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# The effects of ageing on the biomechanical properties of root dentine and fracture





### Haiping Xu<sup>a</sup>, Qinghua Zheng<sup>a</sup>, Yingfeng Shao<sup>b,\*\*</sup>, Fan Song<sup>b</sup>, Lan Zhang<sup>a</sup>, Qian Wang<sup>a</sup>, Dingming Huang<sup>c,\*</sup>

<sup>a</sup> State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, China

<sup>b</sup> The State Key Laboratory of Nonlinear Mechanics, Institute of Mechanics, Chinese Academy of Sciences, Beijing 100190, China

<sup>c</sup> Department of Conservative Dentistry and Endodontics, West China School of Stomatology, Sichuan University, Chengdu 610041, China

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#### ABSTRACT

*Objectives*: Knowledge of the mechanical behaviour of root dentine can facilitate better understanding of spontaneous vertical root fracture (VRF), an age-related disease initiated mainly at the root apex. We tested the hypothesis that the biomechanical properties of root dentine change with ageing.

*Methods*: Sixteen human premolars were divided into "old" (17–30 years) and "young" (50– 80 years) groups. The elastic modulus, nano-hardness, micro-hardness, elemental contents, tubular density/area of root dentine in cervical, middle and apical root regions were evaluated using atomic force microscopy-based nano-indentation, Knoop indentation, scanning electron microscopy and energy dispersive X-ray spectroscopy, respectively.

Results: The apical dentine showed a lower nano-hardness, a lower elastic modulus, a lower calcium content, a lower calcium-to-phosphorus ratio and a smaller tubular density/area than the cervical dentine in both age groups, whereas spatial differences in micro-hardness were observed only in old roots. Compared with young dentine, old dentine showed a greater hardness, a higher elastic modulus, a greater mineral content and a smaller tubular size in the cervical portion, whereas the age-induced changes in tubular density were insignificant. Finite element analysis revealed that due to its higher elastic modulus, old apical dentine has a higher stress level than young dentine.

Conclusions: The intrinsic material properties of root dentine have spatial variations, and they are altered by ageing. The higher stress level in old apical dentine may be one reason, if not the most important one, why spontaneous VRFs are more likely to occur in the elderly population.

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<sup>\*</sup> Corresponding author at: 14#, 3rd Section, Ren Min Nan Lu, West China School of Stomatology, Sichuan University, Chengdu, Sichuan 610041, China. Tel.: +86 28 85501439; fax: +86 28 85582167.

<sup>\*\*</sup> Corresponding author. Tel.: +86 10 82544089.

E-mail addresses: shaoyf@lnm.imech.ac.cn (Y. Shao), dingminghuang@163.com (D. Huang). 0300-5712/\$ – see front matter © 2013 Elsevier Ltd. All rights reserved. http://dx.doi.org/10.1016/j.jdent.2013.11.025

#### 1. Introduction

Vertical root fracture (VRF), a longitudinal fracture line extending from the root canal to the periodontium, can involve both endodontically and non-endodontically treated teeth.<sup>1</sup> Troubled by the poor prognoses of VRFs, many researchers have attempted to investigate the aetiology of VRF for early-stage prevention and to explore new therapies. Iatrogenic factors, such as tooth structural loss<sup>2</sup> and the effects of restorative procedures,<sup>3</sup> are considered to be the main causes of fractures after endodontic treatment. However, VRFs in non-endodontically treated teeth, known as spontaneous VRFs, have received little attention. Indeed, they have a high frequency in Chinese people, who constitute a population with a dietary pattern of chewing hard foods,<sup>4,5</sup> and 40% of VRFs occur in non-endodontically treated teeth.<sup>6</sup>

Clinical studies have found that spontaneous VRFs always occur in severely attrited first molars.<sup>4</sup> It is noteworthy that cracks initiate at the root apex and propagate coronally.<sup>4,5</sup> On the one hand, stress might be concentrated at the apical area during mastication for root geometry; on the other hand, the intrinsic properties of the apex itself might constitute the weakest point of the root.<sup>6</sup> However, to our knowledge, there has been no scientific research that has demonstrated the variations in mechanical behaviour of different portions of root materials and their effects on VRFs.

