JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 26 (2013) 148–154

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Spatial distribution of the human enamel fracture toughness with aging

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ARTICLE INFO

Article history:
Received 21 January 2013
Received in revised form
27 April 2013
Accepted 30 April 2013
Available online 24 May 2013

Keywords:
Fracture toughness
Mechanical property
Enamel
Aging
Mineral content

ABSTRACT

A better understanding of the fracture toughness (KIC) of human enamel and the changes induced by aging is important for the clinical treatment of teeth cracks and fractures. We conducted microindentation tests and chemical content measurements on molar teeth from "young" ($18 \leq age \leq 25$) and "old" ($55 \leq age$) patients. The KIC and the mineral contents (calcium and phosphorus) in the outer, the middle, and the inner enamel layers within the cuspal and the intercuspal regions of the crown were measured through the Vickers toughness test and Energy Dispersive X-Ray Spectroscopy (EDS), respectively. The elastic modulus used for the KIC calculation was measured through atomic force microscope (AFM)-based nanoindentation tests. In the outer enamel layer, two direction-specific values of the KIC were calculated separately (direction I, crack running parallel to the occlusal surface; direction II, perpendicular to direction I). The mean KIC of the outer enamel layer was lower than that of the internal layers ($p < 0.05$). No other region-related differences in the mechanical properties were found in both groups. In the outer enamel layer, old enamel has a lower KIC, II and higher mineral contents than young enamel ($p < 0.05$). The enamel surface becomes more prone to cracks with aging partly due to the reduction in the interprismatic organic matrix observed with the maturation of enamel.

1. Introduction

Enamel is the hardest and stiffest tissue in the human body because it has to withstand the chewing force to protect the internal soft dentin and pulp. Similar to most biological materials, enamel exhibits a range of hierarchical structures from the macro-scale to the nanoscale (Niu et al., 2009). At the micro-scale, enamel is composed of defective carbonate-rich apatite crystals arranged in enamel rods (~5 μm in diameter) or prisms. Each prism spans a surface that is nearly perpendicular from the dentino-enamel junction (DEJ) to the tooth, and the prisms are separated from each other by a thin layer of protein-based organic matrix (Habelitz et al., 2001).

The mechanical properties of enamel have been discussed for decades due to their importance in the clinical treatment and development of tooth-like restorative materials (Ang et al., 2012; An et al., 2012). Enamel has generally been considered a brittle material, similar to ceramic

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http://dx.doi.org/10.1016/j.jmbbm.2013.04.025

(Robinson et al., 2004). The fracture toughness, which describes a material's ability to resist the propagation of an existing crack under a particular state of stress (Park et al., 2008a), is an important parameter in the evaluation of the fracture behavior of enamel. As a hierarchical material, a profound understanding of the structure–behavior relationships of enamel necessitates its mechanical characterization at all hierarchical levels (Ang et al., 2012). However, due to the limited volume of tissue available for examination, conventional tests for fracture toughness, such as the single-edge notched beam (SENB) technique, cannot reflect the changes in the enamel at the micro-scale. Therefore, the fracture toughness of human enamel has been primarily evaluated using indentation methods. The reported micro-indentation fracture toughness of human enamel has been found to be 0.4–1.5 MPa $m^{1/2}$ (Hassan et al., 1981; White et al., 2001; Park et al., 2008a; Hayashi-Sakai et al., 2012). The variability of these results is partly attributed to the type of tooth (e.g., canine, premolar, and molar), the tested location, and the enamel microstructure.

Enamel has been found to have inhomogeneous properties (Habelitz et al., 2001). Studies have reported that both the hardness and the elastic modulus increase from the DEJ toward the tooth surface (Cuy et al., 2002, He and Swain, 2009). Moreover, the H and E values obtained from an occlusal section of enamel are generally higher than those obtained from an axial section (Xu et al., 1998, He and Swain, 2008). The orientation of the prism, the spatial variations in the enamel chemistry, and the mineral contents are hypothesized to contribute to these variations (Kodaka et al., 1992; Cuy et al., 2002). As a result, the fracture toughness of enamel also exhibits variations. Hassan et al. (1981) reported that the fracture toughness of enamel has a tendency to increase from the incisal to the cervical sections. The micro-indentation tests performed on cross-sections of enamel by Xu et al. (1998) showed that the fracture toughness of enamel is not single-valued but varies based on the enamel rod orientations. Additional studies have attempted to determine the fracture toughness of human enamel (White et al., 2001). However, only a limited number of studies have concentrated on the spatial distribution (e.g., from the outer to the inner layer or from different regions) of the enamel fracture toughness (Park et al., 2008a). Moreover, most of the indentation studies have calculated the fracture toughness based on the average elastic modulus of the enamel, which may not precisely reflect the spatial distribution of the fracture toughness.

