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# Differences in the microstructure and fatigue properties of dentine between residents of North and South America<sup>☆</sup>

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## ABSTRACT

Spatial variations in the microstructure of dentine contribute to its mechanical behaviour. **Objective:** The objective of this investigation was to compare the microstructure and fatigue behaviour of dentine from donors of two different countries.

**Methods:** Caries-free third molars were obtained from dental practices in Colombia, South America and the US to assemble two age-matched samples. The microstructure of the coronal dentine was evaluated at three characteristic depths (i.e. deep, middle and superficial dentine) using scanning electron microscopy and image processing techniques. The mechanical behaviour of dentine in these three regions was evaluated by the fatigue crack growth resistance. Cyclic crack growth was achieved in-plane with the dentine tubules and the fatigue crack growth behaviour was characterized in terms of the stress intensity threshold and the Paris Law parameters.

**Results:** There was no difference in the tubule density between the dentine of patients from the two countries. However, there were significant differences ( $p \leq 0.05$ ) in the tubule lumen diameters between the two groups in the deep and peripheral regions. In regards to the fatigue resistance, there was a significant increase ( $p \leq 0.05$ ) in threshold stress intensity range, and a significant decrease in fatigue crack growth coefficient with increasing distance from the pulp in teeth from the US donors. In contrast, these properties were independent of location for the dentine of teeth from the Colombian donors.

**Conclusions:** The microstructure of dentine and its mechanical behaviour appear to be a function of patient background, which may include environmental factors and/or ethnicity.

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## 1. Introduction

Amongst the three hard tissues occupying human teeth, dentine occupies the majority by both weight and volume. Both coronal and radicular dentine are traversed by a network of tubules (approx. 0.5–1.5  $\mu\text{m}$  in diameter) that radiate outward from the pulp cavity to either the dentine enamel junction (DEJ) or cementum.<sup>1</sup> Regarded as the dentine tubules, each consists of a central lumen that is lined by a collagen-free, hyper-mineralized cuff of peritubular dentine, sometimes called intratubular dentine. Intertubular dentine occupies the interstitial space between the tubules and consists of a collagen fibril matrix reinforced by nanoscale crystals of apatite.<sup>2</sup> Owing to its composition and presence of the tubules, the microstructure of dentine is unique from that of the other hard tissues. Understandably, dentine is often regarded as a hierarchical biological composite.<sup>3</sup>

Microscopic evaluations of dentine have been largely focused on characteristics of tubule densities. Overall, the density ranges from roughly 10,000 to over 60,000 tubules/ $\text{mm}^2$ .<sup>4–7</sup> There are changes in the number of tubules and their diameter in the tooth crown, which results in spatial variations of the microstructure. These characteristics vary primarily with distance from the pulp, but also depend on the physiology and traumatic history of the tooth. Nevertheless, the tubule density and the tubule diameters are lowest at the DEJ and highest in deep dentine, nearest the pulp chamber.<sup>4</sup> Pashley<sup>6</sup> estimated that the tubule lumens occupied approximately 22% of the evaluated cross-sectional area near the pulp, and only about 1% near the DEJ.

According to the large variations in microstructure, the mechanical behaviour of dentine within the crown would be expected to be site-specific. Indeed, experimental studies have identified that there are variations in mechanical properties within the tooth crown, including hardness,<sup>8–10</sup> ultimate tensile strength<sup>11–13</sup> and shear strength.<sup>14,15</sup> Pashley et al.<sup>8</sup> found that the reduction in dentine microhardness from the DEJ towards the pulp was correlated with the increase in tubule density. That reduction in hardness was attributed to the increase in tubule diameter and the reduction in volume of intertubular dentine. Kinney et al.<sup>9</sup> reported that the spatial variations in macroscopic hardness are also related to intrinsic changes to the intertubular dentine with location and greater hardness near the DEJ. The increase in tubule density with depth is also important to the tensile strength of dentine. Deep coronal dentine is weaker than dentine located near the DEJ, and the differences have been attributed to tubule density.<sup>11,13</sup> A similar dependence of strength on tubule density has been identified in the root.<sup>16</sup>

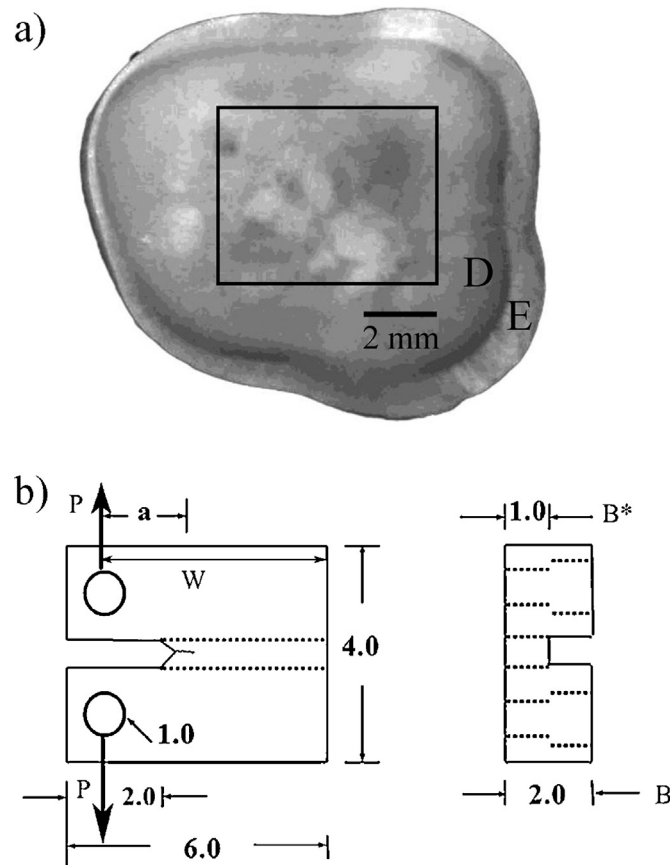
While the hardness and strength are important measures of mechanical behaviour, both being relevant to the success of dental practice, most mechanical forms of tooth failures are expected to occur by fatigue and fracture.<sup>17,18</sup> Consistent with the bulk properties, the fatigue and fracture resistance of dentine are strongly influenced by spatial variations in the microstructure. Ivancik et al.<sup>19</sup> identified that there is a significant decrease in the fatigue crack growth resistance of coronal dentine with increasing distance from the DEJ. Cracks in deep dentine (close to the pulp) begin to undergo cyclic

