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Regulated fracture in tooth enamel: A nanotechnological strategy from nature

Elnaz Ghadimi [a,1], Hazem Eimar [a,1], Jun Song [b], Benedetto Marelli [b], Ovidiu Ciobanu [a], Mohamed-Nur Abdallah [a], Christoph Stähli [b], Showan N. Nazhat [b], Hojatollah Vali [a], Faleh Tamimi [a,*]

[a] Faculty of Dentistry, McGill University, Montreal, QC, Canada H3A 0C7
[b] Department of Mining and Materials Engineering, McGill University, Montreal, QC, Canada H3A 2A7

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ABSTRACT

Tooth enamel is a very brittle material; however it has the ability to sustain cracks without suffering catastrophic failure throughout the lifetime of mechanical function. We propose that the nanostructure of enamel can play a significant role in defining its unique mechanical properties. Accordingly we analyzed the nanostructure and chemical composition of a group of teeth, and correlated it with the crack resistance of the same teeth. Here we show how the dimensions of apatite nanocrystals in enamel can affect its resistance to crack propagation. We conclude that the aspect ratio of apatite nanocrystals in enamel determines its resistance to crack propagation. According to this finding, we proposed a new model based on the Hall–Petch theory that accurately predicts crack propagation in enamel. Our new biomechanical model of enamel is the first model that can successfully explain the observed variations in the behavior of crack propagation of tooth enamel among different humans.

1. Introduction

Tooth enamel is the most highly mineralized and hardest tissue in mammals (Chai et al., 2009; Imbeni et al., 2005; Xu et al., 1998). Enamel inorganic consists of crystal key-hole shape structures composed of prisms (~6–8 μm) that are made of carbonate apatite (CAP) nanocrystals (Cui and Ge, 2007). Enamel organic is highly birefringent and is mainly composed of proteins and minor amounts of proteoglycans and lipoids that fill the spaces between the crystals (Cerny et al., 1996).

Enamel suffers from continuous mechanical stress that causes the formation of cracks favoring bacteria growth, caries and tooth fracture (Huang et al., 2010; Imbeni et al., 2005). Enamel has certain damage tolerance to sustain cracks (Chai et al., 2009) that could be an important factor in the adaptation of tooth to diet in evolution (Ang et al., 2010; Barani et al., 2011; Janis and Fortelius, 1988). It is also of interest for biomaterials research in the development of biomimetic materials (Ang et al., 2010). It has been suggested that geometrical and microstructural characteristics of enamel have an effect on its damage tolerance (Ang et al., 2010; Bajaj and Arola, 2009; Chai et al., 2009; Imbeni et al., 2005; Lawn and Lee, 2009; O'Brien et al., 2013; Xu et al., 1998).

The fact that tooth enamel is tougher than geologic-hydroxyapatite seems to indicate that the specific characteristics of enamel such as the organic content, enamel prisms and crystals might have an effect on defining its mechanical properties (An et al., 2012; He and Swain, 2007; White et al., 2001; Yahyazadehfar et al., 2013). Removal of enamel organic content reduces its fracture resistance (Baldassarri et al., 2008; Zheng et al., 2013). Prisms degree of decussation and its crystal orientation affect tooth enamel mechanical properties (An et al., 2012; Bajaj and Arola, 2009; Chai et al., 2009; Xu et al., 1998; Yahyazadehfar et al., 2013).

However, the specific contribution of enamel crystal size on crack propagation remains largely unknown. Crack propagation in polycrystalline materials is known to be regulated by their crystallographic dimensions. Increasing the crystal size in polycrystalline materials results in lower resistance to crack propagation (Mercer and Soboyejo, 1996; Wang and Shaw, 2009; Yusheng et al., 2004; Zhou et al., 2011), because cracks can propagate more easily around bigger crystals than around smaller ones (Yusheng et al., 2004).