The effects of aging and its related changes on the physical properties of biomaterials, such as dentine, have been noticed (Kinney et al., 2005). Similar changes in the mechanical properties of enamel have been found with aging (Park et al., 2008b; Cardoso et al., 2009). Cardoso et al. (2009) revealed that the Knoop hardness of a cross-section of human enamel significantly increased with an increase in the posteruptive age. Park et al. (2008a, 2008b) reported that older human enamel is more brittle and that the mean hardness and elastic modulus of older enamel near the occlusal surface are greater than that of younger enamel. These changes in the enamel properties are presumed to be caused by the higher mineral content in older enamel, but no further research has been conducted to investigate the influence of these factors on the enamel fracture toughness.

The aim of this study was to obtain an interpretation of the variations in the enamel fracture behavior with aging and location. We thus conducted a microindentation study to identify the cross-sectional fracture toughness of human enamel in specific regions based on the elastic modulus, which was determined in the same study. These studies were conducted in both young and old age groups. The accompanied alterations in the mineral contents of the tested regions were also examined. The fracture toughness, the elastic modulus, and the mineral contents were measured through the Vickers hardness test, atomic force microscopy (AFM)-based nanoindentation, and scanning electron microscopy (SEM) coupled with Energy Dispersive X-Ray Spectroscopy (EDS), respectively.

2. Materials and methods

2.1. *Specimen preparation*

Extracted human mandibular third molars were obtained from West China Hospital of Stomatology. The teeth were collected anonymously with full patient consent and approval from the Ethics Committee of West China Hospital. Thirty teeth were divided into two groups: “young” ($18\leq age\leq 25$; $N=15$) and “old” ($55\leq age$; $N=15$). The average age and the standard deviation of the young and old groups were 21 ± 3 and 68 ± 10 years, respectively. The teeth were stored fully hydrated at 4 °C in Hank's balance salt solution (HBSS) with 0.5% (w/v) thymol until use.

Sections were cut perpendicular to the buccolingual division line using a water-cooled diamond saw (Struers Minitom; Struers, Copenhagen, Denmark) (Fig. 1), polished with progressively finer grades of silicon carbide (waterproof silicon carbide paper, Struers, Germany), and finished with diamond abrasives (diamond paste, Struers) in decreasing grit size (2.5, 1.5, 1, and 0.5 μm). The samples were ultrasonically cleaned for 10 s and maintained moist during the testing through frequent spraying with HBSS. The mesial halves of the crowns were used for the nanoindentation test and the measurement of the mineral contents, and the distal haves were used for the microindentation test.

2.2. *Nanoindentation*

The nanoindentation experiments were performed using a Triboscope indenter system (Hysitron, USA) mounted with a Berkovich diamond indenter (Hysitron, USA). The elastic modulus and the hardness of the specimens were measured according to the protocols described by previous studies (Oliver and Pharr, 1992). A standard load/unload procedure was used with a rate of loading and unloading of 500 μN/S, and a maximum load of 5000 μN was maintained for 5 s. The indentations were measured in the outer, the middle, and the inner layers of the enamel, as defined by Kodaka et al. (1992) at both the buccal cuspal and the intercuspal regions (Fig. 1b). The indentations were made at a distance of at most 200 μm from the DEJ (inner enamel), midway between the DEJ and