extension at stresses nearly 40% lower than that in tissue nearer to the DEJ. According to microstructural analysis, the average fatigue crack growth rates increased significantly with increasing tubule density. The fracture toughness of dentine is a function of location as well, with an increase in fracture toughness of nearly 50% from deep dentine to the outer regions.<sup>19</sup> There is substantial clinical significance to the aforementioned findings, namely that restorations extended into deep dentine are much more likely to facilitate tooth fracture. Flaws resulting from cavity preparations in deep dentine will undergo more rapid cyclic crack growth, and will reach a critical length much sooner than that in the peripheral regions.

Although the spatial variations in microstructure of dentine are well-recognized, to the authors' knowledge no study has questioned whether the microstructural variations are equivalent amongst all individuals, or if they are dependent on the patient background, e.g. living environment or ethnicity. A comparison of the fatigue properties of dentine obtained from senior residents in the US and China reported a significant difference in fatigue crack growth resistance between the two groups.<sup>20</sup> However, that study did not perform a thorough comparison of the microstructures. Environmental and/or ethnic factors could be important to the microstructure of dentine, thereby resulting in differences between patients of disparate groups. In recognition of past studies, differences in the microstructure would reflect on the mechanical behaviour of dentine. Thus, the objective of this investigation was to compare the microstructure and corresponding fatigue crack growth resistance of dentine from age-matched residents of North and South America. The null hypothesis of the investigation is that there are no differences in the microstructure of dentine or its fatigue resistance from these two populations.

## 2. Materials and methods

Non-carious third molars were obtained from donors in two locations, namely patients visiting private practices in the state of Maryland and patients visiting practices in the city of Medellín, which is located in the Department of Antioquia, Colombia. These two locations were chosen for the collaborative relationship that has been established between EAFIT University and CES University of Medellín and the University of Maryland. Details regarding the potential differences between donor groups in these two locations will be discussed later. In both countries, the teeth became available as a result of routine extractions necessary for maintaining oral health of the patients. All collected teeth were obtained from donors between the ages of 18 and 35 years old, an age span defined by earlier studies to exhibit consistent fatigue and fracture behaviour.<sup>21–23</sup> Those teeth from donors in the US were obtained in accordance with an approved protocol issued by the Institutional Review Board of the University of Maryland County (Approval Y04DA23151). In Colombia, the teeth were obtained with written consent and followed the protocols required by both the Cooperative University of Colombia (UCC) Dental Clinic and EAFIT University. In both locations the teeth were placed in Hanks Balanced Salt Solution (HBSS)



**Fig. 1 – Preparation of a compact tension (CT) specimen from the coronal dentine of a 3rd molar. (a) View of an axial section from a tooth crown and potential location of a specimen. E and D indicate the enamel and dentine, respectively; (b) final geometry and dimensions of the CT specimen. Loading (P) is applied from pins placed within the two holes.**

immediately after extraction along with record of donor age and gender.

Evaluations of the microstructure and fatigue resistance for both groups of teeth were conducted within the US. Within one month of extraction, the teeth were sectioned using a numerical controlled slicer/grinder (Chevalier, Model SMART-H81811, Taiwan) under water-based coolant using diamond abrasive slicing wheels. Serial sections were made perpendicular to the tooth's axis to obtain a single 2 mm thick section (Fig. 1(a)). Secondary sectioning was then performed to obtain Compact Tension (CT) specimens as shown in Fig. 1(b). The specimens were obtained as a function of relative distance from the DEJ in three general regions, including deep dentine (nearest the pulp), middle dentine (tissue located centrally between the pulp and DEJ) and superficial dentine (the peripheral region closest to the DEJ). It is important to note that only one specimen was obtained from each tooth. In some of the largest teeth more than one sample could be prepared from the crown tissue (e.g. from the deep and superficial dentine). But this is rare, and seldom possible with the teeth from Colombia due to the smaller size. Thus, to minimize the chances for conflicting degrees of independence between the two sample groups, only one sample was prepared from each tooth. Based on the location and manner of sectioning, the dentine tubules were oriented in-plane with

the fatigue crack surface, and perpendicular to the direction of crack extension. A total of  $N = 43$  teeth were evaluated from donors of the two countries. In terms of the three regions of evaluation, the investigation consisted of 14 specimens of deep dentine (US  $n = 10$ ; Colombia  $n = 4$ ), 16 specimens of middle dentine (U.S.  $n = 12$ ; Colombia  $n = 4$ ) and 13 specimens from the superficial region (US  $n = 9$ ; Colombia  $n = 4$ ).

Cyclic loading of the CT specimens was performed using a BOSE ElectroForce 3200 (Eden Prairie, MN, USA) universal testing system using routine methods developed and applied in previous studies.<sup>19,24</sup> Briefly, the specimens were subjected to cyclic loading at a frequency of 5 Hz and under load control displacement. All testing was conducted while the specimens were immersed in HBSS maintained at room temperature (22 °C). The loads were applied in opening mode (Mode I) in which two pins were placed within the opposing holes of the CT specimen (Fig. 1(b)). Crack initiation was achieved using a stress ratio ( $R$ ) of 0.5, which defines the ratio of minimum and maximum load. Once the crack was initiated, cyclic crack growth was conducted using  $R = 0.1$ . Thereafter, the specimens were subjected to increments of cyclic loading, followed by an assessment of the crack extension. Crack length measurements were achieved using a digital microscope (Navitar IEEE 1394) at a magnification of 60 $\times$ . The number of cycles between measurements ( $\Delta N$ ) was chosen according to

the observed crack growth rate and typically ranged between 5 and 20 kcycles; the average increment of extension was between 60 and 120  $\mu\text{m}$ . The incremental crack growth rates ( $da/dN$ ) were quantified by dividing the measured incremental crack extension ( $\Delta a$ ) by the increment of loading cycles ( $\Delta N$ ). These methods are consistent with those used in previous studies concerning fatigue crack growth behaviour of dentine.<sup>19,23,24</sup>

