentation loads in excess of 1 N. However, the apparent hardness also appears to be a function of the microstructural dimension. At the load of 0.1 N the indentation is on the order of a single prism (Fig. 3a), while the indentations made at much larger loads reflected a continuum measure of indentation resistance and not a discrete measure of single prisms (Fig. 3b). Also relevant, the hardness for loads of 0.1 N were not all centered on single prisms, hence resulting in greater hardness variation than noted for higher loads. Therefore, the hardness of a single prism is close to 7 GPa, while the overall hardness of enamel is approximately 3 GPa, which agrees with previous studies [Balooch et al., 2004; Park et al., 2008a; Willems et al., 1993; Meredith et al., 1996].

The apparent fracture toughness of enamel is shown as a function of normalized distance from the occlusal surface (x/d) in Figure 4(a). There is an increase in $K_{c(app)}$ from the occlusal surface to the DEJ of approximately 10%. The results indicate that enamel exhibits an increased ability to resist crack growth beneath the occlusal surface. There was no significant difference ($p>0.05$) between the results obtained for the enamel of male and female patients. Results for the $K_{c(app)}$ were used in determining the indentation brittleness according to Eqn. 3, which is shown in Figure 4(b). There is a significant ($p=0.01$) decrease in brittleness from the occlusal surface to the DEJ. A lower brittleness value denotes that deep enamel has greater preference for inelastic deformation than fracture [Quinn et al., 1997]. The values of brittleness and $K_{c(app)}$ presented in Figure 4 are in close agreement with those reported by Park et al. [2008b] for the enamel from third molars of patients in the US. Therefore, there is no difference in the mechanical properties of young enamel between the teeth of patients from the US and Colombia. Differences in the fatigue crack growth behavior have been found between human dentin of patients from the US and Asia [Arola et al., 2010]. Hence, while ethnic background does not appear to be important to the mechanical behavior of enamel for young patients, there may be important changes related to the aging process that remain to be addressed.

After the introduction of indentation cracks, it was noticed that they began to undergo a reduction in length (Figure 2(b)) as if they were repairing themselves. This process continued to increase with time after which portions of the cracks were no longer visible (Figure 5a). The degree of crack repair is presented in terms of the repair efficiency (Eqn. 4) in Figure 5b, at the occlusal surface ($x/d=0$), approximately midway across the enamel thickness ($x/d=0.5$) and adjacent to the DEJ ($x/d=1$). Evident in this figure, the degree of repair is a strong function of location and time, with the largest extent of repair occurring near the DEJ ($x/d=1$). Over 90% of the repair takes place in the first 24 hours. There also

appears to be a difference in repair efficiency with gender. At the occlusal surface the reduction in crack length was greater in the enamel of male patients, while as depth increased the repair efficiency for female patients was higher than for male patients. The extent of repair also increased with depth, regardless of gender.

Results of the evaluation showed that there was a reduction in crack length with time, indicative of a repair process, and that there were spatial variations in the degree of repair. But what are the contributing mechanisms? The reduction in crack length could be a result of viscoelastic recovery and/or via the operation of crack closure stresses. An SEM image of the crack front of an indentation soon after it was performed is shown in Figure 6. There is evidence of organic proteins acting to bridge the crack and assist in the crack closure process. This organic matter possesses viscoelastic characteristics [Habelitz et al., 2002; Sognnaes, 1949; Svensson et al., 2010; Wu et al. 2012; Yang et al., 2012] and as evident in Figures 2b and 6, creates closure stresses, facilitating crack closure and the process of repair. The time necessary to reach the threshold efficiency decreases with depth, going from approximately 48 hours for external and mid enamel, to approximately 24 hours for inner enamel. This trend may be related to the extent of protein, as there is an increase in the organic content of enamel approaching the DEJ [Ten Cate, 2008]. Indeed, as the organic matter is responsible for crack bridging and the viscoelastic behavior, a relation between the amount of organic matter and crack repair would be expected. The differences in repair efficiency with gender (Fig. 5) suggest that there could be a higher organic matter in the enamel of young females in comparison to young males. The crack repair dependence on gender is consistent with the results in Figure 4, where the apparent fracture toughness for female enamel is higher than that for males, and the brittleness is lower, both suggesting a higher organic content in the enamel of females.