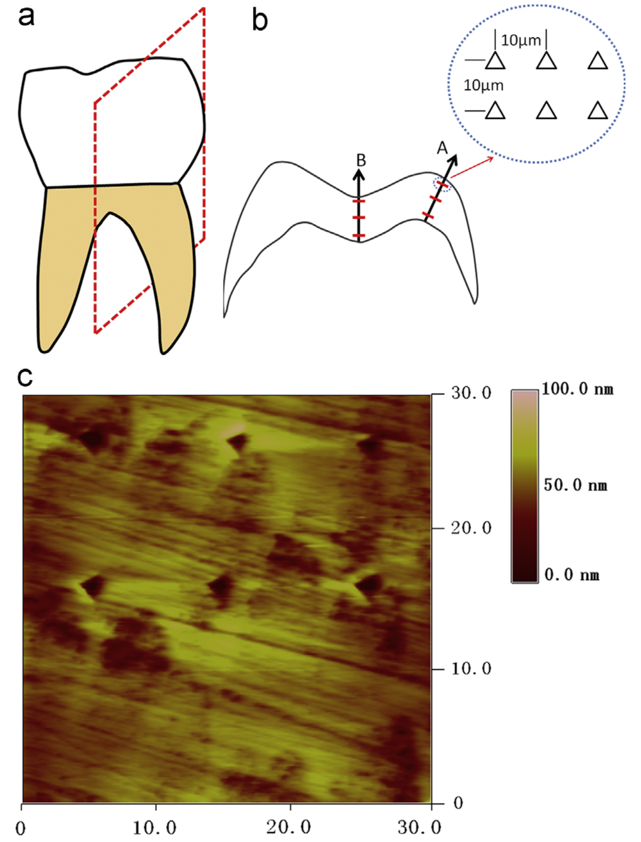

Fig. 1 – (a) The mandibular third molar was sectioned perpendicularly at the mesial cusp top in the buccolingual direction to divide the tooth into the mesial and distal halves. (b) Schematic diagram of a sectioned tooth crown and the two paths of evaluation. A and B represent the buccal cusp path and the intercuspal path, respectively. The red section denotes the measured region at the outer, the middle, and the inner enamel layers. The outer enamel layer was approximately 100 µm from the natural surface, the middle enamel layer was located between the surface and the DEJ, and the inner enamel layer was approximately 100 µm from the DEJ. AFM was used to investigate the topography of the indented enamel surface. (c) An AFM image of the indentations. Six indentations were made at each examined site from a 2 × 3 array.

the occlusal surface (middle enamel), and at a distance of at most 200 µm from the occlusal surface (outer enamel). We choose a distance of 200 µm (Kodaka et al. used 100 µm) to avoid the boundary effect of the DEJ and the occlusal surface during the following fracture toughness test.

In each enamel layer, two sites were randomly selected, and six indents were made at each site to obtain a total of 36 indents for each sample.

2.3. *SEM-EDS examination*

After the nanoindentation test, the samples were ultrasonically cleaned for 10 s, dried, and fixed on metal backings. After gold sputtering, the calcium (Ca) and phosphorus (P) contents of the enamel surfaces were evaluated using a SEM (Sirion400NC, Holland) equipped with a UTW-type EDS detector (Oxford INCA Penta Fetx 3, England). All of the EDS analyses were recorded for the outer, the middle, and the inner enamel layers. Six spot scans were performed for each layer.

2.4. *Micro-indentation fracture*

The Vickers hardness and indentation toughness tests were performed on a microhardness tester (MH-6, HengYi, China) at 9.8 N with a holding time of 20 s. The K_{IC} was calculated by the following equation (Niihara et al., 1982):

$$K_{IC} = 0.0084\left(\frac{E}{H_v}\right)^{2/5}\left(\frac{P}{a}\right)\frac{1}{c^{1/2}} \tag{1}$$

where E is the elastic modulus measured in the nanoindentation test, H_V is the Vickers microhardness, P is the applied indentation load (kg), a is half of the indentation diagonal length (m), and c is the indentation crack length (m).

For each sample, four indents were made in the inner, the middle, and the outer enamel. One diagonal of the Vickers indentation impression was aligned approximately perpendicular to the occlusal surface. Two values of the K_{IC} (in two directions) were calculated separately in the outer enamel: $K_{IC,I}$, which is the toughness associated with a crack running approximately parallel to the occlusal surface, and $K_{IC,II}$, which is the toughness associated with a crack in the direction perpendicular to the occlusal surface. In the middle and the inner enamel, where the prisms become winding, the mean values of K_{IC} was calculated for each indent.

2.5. *Statistical analysis*

The data obtained for the three enamel layers were analyzed using one-way ANOVA. The differences between the two age groups were analyzed using student's t test. Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. *Nanoindentation test*

The elastic modulus and the hardness distribution of enamel in young and old molars are shown in Table 1. The outer enamel has a higher elastic modulus and a greater hardness than the inner enamel ($p < 0.05$). There was no significant difference ($p > 0.05$) in the properties between the cuspal and the inter-cuspal regions in both age groups. In the outer layer, old enamel has a greater elastic modulus and a greater hardness than young enamel ($p < 0.05$).