Most of the fatigue crack growth responses exhibited three characteristic regimes of fatigue behaviour including initiation, steady-state incremental growth, and the tertiary regime of unsteady crack extension to fracture. The incremental fatigue crack growth rate ( $da/dN$ ) within the region of steady state (Region II) response was quantified using the Paris Law<sup>25</sup> according to

$$\frac{da}{dN} = C(\Delta K)^m \quad (1)$$

where  $\Delta K$  is the stress intensity range, and the quantities  $C$  and  $m$  are the fatigue crack growth coefficient and exponent, respectively. The stress intensity range ( $\Delta K$ ) is determined from the difference in stress intensity at the minimum and maximum loads.<sup>26</sup> Due to the unique specimen size, the stress intensity resulting from cyclic loading was estimated according to the work of Bajaj et al. (2006).<sup>24</sup> Using the incremental crack length measurements and the corresponding stress intensity, the fatigue crack growth rate ( $da/dN$ ) were plotted in terms of  $\Delta K$  to estimate the quantities  $m$  and  $C$  for each specimen. In addition, the apparent stress intensity threshold range ( $\Delta K_{th}$ ) was estimated from the intercept of the responses at an apparent fatigue crack growth rate of  $1 \times 10^{-7}$  mm/cycle. Thus, results obtained from each specimen include measures of the  $\Delta K_{th}$  and the two Paris Law parameters ( $C$  and  $m$ ). The cumulative responses for each parameter corresponding to the two different groups of donors were defined in terms of the average response from individual specimens of each region. A comparison of the fatigue crack growth parameters for specimens obtained from each donor group was conducted using an Analysis of Variance (ANOVA) with significant differences identified by  $p \leq 0.05$ . A comparison of the responses from amongst the three depths was performed using Tukey's Honestly Significant Different (HSD) test.

After completion of the fatigue crack growth experiments, the CT specimens were dehydrated in air for a day, and then sputter-coated (Model LLC Desk II, Denton vacuum, Moorestown, NJ, USA) with gold-palladium alloy. The fracture surfaces and the overall microstructure were evaluated using scanning electron microscopy (SEM; JEOL JSM 5600, JEOL Inc., Peabody, MA) under secondary electron imaging and/or back scatter emission modes. The microstructural characteristics of the dentine from the two donor groups were examined quantitatively to identify if there are any fundamental differences that could contribute to the fatigue crack growth behaviour. To facilitate clear identification of the lumen dimensions and peritubular cuff, the samples were polished briefly using diamond particle suspensions (Buehler) of sizes 9, 3, and 0.04  $\mu\text{m}$ . All polishing was performed by hand on a standard cloth wheel mounted on top of a granite surface plate. The polishing was performed without application of pressure. A commercial image analysis software (Image) 1.42,

National Institute of Mental Health, Bethesda, Maryland, USA) was used to count the number of lumens per unit area, and in quantifying the average lumen dimensions in each of the three regions of evaluation. Significant differences in the microstructure were identified using an ANOVA with  $p \leq 0.05$

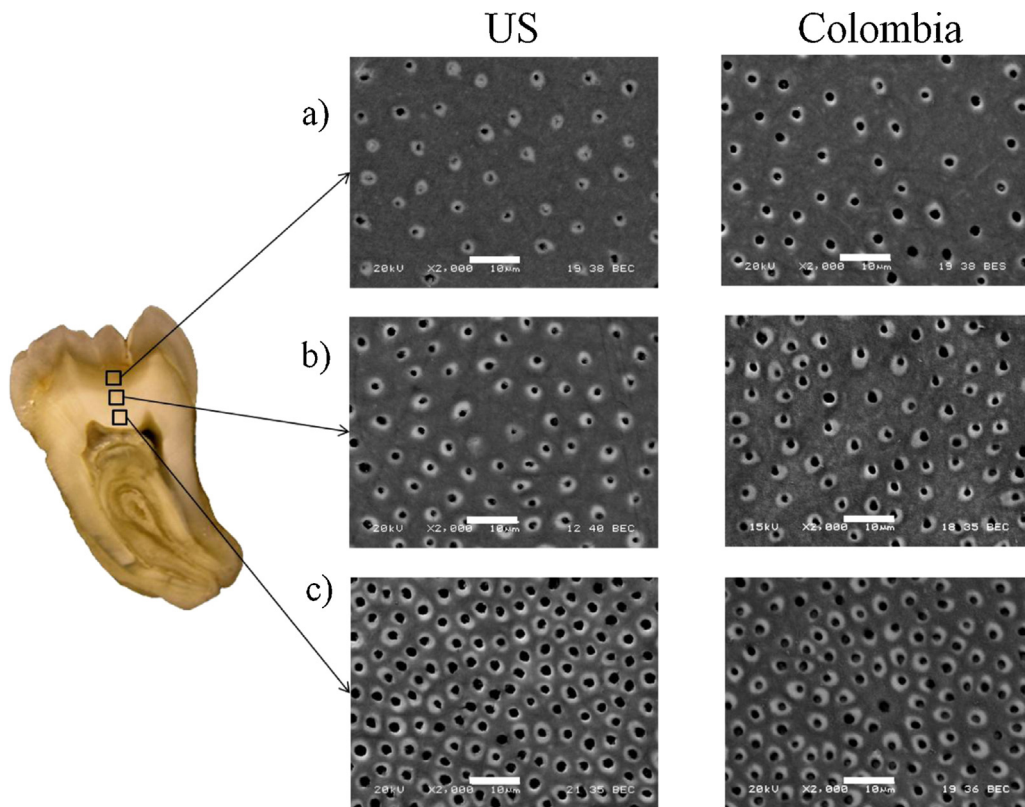
### 3. Results

A comparison of the microstructure of dentine specimens representing the two groups of donors is presented in Fig. 2. Note that pairs of micrographs are shown for superficial or peripheral dentine (Fig. 2(a)), central or middle dentine (Fig. 2(b)) and deep dentine (Fig. 2(c)), with one image from each of the two donor groups. As evident from these images, there is a reduction in the tubule density with increasing distance from the pulp for both donor groups. Overall, the lumen density ranged from approximately 10,000 to 60,000 lumens per  $\text{mm}^2$  and 15,000 to 43,000 lumens per  $\text{mm}^2$  for the teeth of donors from the US and Colombia, respectively.