We hypothesize that crack propagation in enamel might be influenced by its crystallographic dimensions. This study was designed to analyze the associations of enamel crystallographic

* Correspondence to: McGill University, Faculty of Dentistry, Strathcona Anatomy & Dent, 3640 University Street, Montreal, QC, Canada H3A 0C7.
Tel.: +1 514 398 7203x09654; fax: +1 514 398 8900.
E-mail address: faleh.tamimimarino@mcgill.ca (F. Tamimi).
[1] Both authors contributed equally to this work.

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nanostructure and chemical content with crack propagation in human teeth.

2. Materials and methods

2.1. Study sample

After obtaining ethical approval from McGill University Health Center ethical-committee, 36 extracted human upper-anterior teeth were collected from McGill Undergraduate-Dental Clinic. Teeth were cleaned and stored as previously described (Eimar et al., 2012a, 2011; Ghadimi et al., 2013).

2.2. Hardness and enamel crack propagation

A sagittal section was obtained from each tooth and fixed in clear resin (DP-Ortho-F, DenPlus, Montreal, QC). In our study and similar to previous studies (Baldassarri et al., 2008; Hassan et al., 1981; Hayashi-Sakai et al., 2012; Park et al., 2008a; Zheng et al., 2013), we evaluated cracks propagation in enamel using Vickers indenter (Clark-CM100AT, HT-CM-95605, Shawnee Mission, KS) since it provides direct measurements of cracks without causing a catastrophic failing of tooth enamel (Fett and Munz, 2006).

Indentations were applied on tooth enamel incisal third because enamel is thicker there than areas cervical two thirds and its prisms follow roughly a straight path from the DEJ towards the tooth surface (Roberson et al., 2002). All pyramid-shape indentation were conducted in regions that are not close to DEJ or tooth surfaces since previous studies have shown that these regions have toughening effects that resist cracks propagation (Imbeni et al., 2005; Jia and Xuan, 2012).

The diagonals (d_1 and d_2) of each indentation were parallel and perpendicular to DEJ, respectively. For hardness, 7 indentations of 100 g with 10 s loading time and a minimum distance of 50 μm between the successive indentations were conducted. This low level of load was applied to enhance the efficiency in measuring enamel hardness by preventing microcrack formation (Quinn and Quinn, 1997). For crack measurement, another high load (500 g) 7 indentations with 20 s loading time and a minimum distance of 100 μm between successive indentations were applied. Each indentation created measurable cracks that propagated from its corners. Then, samples were sputter-coated with gold and images of the cracks were captured at 500× magnification using a low vacuum VP-SEM (Hitachi S-3000N VP, Japan) to minimize the influence of dehydration on crack growth (Kruzic and Ritchie, 2008). We also followed a previous protocol for sample dehydration conducted on bone in which it was demonstrated that the followed protocol did not influence the crack behavior (Burr and Hooser, 1995; Mullins et al., 2008).

Crack length was measured using the ImageJ software (US National Institutes of Health, Bethesda, MD). The average crack length for each indentation was calculated by summing up the length of cracks and dividing by the number of cracks (Ang et al., 2011; Chicot et al., 2009).

Two kinds of cracks were observed around each indentation, cracks emanating from the indenter-pyramid-diagonal (d_1) that was parallel to DEJ and cracks originated at the indenter-pyramid-diagonal (d_2) that was perpendicular to DEJ. The average of the cracks lengths extended from d_1 and d_2 were calculated separately.

2.3. XRD

X-ray diffraction (XRD) (D8-Discover/GADDS, Bruker, Karlsruhe, Germany) was used to determine the crystallographic dimension of enamel apatite as previously described (Eimar et al., 2012a). Average crystal dimensions along *c*-axis and *a*-axis for each enamel sample were calculated using Scherrer's-formula on the (0 0 2) and (3 1 0) Bragg peaks.

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (1)$$

where D is the average diameter, k is the shape factor, λ is the X-ray wavelength β is the line broadening at half the maximum intensity (FWHM) and θ is the Bragg angle.