3.2. *Fracture toughness*

Fig. 2 shows a representative indentation impression. No significant differences in the mean K_{IC} values were found between the cuspal and the inter-cuspal regions in both age groups. The $K_{IC,II}$ value was significantly lower than the corresponding $K_{IC,I}$ value in both age groups. Older enamel

Table 1 – Indentation results of the mechanical properties of enamel (H and E, in GPa; K_{IC}, in MPa m$^{1/2}$).

				Outer①		Middle②	Inner③
Cusp	E	Old		101.33 ± 3.51^a		92.95 ± 2.26	79.28 ± 2.19^b
		Young		95.69 ± 1.66^a		90.56 ± 3.62	75.67 ± 3.01^c
	H	Old		4.48 ± 0.36^a		4.01 ± 0.15^a	3.24 ± 0.25^b
		Young		3.89 ± 0.22^a		3.44 ± 0.31^a	3.10 ± 0.23^b
			$K_{IC,I}$	$K_{IC,II}{}^a$	Mean value		
	K_{IC}	Old	1.27 ± 0.11	0.76 ± 0.06^d	1.00 ± 0.14^e	1.23 ± 0.16	1.22 ± 0.21
		Young	1.30 ± 0.08	0.92 ± 0.05^d	1.11 ± 0.12^f	1.27 ± 0.22	1.23 ± 0.23
Intercuspal	E	Old	99.47 ± 1.57^a			89.93 ± 3.90	77.02 ± 3.44^b
		Young	93.24 ± 2.00^a			90.26 ± 4.53	80.85 ± 3.30^c
	H	Old	4.71 ± 0.27^a			3.99 ± 0.25	3.32 ± 0.29^b
		Young	3.79 ± 0.26^a			3.74 ± 0.29	3.26 ± 0.13^c
	K_{IC}		$K_{IC,I}$	$K_{IC,II}{}^a$	Mean value		
		Old	1.28 ± 0.10	0.72 ± 0.17^d	0.99 ± 0.10^e	1.25 ± 0.15	1.21 ± 0.30
		Young	1.29 ± 0.20	0.97 ± 0.14^d	1.15 ± 0.13^f	1.28 ± 0.20	1.22 ± 0.26

Notes:[a] $p < 0.05$ between the old group and the young group.
[b] $p < 0.05$ between ① and ②; $p < 0.05$ between ② and ③.
[c] $p > 0.05$ between ① and ②; $p < 0.05$ between① and ③; $p < 0.05$ between ② and ③.
[d] $p < 0.05$ between $K_{IC,I}$ and $K_{IC,II}$.
[e] $p < 0.05$ between ① and ②; $p < 0.05$ between ① and ③; $p > 0.05$ between ② and ③.
[f] $p < 0.05$ between ① and ②; $p > 0.05$ between ① and ③; $p > 0.05$ between ② and ③.

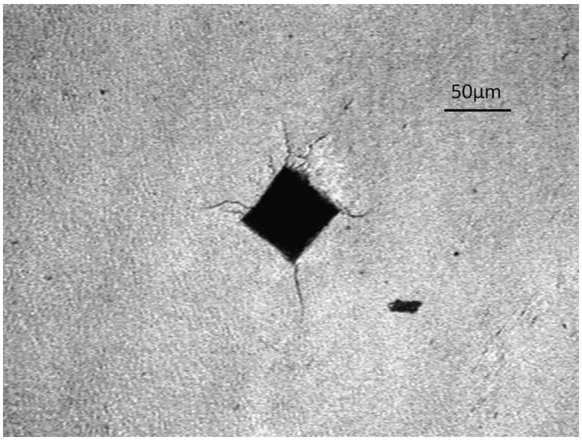

Fig. 2 – Optical micrographs of a representative indentation impression in a polished enamel occlusal section.

has a lower $K_{IC,II}$ value than young enamel. The mean K_{IC} value of the outer enamel layer was smaller than that obtained for the underlying enamel layers, and a more obvious reduction was observed in old enamel ($p < 0.05$). The detailed results are shown in Table 1.

3.3. *EDS analysis*

The Ca and P contents exhibited a downward trend from the outer to the inner layer of both old and young enamel. The mineral content distributions in the cuspal and the intercuspal regions were similar ($p > 0.05$). In the outer layer, old enamel has higher Ca and P contents than young enamel. The data are summarized in Fig. 3 and Table 2.

4. Discussion

An overall understanding of the aging-induced changes in the fracture-related properties of human enamel facilitates the design of a better clinical treatment and the prevention of tooth fracture, especially in older people, who are more prone to fractures.