A quantitative comparison of the microstructural characteristics of dentine from the two donor groups is shown in Fig. 3. In Fig. 3(a) the average lumen density is presented within the three separate regions of evaluation. There was a significant reduction in the lumen density with depth ( $p \leq 0.05$ ), for both of the donor groups. However, there was no significant difference ( $p > 0.05$ ) in the tubule density between the two donor groups within any of the three regions. A comparison of the average lumen diameters within the three regions of evaluation and for the two donor groups is shown in Fig. 3(b). For the donor group of the US, there was a significant decrease in lumen diameter from the peripheral region to the deep dentine, with average lumen diameters in these regions of approximately 1.0 and 1.8  $\mu\text{m}$ , respectively. However, for the teeth from Colombian donors there was no significant change in the lumen diameter with depth. The average diameter for this donor group considering all three regions was roughly  $1.4 \pm 0.2 \mu\text{m}$ ; the largest variation in this dimension was noted in the deep dentine.

A typical fatigue crack growth history from the dentine of a single donor is shown in Fig. 4(a). This response is annotated to highlight the three regions of responses, as well as the three measures of cyclic extension obtained for each specimen. The cumulative fatigue crack growth responses for all specimens obtained from the Colombian donors are shown as a function of the stress intensity range in Fig. 4(b). A comparison of these responses with those obtained from the dentine of donors in the US is shown in Fig. 4(c). Apparent from this comparison, the overall fatigue crack growth behaviour of the two donor groups is consistent. However, the responses obtained from the donors of Colombia appear to exhibit a much smaller range of fatigue resistance, and show less dependence on location in which the tissue was obtained.

A quantitative assessment of the fatigue crack growth results for the individual specimens provided the stress intensity threshold range, and the two Paris Law parameters describing the steady-state growth behaviour. The average values of these parameters were estimated as a function of dentine depth and are shown in Fig. 5. The importance of location on the stress intensity threshold range for the two

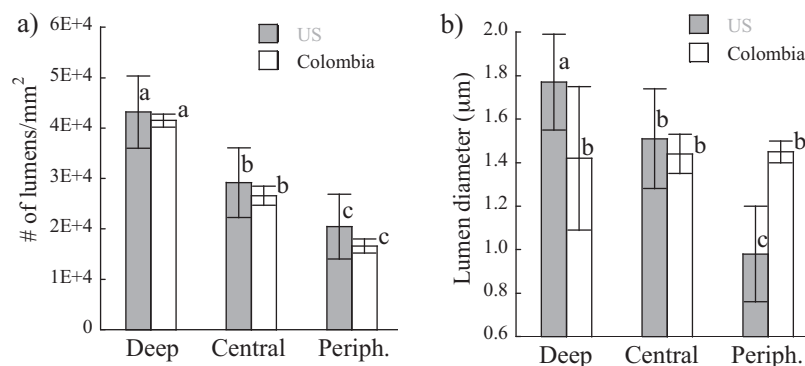


**Fig. 2 – SEM micrographs of the dentine microstructure for specimens of selected US and Colombia donors. (a) Peripheral dentine; (b) central dentine; (c) deep dentine. The scale markers in each micrograph represent 10  $\mu\text{m}$ . Note that both patient groups were found to have comparable lumen densities for the three evaluated regions. There is an increase in lumen diameter with depth evident in the dentine from US donors, whereas the average lumen diameter appears nearly constant for the Colombian dentine in the three regions of the crown.**

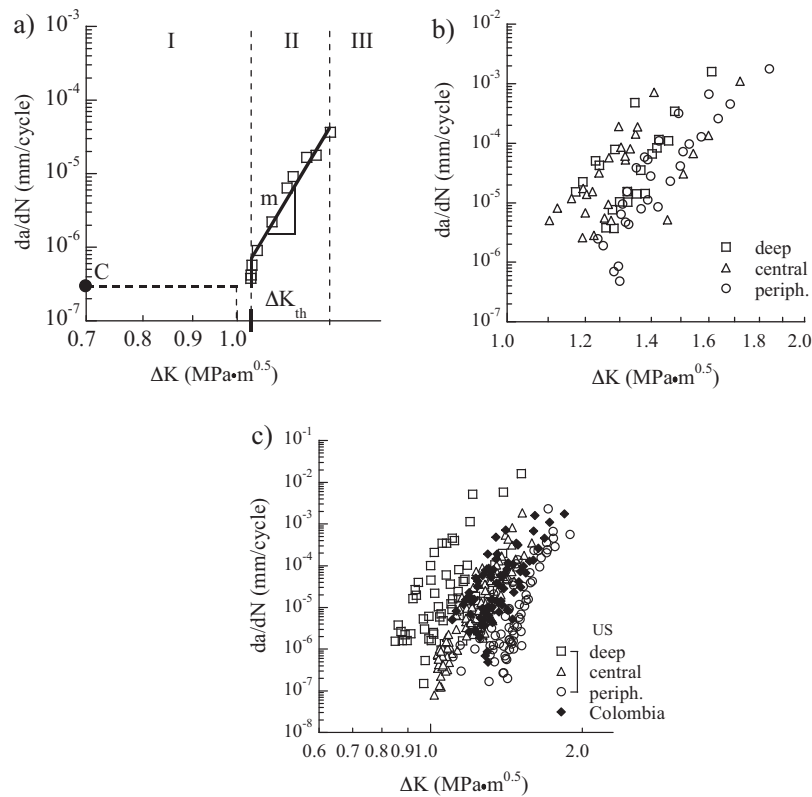
donor groups is shown in Fig. 5(a). In comparing results from the two donor groups, there were differences in the fatigue crack growth initiation resistance including the magnitude and spatial dependence. For dentine obtained from US donors the stress intensity threshold range from the inner ( $0.8 \pm 0.1 \text{ MPa m}^{0.5}$ ) dentine was significantly lower ( $p \leq 0.0001$ ) than that from the peripheral region ( $1.2 \pm 0.1 \text{ MPa m}^{0.5}$ ). However, for dentine of Colombian donors, there was no significant difference in the average stress intensity threshold range obtained for the three

regions; the overall average value for this group was  $1.1 \pm 0.1 \text{ MPa m}^{0.5}$ . In comparing results obtained by region, there were significant differences ( $p \leq 0.05$ ) between the two groups in the deep and peripheral dentine.