Enamel crystal cell lattice parameters, *a*-axis and *c*-axis, were calculated from the XRD (002) and (300) Bragg peaks, relying on the following equation (Hong et al., 2006):

$$\frac{1}{d^2} = \left(\frac{4}{3}\right) \times \left(\frac{h^2 + hk + k^2}{a^2}\right) + \left(\frac{l^2}{c^2}\right) \quad (2)$$

where d is the spacing between adjacent planes in the crystal, hkl are the miller indices that are the reciprocal intercepts of the plane on the unit cell axes, a is the *a*-axis and c is the *c*-axis.

2.4. Raman spectroscopy

Crystallinity index of tooth enamel samples was analyzed using a Raman spectrometer coupled with a 785 nm diode laser (Senterra, Bruker, Karlsruhe, Germany) and an Olympus BX51 microscope (Olympus, Melville, NY). Seven different spots were analyzed half-way between DEJ and outer enamel surface. Crystallinity index was quantified relying on the bandwidth at FWHM of the phosphate peak (ν_1PO_4) at ~960 cm^{-1} (Eimar et al., 2012b).

2.5. FTIR

Enamel chemical composition was investigated by a Perkin-Elmer FTIR Spotlight-400 (Waltham, MA) equipped with an ATR imaging accessory. The machine was adjusted at 6.25 × 6.25 μm pixel size, and 64 spectra were accumulated within a surface of 50 × 50 μm entirely inside the enamel region. FTIR studies were carried out in the range 750–1800 cm^{-1} with a spectral resolution of 2 cm^{-1}. Collected spectra were normalized according to the absorbance of ν_3PO_4 at 1013 cm^{-1}.

Organic content of enamel was estimated from the Amide I-to-ν_3PO_4 ratio (Bartlett et al., 2004). Carbonate content was estimated from the following ratios of ν_2CO_3 type A (~878 cm^{-1}) and B (~872 cm^{-1}) to the ν_3PO_4 and ν_1PO_4 (~960 cm^{-1}) absorption bands (Eimar et al., 2012a).

2.6. Statistical analysis

The data obtained was used to analyze the correlation of crack propagation within the tooth enamel with the characteristics of enamel apatite crystals and enamel protein content. The correlation coefficient "*R*", the regression coefficient "*B*" and the significance of the correlation "*P*" were calculated for each correlation analysis. The statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. Enamel crystallographic dimension, obtained by XRD, and protein content, obtained by FTIR

In this study, XRD data indicated that enamel apatite crystal size ranged between 9.4 and 22.2 nm along the *a*-axis and between 17.0 and 28.1 nm along the *c*-axis (Supplementary information, Fig. S2a and b). The crystal size dimensions reported in our study are similar to those measured by dark field electron microscopy (Grove et al., 1972). Similar to previous studies, it was found that the length of crystal lattice parameters along the *a*-axis and *c*-axis were ~9.43 Å and ~6.86 Å, respectively (Eimar et al., 2012a; Glas and Omnell, 1960; Legeros et al., 1983) (Supplementary information, Fig. S2c and d).

The protein content of the tooth enamel was estimated by FTIR spectroscopy. The relative protein content was highly varied among the examined teeth and followed a distribution that was not normal (Supplementary information, Fig. S3).

3.2. Cracks propagation

The average crack length in each enamel indentation varied between 10.9 and 66.93 μm (mean = 31.08 ± 15.69 μm) (Supplementary information, Fig. S1). Average length of cracks parallel to d_1 varied between 0 and 57.88 μm (mean = 19.95 ± 15.17 μm) and the average length of the cracks parallel to d_2 varied between 21.83 and 92.14 μm (mean = 42.14 ± 19.18 μm).

Differences in crack propagation along the directions of indenter pyramid-two-diagonals can be attributed to the anisotropic structure of the enamel detailed below. Enamel prisms are arranged in rows originating from DEJ to tooth surface (Daculsi et al., 1984), and are bound together across weak interfaces consisting of organic material (e.g., protein-rich rod sheaths) (Bajaj and Arola, 2009) as shown in Fig. 1c. During enamel fracture, cracks propagate along these weak interfaces following a straight path along the direction parallel to the prisms (indicated by red line in Fig. 1d), and a zigzag/bifurcated path perpendicular to the prisms (indicated by the blue line in Fig. 1d) (Bajaj and Arola, 2009;

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