Dentine is the major constituent of the tooth root. Similar to most bio-mineralization materials, such as enamel and bone, dentine has a hierarchical structure, in which collagen and apatite molecules cross each other on a nano-scale. Subsequently, these composites organize into a microscopic morphology, forming tubules with different sizes and densities. The specific arrangement and order of the nano- and micro-structures produce a macroscopic anisotropy with certain functional behaviours.<sup>7</sup> The properties of coronal dentine have been researched in many studies,<sup>8,9</sup> but root dentine, which is closely related to the fracture behaviour of the root, has not been comprehensively researched.

Previous research has revealed that dentine undergoes gradual spatial transition in its material properties.<sup>10</sup> In the crown, the hardness and elastic modulus of intertubular dentine decrease towards the dentino-enamel junction,<sup>8</sup> which has been deduced to be related to the degree of mineralization.<sup>11</sup> In the root, a decreasing trend in mineral content from the cervical dentine to the apical dentine has been found.<sup>12</sup> Hence, the mechanical properties of root dentine are suspected to vary with location.

In addition, spontaneous VRFs tend to occur mainly in patients older than 40 years old.<sup>1,5</sup> Ageing problems and agerelated changes in the mechanical behaviour of dentine have attracted attention. In coronal dentine, there is a significant reduction in the strength, energy required to fracture and fracture toughness with increasing age.<sup>13–15</sup> However, little research has been undertaken on root dentine until now, except for Kinney et al., <sup>12</sup> and Porter et al.,<sup>16</sup> who found that the mineral concentrations and crystallite size of root dentine changed with age. Whether there are changes in the mechanical properties of aged root dentine is unclear. Because material properties can vary with spatial scales, root dentine should be studied at all hierarchical levels.<sup>17</sup> Microscopic tests are technically difficult to perform due to the limited volume of human root dentine. In addition, dentinal tubules are known to vary in diameter, which might cause different properties among rectangular beam specimens. Thus, the indentation technique has been increasingly applied in small areas of potentially anisotropic composite materials, such as dentine.

To investigate the changes in the mechanical properties of root dentine associated with ageing and the effects of ageing on VRFs, Knoop indentations and atomic force microscopy (AFM)-based nano-indentations were utilized. The corresponding tubular density/area and elemental composition were examined by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). We hypothesized that the biomechanical function of the root would change with age.

#### 2. Materials and methods

#### 2.1. Specimen preparation

Human mandibular premolars with single roots and vital pulp were obtained following a protocol approved by the Ethics Committee of the West China Hospital of Stomatology. The collection of teeth did not change the course of any patient's treatment plan, including orthodontic treatment, periodontal treatment, and so on. The teeth were sterilized by gamma radiation<sup>18</sup> and were stored in Hanks' balanced salt solution (HBSS) at 4 °C before examination.<sup>19</sup> All teeth were integral and examined using a stereomicroscope (SMZ1000, Nikon Corp., Tokyo, Japan) to ascertain the absence of any root fractures or craze lines. The collected teeth were divided into "young" (17-30 years old, n = 8) and "old" (50-80 years old, n = 8) groups.<sup>13</sup> The average age and the standard deviation of the young and old groups were 24  $\pm\,3$  and  $64 \pm 7$  years, respectively. The crowns were removed at the cemento-enamel junction, using a water-cooled diamond saw (Struers Minitom, Struers, Copenhagen, Denmark). The roots were then sectioned bucco-lingually through the centre of the root canal (Fig. 1A). The mesio-segments were embedded and polished using a series of SiC papers to 4000 grit and were finished with diamond pastes (1- and 0.25-µm). The specimens were then cleaned ultrasonically and were maintained in HBSS until used.

#### 2.2. AFM-based nano-indentation

Nano-indentation was performed using a Nanoscope III AFM (Digital Instruments, Santa Barbara, CA, USA), modified with a Triboscope system and a Berkovich diamond indenter (Hysitron Inc., Minneapolis, MN, USA) under wet conditions. Measurements were obtained at the cervical, middle and apical root regions along the centre of the root canal (Fig. 1A). Each region was tested at two sites. For each site, six indentations, approximately 10  $\mu$ m apart, were performed in intertubular dentine. The maximum load was 2500  $\mu$ N and was held for 3 s, with loading/unloading

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