Results obtained for the steady-state region of fatigue crack growth are presented in Fig. 5(b) and (c). Specifically, the importance of location on the average fatigue crack growth exponents for the two groups of donors is shown in Fig. 5(b). There was no significant difference ( $p > 0.05$ ) in the exponents with respect to location or donor group. The average Paris Law



**Fig. 3 – A comparison of the microstructure as a function of location in the coronal dentine from donor teeth of the US and Colombia. (a) Lumen density; (b) lumen diameter. Columns with different letters are significantly different ( $p \leq 0.05$ ).**



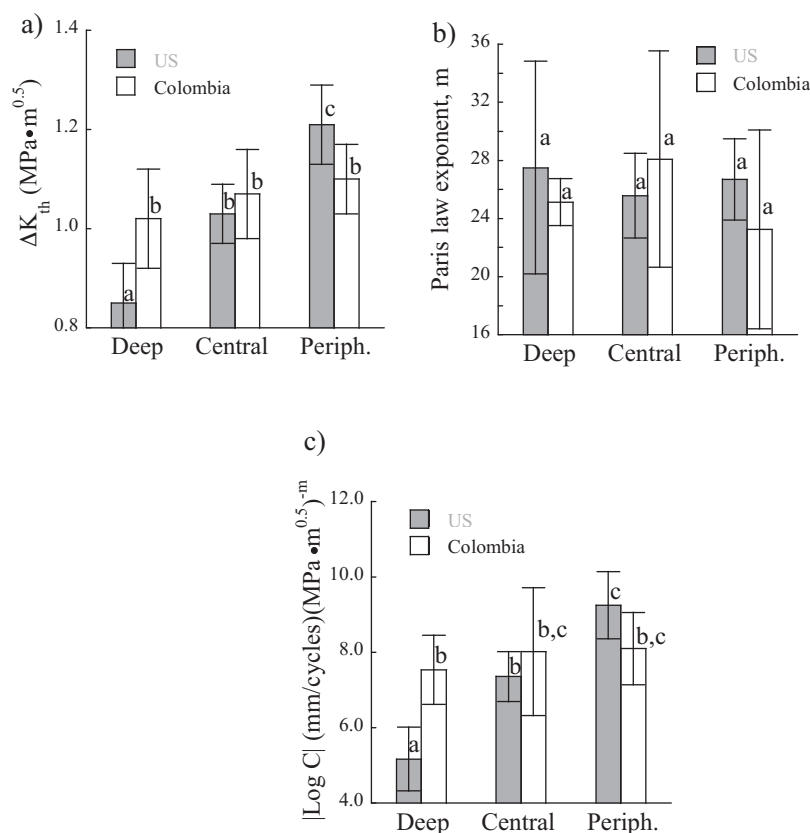
**Fig. 4 – Fatigue crack growth (FCG) resistance of coronal dentine. (a) A typical fatigue crack growth response distinguishing all three regions of cyclic extension; (b) a comparison of the resistance to cyclic extension for coronal dentine obtained from the Colombian donors. The responses are divided into specimens obtained from the inner, central and peripheral regions. (c) Comparison of the fatigue crack growth responses of specimens obtained from both donor groups (US and Colombia). Results from (b) for the Colombian donors are not denoted by region in (c). Note the consistency in results for the Colombian responses in comparison to those for the US donor group.**

exponent for dentine of the US and Colombian donors was approximately  $27 \pm 8$  and  $25 \pm 9$ , respectively. The fatigue crack growth coefficients obtained from specimens at the three depths of evaluation are shown in Fig. 5(c). For clarity of presentation, the average values at each depth are presented in terms of absolute value of the log base 10. In evaluation of results for the US donor group, there was a significant increase ( $p \leq 0.01$ ) in the fatigue crack growth coefficient with depth, indicating that the greatest incremental fatigue crack growth rate occurred in deep dentine. However, for the donor group of Colombia, there was no significant difference in the fatigue crack growth coefficient amongst the three locations. In comparing results from the two donor groups by region, there was a significant difference ( $p \leq 0.05$ ) in the fatigue crack growth coefficients of the deep dentine only.

During analysis of the fatigue crack growth behaviour, it was noted that some aspects of cyclic extension were not directly represented by the quantitative responses. For example, some of the specimens exhibited locations with an irregular tubule distribution, or locations without lumens. These observations were most common in dentine from the Colombian donors. While regions with this characteristic were not included in the quantitative estimates of tubule density and lumen diameter, they were important to the fatigue crack growth responses. Selected specimens with this characteristic

are shown in Fig. 6(a) and (b). A crack path corresponding to cyclic crack extension within a dentine specimen obtained from a Colombian donor is shown in Fig. 6(c); the direction of crack extension is from left to right. In regions with sparse or irregular lumens, the crack growth process was temporarily interrupted. In these locations the crack would undergo a local change in orientation that followed a more favourable path consisting of greater lumen density, as evident in Fig. 6(c). Highlighted in this micrograph are the locations where the crack extended uniformly, in regions of evenly distributed lumens (encircled in white), and a region of interruption (encircled in yellow) where the crack extended into an area of sparse tubules (yellow). In regions of low lumen density, it was noted that the crack underwent a decrease in the growth rate or even temporary crack arrest.