The reduction in the stress intensity associated with bridging by the organic matrix can be estimated using the uniform traction Dugdale-zone model [Evans and McMeeking, 1986] defined as

$$K_{b(\text{protein})} = 2\sigma_b f_p \left(\frac{2\ell_b}{\pi} \right)^{1/2} \quad (5)$$

where σ_b is the nominal bridging stress on the protein matrix (assumed to be equivalent to the yield strength of protein ~ 20 MPa [Ji and Gao, 2004], f_p is the area fraction of protein matrix bridging ligaments (0.2 for dentin and enamel, from observation of crack path), and ℓ_b is the bridging zone length (10–200 μm , from observation of crack path). Based on calculations using Eqn. (5), it was estimated that $0.02\text{MPa}\sqrt{\text{m}} \leq K_{b(\text{protein})} \leq 0.09\text{MPa}\sqrt{\text{m}}$ which is approximately 10% of the apparent fracture toughness measured for enamel (Fig. 4a) and consistent with the degree of repair observed in the enamel specimens (Fig. 5). Despite the consistency in the order of contribution and degree of repair observed, it should be recognized that the aforementioned estimate of bridging stress intensity was based on a model and additional experimental evidence of the contributions of the protein bridges is needed.

Based on the estimate of the bridging stress contributions to the toughening behavior, it would appear that the repair process could be a function of both the viscoelastic recovery and the intrinsic toughening mechanisms offered by the protein ligaments. While both of these mechanisms may contribute to crack closure, they would not necessarily repair the crack. The organic proteins do potentially offer another “bridge” in addition to the mechanical component. In the oral environment the bridging proteins play an important role in transfer of ions and the remineralization process, which could result in repair of the crack

and thereby reduce the local stress intensity. But in order to fully understand the underlying healing mechanisms of enamel and its relation to the organic content, it will be necessary to perform crack growth resistance evaluations using a conventional fracture approach and measurements of toughness after periods of repair (e.g. Bajaj and Arola, 2009 or Bechtle et al., 2010). In that manner the degree of crack repair can be evaluated as a function of organic matter content, and the increase in toughness can be quantified. This is beyond the present scope and is reserved as a topic for future work. Nevertheless, the present study has provided the first quantitative evidence that enamel undergoes mechanical processes essential to repair and the eventual healing of mechanical damage. Future study of this important topic may provide further bioinspiration for design and fabrication of high performance composite materials.

4. Summary

Using microindentation techniques, the hardness, apparent fracture toughness and brittleness of enamel from Colombian patients was evaluated. The apparent fracture toughness increased with increasing proximity to the DEJ, whereas the indentation brittleness increased with proximity to the occlusal surface. Both properties and their distributions were in agreement with those of previous studies performed on enamel from patients of the US, suggesting that there are no ethnic or dietary factors contributing to the properties of young enamel. In addition, the indentation crack lengths decreased with time, suggesting that the microstructure of enamel promotes the repair of cracks. The repair process reached saturation after approximately 48 hours, and resulted in up to 10% reduction in crack length. The repair efficiency was greater close to the DEJ than near the occlusal surface, which appears to be due to the larger content of organic proteins near the DEJ. The repair efficiency was also significantly greater in the enamel of female patients.

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Figure 1. Schematic diagram of a sectioned molar embedded in cold cured epoxy for the indentation analysis. Note that this is a portion of the tooth crown that is mounted to permit the axis of indentations to be parallel to the enamel prism axes.

a) Vickers indentation on enamel with cracks emanating along the diagonals

b) healing studies involve crack length measurements immediately after indentation (c_o) and after the designated period of repair (c_t)

Figure 2.
Indentation of the enamel for determination of the apparent fracture toughness and crack repair.

Figure 3.

Effect of indentation load on the Vickers hardness of enamel. a,b) indentations at 0.1 N and 3 N, respectively, and viewed after etching to reveal enamel prisms. Note that the relative size of the indent at loads of 0.1 μN (a) is on the order of the prism diameter, whereas those at larger loads (b) span a group of prisms. c) indentation size effects on the hardness distribution

a) apparent fracture toughness.

b) indentation brittleness distribution.

Figure 4. Properties related to the indentation fracture resistance of enamel. The distributions are shown from the occlusal surface ($x/d = 0$) to the DEJ ($x/d = 1$).

a) crack length for selected samples.

b) repair efficiency.

Figure 5. Crack length and repair efficiency of enamel from male and female patients depending on time after introduction of the indent and depth.

Figure 6. SEM image of a selected indentation crack. Arrows point towards organic ligaments spanning the crack faces which help on the crack repair process.