In this study, we used AFM-based nanoindentation tests to determine the elastic modulus, which is required for the calculation of the K_{IC}. The use of AFM provided a convenient in situ observation of the indentation, which allowed us to improve the reliability of the data based on the indentation morphology. The mean values of the elastic modulus and the hardness and their aging- and location-induced changes were consistent with previous investigations (Cuy et al., 2002; Braly et al., 2007; Park et al., 2008b).

The mean K_{IC} value of young enamel measured in this study was 1.18 ± 0.27 MPa·m$^{1/2}$, which was in accordance with that reported in previous studies (Hassan et al., 1981; Xu et al., 1998; White et al., 2001). In both old and young enamel, the mean K_{IC} value increased from the outer to the middle layer and then exhibited a slight decrease from the middle to the inner layer, and the changes from the outer to the middle layer were more pronounced ($p < 0.05$).

Bajaj and Arola (2009) performed the most comprehensive quantification of the toughness of human enamel. Their results showed an increase in the enamel toughness as a function of the distance from the Dentin Enamel Junction (DEJ),

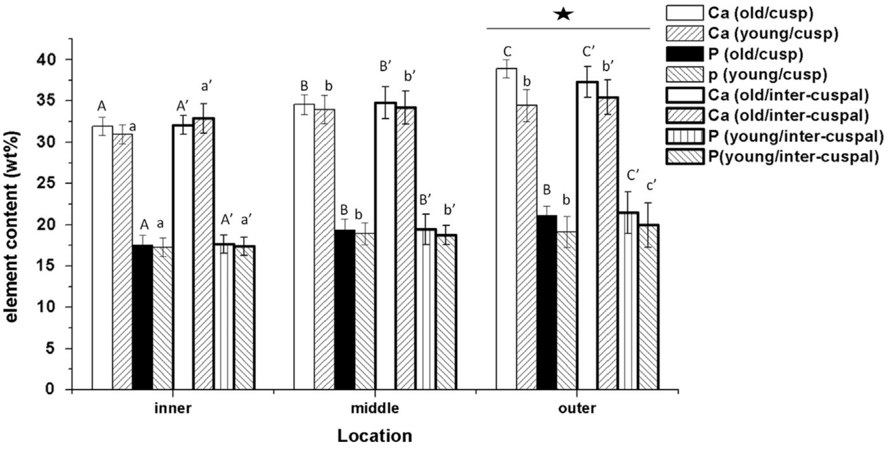

Fig. 3 – Element contents of the outer, middle, and inner enamel layers in both the cuspal and the intercuspal regions. The different capital letters (A–C) denote significant differences in the Ca or P content between the different enamel layers in the old age group, and the different lowercase letters (a–c) denote significant differences in the Ca or P content between the different enamel layers in the young age group. The letters without quotation marks represent the cuspal region, whereas the letters with quotation marks represent the intercuspal region. * indicates that there are significant differences in the Ca and P contents in the outer enamel layer between the young and the old age groups.

Table 2 – Ca and P contents in the different enamel layers and paths for teeth from two age groups (wt%).

Region		Old age group		Young age group	
		Ca	P	Ca	P
Cusp	Outer	38.91±1.31	21.56±1.44	34.45±1.44	19.12±1.89
	Middle	34.55±1.22	19.40±1.34	33.95±1.32	18.92±1.32
	Inner	31.55±1.09	17.36±1.22	32.07±1.25	17.29±1.12
Intercuspal	Outer	37.29±1.89	21.45±2.53	35.40±2.13	19.96±2.68
	Middle	34.76±1.92	19.44±1.86	34.18±2.01	18.76±1.18
	Inner	32.07±1.12	17.66±1.12	32.87±1.76	17.39±1.07

which is similar to the results from the present study. However, these researchers measured a fracture toughness in the inner enamel that reached a maximum value of 2.37 MPa m$^{1/2}$, which is considerably higher than that obtained in the present study. Bajaj and Arola quantified their measurements using incremental crack growth measures, whereas we used microindentation measurements. In addition, to avoid the boundary effects of the DEJ, the indentations on the inner enamel in this study were made at a distance of more than 200 μm from the DEJ, which exhibits a lower fracture toughness than the enamel near the DEJ (Imbeni et al., 2005). Therefore, it is possible that the techniques and the locations caused the differences in the measured toughness.