Based on observations of cyclic crack growth from the surface used in viewing and measuring the crack length, cyclic extension occurred through a connection of fracture events that developed in adjacent lumens. Generally the crack would extend from lumen to neighbouring lumen along a path of large tubule density, where the distance between lumens was shortest. The extension to adjacent lumens did not occur every cycle, but rather after tens of cycles or more. There was consistency in this behaviour for both donor groups, suggesting that the toughening mechanisms contributing to the crack



**Fig. 5 – A comparison of the Paris Law parameters for the two donor groups and differences with location. (a) Stress intensity threshold. (b) Paris law exponent  $m$ . (c) Log of the Paris law coefficient  $C$ . Columns with different letters are significantly different ( $p \leq 0.05$ ).**

growth resistance were similar. These included crack bridging posed by unbroken ligaments of tissue and collagen fibrils of matrix that were active behind the crack tip, microcracking of the peritubular cuffs within the frontal zone of the crack-tip, and also localized crack curving. The latter mechanism was found to be highly influenced by the tubule density, and was most commonly noted in regions of irregular tubule distribution, as shown in Fig. 6(c).

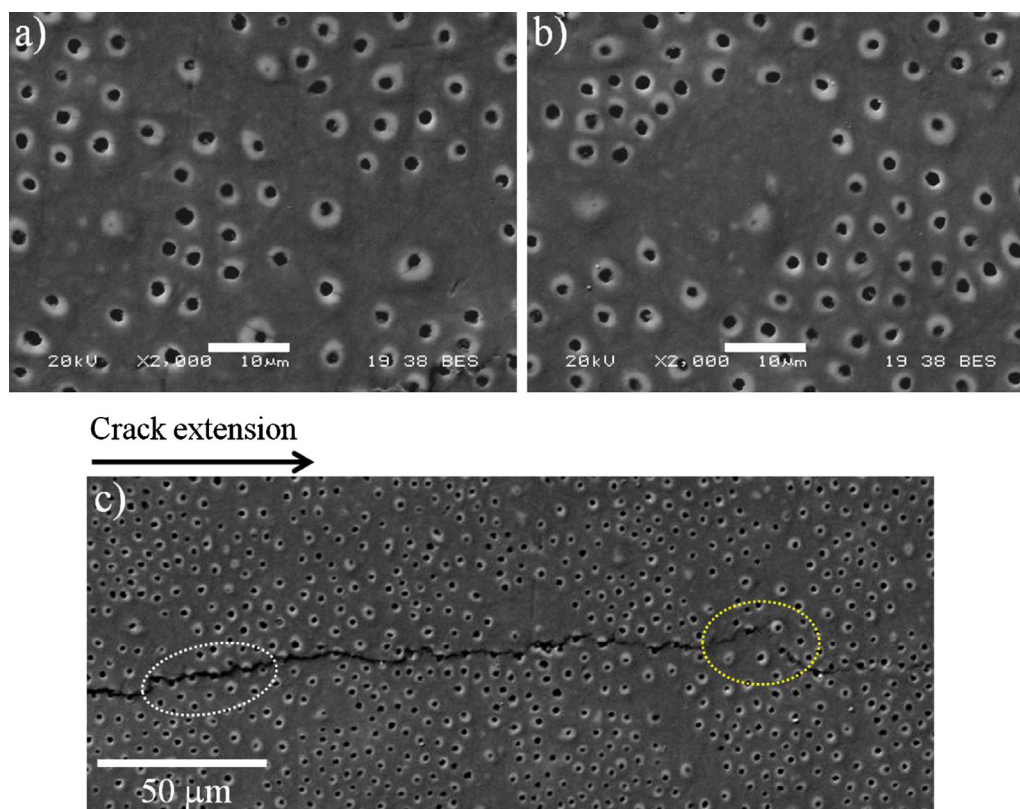
#### 4. Discussion

In this investigation, the microstructure and fatigue crack growth resistance of dentine from donor teeth of patients living in selected countries of North and South America were evaluated. To the author's knowledge, the present investigation is the first to compare the microstructure of dentine from patients of different countries, and to consider this information in an exploration of the mechanical behaviour relevant to the success of restorative dentistry.

The three most common forms of restored tooth failure are recurrent caries, deterioration of the margins and tooth fracture. Approximately one-half of all dental restorations fail within 10 years due to secondary caries and fracture.<sup>27–29</sup> Cracking and tooth fractures are an impediment to lifelong oral health.<sup>30,31</sup> The incidence of tooth fractures is not only a concern in the US, but also in the countries of South

America.<sup>32,33</sup> As tooth fracture is generally facilitated by cyclic crack extension of existing flaws in dentine,<sup>17,18</sup> the fatigue crack growth resistance is of substantial relevance. Furthermore, teeth with cracks are difficult or even impossible to repair – they are often extracted! The best approach for preventing tooth fracture today is the development of clinical practices guided by knowledge on the fracture resistance of teeth. Hence, this study was motivated in recognition of the importance of tooth fracture to lifelong oral health within the two countries.

For both donor groups, the measures of microstructure were within the ranges previously reported for tubule density<sup>4,6</sup> and tubule diameter.<sup>34,35</sup> Surprisingly, although the average dentine tubule density was consistent between the two donor groups, there were significant differences in the tubule diameters. Thus, the null hypothesis must be rejected. In the coronal dentine of teeth from US donors, the average lumen diameter was found to increase with depth, whereas the lumen diameter was essentially a constant value throughout the crown in Colombian teeth. Similar results have been reported in a microstructural analysis of dentine in Brazilian teeth<sup>35</sup> indicating a constant lumen diameter over the crown depth. The overall average lumen diameter in the teeth of Colombian donors ( $1.4 \pm 0.2 \mu\text{m}$ ) was equivalent to that in the middle region of teeth obtained from US donors. The largest difference in microstructure between the two groups was identified in the peripheral dentine (Fig. 3(b)),



**Fig. 6** – Micrographs detailing the microstructure of dentine from the teeth of Colombian donors. (a) and (b) show examples of non-uniform lumen distribution in two different specimens. The scale markers represent 10  $\mu\text{m}$ . (c) Crack extension from left to right in the central region obtained from a 21-year-old female donor. Note the path of crack extension (enclosed in white) extends from “lumen to lumen” and that a change in path occurs in the region that exhibits a non-uniform tubule pattern (enclosed in yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

where the average lumen diameter of tissue from the Colombian donors was over 40% greater than that from the dentine of US donors.

Trends in the dentine microstructure were reflected in the fatigue crack growth resistance of the two groups of donor teeth. With respect to spatial variations in properties, it was found that there was a significant reduction in the fatigue crack growth resistance with depth in dentine from US donor teeth only. The spatial variations agree with results of previous studies reporting on the fatigue crack growth properties and fracture toughness of dentine.<sup>19,36</sup> These prior studies were conducted on tissue obtained from teeth of US donors. In both loading formats, deep dentine showed the lowest resistance to crack extension. However, in the tissue from Colombian donors there were no significant changes in either the initiation behaviour or the steady-state fatigue crack growth behaviour with location. The  $\Delta K_{\text{th}}$  and Paris Law parameters ( $m$  and  $C$ ) remained constant in this group as evident in Fig. 5. The average values for  $\Delta K_{\text{th}}$  and  $C$  of the three regions were  $1.1 \text{ MPa m}^{0.5}$  and  $1.78\text{E}-08 \text{ (mm/cycle) (MPa m}^{0.5})^{-m}$ , respectively. These values were also consistent with the averages for central dentine obtained from US donors ( $\Delta K_{\text{th}} = 1.03 \text{ MPa m}^{0.5}$ ,  $C = 4.41\text{E}-08 \text{ (mm/cycle) (MPa m}^{0.5})^{-m}$ ). When the stress intensity threshold range of the two groups is

compared by region, there were significant differences ( $p \leq 0.05$ ) between the two groups for both the deep and peripheral dentine. Neither donor group showed spatial variations in the fatigue crack growth exponent (Fig. 5(b)). However, there was a significant difference in  $C$  of the deep dentine between the two groups, which manifested as a larger incremental crack growth rate in dentine of the US donor teeth.

One factor to consider in comparing the fatigue crack growth parameters and average crack growth rates of the two donor groups is the difference between the experimental conditions and those of actual mastication. The experiments were conducted within a hydration bath, which maintained the moisture content of the dentine samples. Nevertheless, the experiments were conducted at room temperature rather than  $37^\circ\text{C}$ . But the difference in temperature does not appear to be an important factor.<sup>37</sup> Another concern is loading frequency. According to Braem et al.<sup>38</sup> the upper range of chewing frequency is  $\sim 2 \text{ Hz}$ , while in this study a frequency of  $5 \text{ Hz}$  was used. The higher frequency was chosen to maintain consistency with earlier studies on fatigue crack growth in human dentine;<sup>19,24</sup> and to balance the concerns associated with clinical relevance and the time required to achieve meaningful data. A single experiment at  $5 \text{ Hz}$  typically

extends over a period of one week, and a reduction of frequency results in substantial changes in time required for the investigation. Kruzic et al.<sup>39</sup> showed that higher frequencies can promote slower crack growth rates within dentine. While these studies were conducted on elephant tusk dentine, the findings suggest that the measured growth rates obtained at 5 Hz may be slightly lower than those resulting from mastication.

Results from the experiments showed that the fatigue crack growth responses of dentine from the Colombian donors did not depend on location. Spatial variations were only found in dentine of the US donors. Reflecting on the microstructural measurements, the only notable difference in microstructure between the two groups is the lumen diameter. That would suggest that the lumen diameter is the primary factor regulating the spatial aspects of fatigue crack growth resistance, and that it decreases with increasing lumen diameter. Indeed, the mechanism of cyclic crack extension in this orientation is from lumen to lumen, and the larger lumen size results in an elevated average stress due to the stress concentration. The relatively constant lumen diameter of dentine from the Colombian teeth (Fig. 3(b)) appears to homogenize the fatigue responses. While the influence of microstructure on fatigue crack growth resistance is interesting, there are clear implications to the propensity for restored tooth fracture. For shallow restorations extending only into peripheral dentine, there is greater probability for fatigue crack growth to cause tooth fractures in the teeth of Colombian patients. However, for restorations extending into the deep dentine, there is a greater probability of fatigue crack growth and consequent tooth fracture in the patients of the US.

The microstructural evaluation concentrated on the dimensional characteristics of the dentine. That has been the most common method to characterize the variations in microstructure of dentine over the tooth.<sup>4–7,34</sup> But there are other aspects of the tissue that exhibit spatial variations as well, including the relative mineral and organic content. For example, Tesch et al.<sup>40</sup> evaluated the spatial variations in intertubular dentine of the crown using a host of quantitative approaches. From a comparison of correlations, it was identified that the relative mineral content and the thickness of the mineral crystals were the best predictors of nanohardness and elastic modulus of intertubular dentine. Ryou et al.<sup>41</sup> evaluated the strength, as well as the strain and energy to fracture of coronal dentine and found that the trends in mechanical behaviour were consistent with the mineral/collagen ratios. Deep dentine exhibited the highest mineral/collagen ratios and highest “brittleness” as indicated by the low strength and strain to fracture. Consistent with the findings of Staninec et al.,<sup>12</sup> deep dentine also exhibited a larger size distribution of contributing intrinsic flaws. Therefore, in addition to differences in lumen dimensions, there may be aspects of the relative mineral and organic content of the donor groups that contributed to the fatigue resistance. Future studies in this area should consider adopting additional methods of characterization for analyzing the chemical composition and its importance to the spatial variations in fatigue resistance.

The identification of differences in the microstructure raises questions pertaining to the principal cause. Environmental influences, nutrition and oral health could be

contributing factors based on their influence to tooth formation and development.<sup>42,43</sup> The environmental conditions of Medellín parallel those of US cities, with air quality three times better than the values suggested by the World Health Organization.<sup>44</sup> The participating clinics serve an area of Medellín supported by public water, which achieves potable standards, chemistry and quality equivalent to those of the western world.<sup>45</sup> Furthermore, the nutritional status of those in Medellín is equivalent to that in the US<sup>46</sup> with average adult intake exceeding 2000 cal/day. The average Colombian diet consists of more maize and beans than in the US, but the seven major food groups are represented. Of relevance, similar results in the lumen diameter were reported for the dentine of third molars from donors in Brazil.<sup>35</sup> Brazil borders Colombia and the general diet and living environment of people in these two countries are very similar. Therefore, disparities in living environment and nutrition do not appear to be contributing factors to the difference in microstructure between the two donor groups.