The variation in the K_{IC} is related to the structure of the enamel in different layers. In the outer enamel layer, the enamel prisms are generally perpendicular to the occlusal surface and are arranged in a more orderly fashion. From a mechanistic point of view, the outer enamel layer lacks the microstructural features to dissipate the fracture energy. In contrast, acute decussation in the middle and the inner enamel layers retards crack growth through the deflection, twist, and fracture of the prisms (Bajaj and Arola, 2009). Furthermore, it is easier for cracks to propagate parallel to the long axis of the prisms than to cut through the enamel rods (White et al., 2001; Hassan et al., 1981; He and Swain, 2007; Hayashi-Sakai et al., 2012). As shown in this study, the $K_{IC,II}$ value of the outer layer of both young and old enamel is lower than the corresponding $K_{IC,I}$ ($p<0.05$), which suggests that the elongation of cracks along the straight prisms of the outer enamel is easier. Therefore, the reduction in the mean K_{IC} value in the outer layer was attributed to the low value of $K_{IC,II}$. Because the prisms in the middle and the inner enamel layers are oriented toward the DEJ in a curved orientation, it is difficult to obtain the separate K_{IC} values for two directions (along and perpendicular to the prisms' long axis). Thus, we only determined the mean K_{IC} value. In particular, there is no obvious difference in the mineral contents (Ca and P) between the outer and the middle enamel in the young group, which indicates that there are no obvious changes in the proportion of the organic substances. Thus, the difference in the fracture toughness between the outer and the middle enamel, which is approximately 0.34 MPa m$^{0.5}$ in the direction perpendicular to the occlusal surface, is mainly due to the structure.

Studies have revealed that the aging process may affect the physical properties of enamel (Park et al., 2008a; Cardoso et al., 2009). In this study, the cross-sectional fracture toughness of enamel was found to change with aging. This variation was mainly observed in the outer enamel layer and was accompanied by changes in the mineral contents. In particular, old enamel exhibited a lower $K_{IC,II}$ in the outer layer than young enamel ($p<0.05$), and the mineral contents

(Ca and P) in the outer layer of old enamel were higher than in young enamel ($p < 0.05$) due to the prolonged exposure to the calcium, phosphate, and fluoride contents of the oral cavity (Brudevold et al., 1982). Thus, with increasing age, there is a reduction in the organic matrix surrounding the prisms in the outer enamel layer (Park et al., 2008b), which leads to the decreased value of $K_{IC,II}$ found in old enamel. Because there is no obvious difference in the microstructural features of the outer layer between young and old enamel, the aging-induced difference in the fracture toughness in the outer enamel layer, which is approximately 0.16 MPa $m^{1/2}$ in the direction perpendicular to the occlusal surface, is mainly due to the mineral contents. Note that there is no obvious difference in the value of $K_{IC,I}$ between the two age groups, which suggests that the effect of the mineral contents on the K_{IC} is concentrated in the interprismatic region.

In addition, the diffusion of mineral ions from the oral environment to the enamel may decrease with an increase in the distance from the enamel surface (Park et al., 2008b; Simmer et al., 2012) because the changes in the mineral contents in the middle and the inner enamel layers are not significant between the two age groups ($p > 0.05$). As a result, there is no obvious difference in the fracture toughness of the interior enamel layers between the old and the young age groups.

Based on the abovementioned results, we conclude that these microstructural changes in the outer enamel layer cause a reduction in the energy dissipation through inelastic deformation and a decrease in the fracture toughness, especially in older enamel with higher mineral contents. To avoid excessive damage to the teeth and to protect them from cyclic contact with dental materials, more attention should be paid to the selection of tooth restoration materials during the treatment of senior patients. In addition, the posteruptive enamel maturation is suggested to occur even 10 years after eruption (Cardoso et al., 2009), but it is unclear whether this mineral deposit continuously increases over time. Thus, to provide more precise instructions to clinicians, further studies should be conducted to identify whether the posteruptive maturation stops at a specific age and whether the decrease in the fracture toughness ceases with aging.

5. Conclusion

The fracture toughness was lower in the outer enamel layer than in the internal enamel layers because of the arrangement of prisms (lack of decussation). The aging-induced changes in the fracture toughness were mainly observed in the outer enamel layer. The deposition of mineral contents in interprismatic region made the older outer enamel more prone to crack elongation along the prisms. More concern should be paid to the design and selection of the tooth restoration materials used in the treatment of senior patients.

Acknowledgments

The work was supported by the National Natural Science Foundation of China (11272226, 11072252 and 11023001). All of the authors declare that there are no conflicts of interest.

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