Other potentially contributing factors may include difference in the diseases and dental care between the donor groups. According to the statistics reported by the World Health Organization<sup>47</sup> the 4 primary noncommunicable diseases in Colombia are cardiovascular disease (28%), cancer (17%), respiratory disease (6%) and diabetes (3%). No connection has been made between these diseases and the microstructure or mechanical properties of dentine. The prevalence of diabetes is increasing in Colombia,<sup>48</sup> but it is far lower than that in the US. In regards to oral health, the primary dental diseases are caries, periodontitis and dental fluorosis.<sup>49</sup> The prevalence of dental fluorosis in children of Medellín (8%)<sup>50</sup> is higher than in the USA (3%).<sup>51</sup> However, dental fluorosis is easy to identify and the teeth were screened at receipt to exclude those with potential evidence of fluorosis. Consequently, there does not appear to be contributions from disease or oral health factors to the findings.

There could be differences in ethnic background. In both countries the teeth were collected with record of patient age and gender only, and without identification of patient ethnic background. For the US donor group the collected teeth are considered of “mixed” ethnic background. Considering the demographics of Maryland, the population of donors consisted of roughly 54% white, 30% black, 9% Hispanic and 6% Asian. The Colombian population overall is primarily of European origin (~86%), Amerind (American Indian, ~3.4%) and Afrocolombians (~10.6%).<sup>52</sup> According to genetic studies,<sup>53,54</sup> the demography of Medellín is ~94% European origin (of Spain), ~5% African and ~1% Amerind. Those in the European category are regarded as white or “mestizos” denoting a mixture of European and indigenous Indian background. Furthermore, the participating clinics are located in the El Poblado district of Medellín (strata between 3 and 5), a centre of commerce largely populated by persons of European descent (i.e. not African or Amerind). Thus, it is not clear if the findings are related to differences in ethnicity of the two groups, but it is a relevant consideration. A report issued by the United States Census Bureau<sup>55</sup> recently provided an evaluation of the Hispanic population in the US. The report details that in 1970 the Hispanic population was comprised of nearly 10 million individuals (approx. 5% of the total US population).

Today that number is reaching 45 million (14%) and is expected to surpass 75 million (20%) by the year 2030. Between 2000 and 2006 the growth rate in Hispanics (24.3%) was more than 3 times the growth rate of the total population. Hispanics are the fastest growing minority in the United States. If the mechanical behaviour between the two donor groups are related to ethnicity, then the substantial rise in the number of Hispanics in the US could have important implications to the field of dentistry.

Results of the present investigation identified that the structure and fatigue resistance of dentine from the teeth of US and Colombian donor groups exhibit significant differences. Similar differences could exist between other donor groups. Previous studies have compared the structure and/or physical aspects of bovine dentine to human dentine, largely to address whether bovine dentine is an adequate substitute for human tissue.<sup>34,56–60</sup> Based on the present findings, there may be equal or greater merit to the field of dentistry in exploring the differences between patient groups than animal species. If those studies are conducted, one factor to consider is the impact of ageing. There are changes in the microstructure of dentine with ageing that result in a reduction of the fatigue crack growth resistance<sup>23,24</sup> and fracture toughness.<sup>22,61</sup> An earlier comparison of the fatigue crack growth properties of tissue from donors of China and the US reported significant differences.<sup>20</sup> If the effects of ageing and/or its rate of progression is not consistent amongst patient groups, that will add another dimension to the complexity of restorative dentistry in the future. If one accepts that premise that type II diabetes leads to “accelerated ageing”, then tooth dentine of US residents may be physiologically older than aged-matched controls from countries with far less type II diabetes.

## 5. Conclusions

On the basis of the results obtained, the following conclusions may be drawn:

1. There was no significant difference in the tubule density of coronal dentine from the two donor groups. The overall tubule density ranged from approximately 10,000 to 60,000 tubules/mm<sup>2</sup>. There was a significant increase in the tubule density from the dentine enamel junction to the pulp, in teeth from both the US and Colombian donor groups.
2. In evaluation of the lumen diameters, there was a significant decrease in the lumen diameter with increasing distance from the pulp in dentine from the US donors. However, the average lumen diameter of dentine from the Colombian donors did not change significantly with depth; the average diameter was  $1.4 \pm 0.2 \mu\text{m}$ . The tubule lumens in the peripheral dentine of the US donors were significantly smaller than those in the corresponding location of the Colombian donors.
3. There were significant differences in the fatigue crack growth resistance between the two donor groups. For the teeth obtained from the US donors, the stress intensity threshold range from deep ( $0.8 \pm 0.1 \text{ MPa m}^{0.5}$ ) dentine was significantly lower ( $p \leq 0.0001$ ) than that from the peripheral region ( $1.2 \pm 0.1 \text{ MPa m}^{0.5}$ ). However, for dentine from the Colombian donors there was no significant difference in the stress intensity threshold range between the three regions of investigation. The average value was  $1.1 \pm 0.1 \text{ MPa m}^{0.5}$ .
4. There were no spatial variations in the Paris Law exponents for either donor group. However, in evaluation of the fatigue crack growth coefficient, there was a significant increase in the coefficient with increasing depth for the donor group of the US. In contrast, there was no significant spatial variation in that parameter for the dentine of the Colombian donor group. These spatial variations in the US donor group resulted in a decrease in the fatigue crack growth resistance with distance from the DEJ.

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## Competing interests

None declared.

## Ethical approval

Those teeth from donors in the US were obtained and evaluated in accordance with an approved protocol issued by the Institutional Review Board of the University of Maryland County (Approval Y04DA23151). In Colombia, the teeth were obtained with written consent and followed the protocols required by both the Cooperative University of Colombia (UCC) Dental Clinic and EAFIT University. A number was not assigned to the evaluation or confirmation of the protocol.

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