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Effect of aging on the microstructure, hardness and chemical composition of dentin

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ABSTRACT

Objective: Understanding the effects of biological aging on human tissues has been a topic of extensive research. With the increase in healthy seniors and quality of life that topic is becoming increasingly important. In this investigation the effects of aging on the microstructure, chemical composition and hardness of human coronal dentin was studied from a comparison of teeth within "young" and "old" age groups.
Methods: The microstructure of dentin within three regions (i.e., inner, middle and outer) was analyzed using electron and optical microscopy. The mineral-to-collagen ratio in these three regions was estimated using Raman spectroscopy and the hardness was evaluated using microindentation.
Results: Results showed that there were significant differences in tubule density, tubule diameter and peritubular cuff diameter with depth. Although there was no difference in tubule density and diameter of the tubules between the age groups, there was a significant difference in the occlusion ratio. A significant increase in hardness between young and old patients was found for middle and outer dentin. An increase in mineral-to-collagen ratio from inner to outer dentin was also found for both groups. In old patients, an increase in mineral content was found in outer coronal dentin as a consequence of tubule occlusion.
Conclusions: An increase in occlusion ratio, hardness, and mineral content was found in the dentin of adult patients with age. This increase is most evident in the outer coronal dentin.

1. Introduction

The effect of aging on the microstructure and mechanical properties of bone has been studied extensively due to its importance to the elderly and their quality of life (e.g., (Currey, Brear, & Zioupos, 1996; Nalla, Kruzic, Kinney, & Ritchie, 2004; Ural & Vashishth, 2006; Ural & Vashishth, 2007; Wang & Puram, 2004; Wang, Shen, Li, & Agrawal, 2002; Zioupos & Currey, 1998; Zioupos, Currey, & Hamer, 1999)). However, the effect of aging on dental hard tissues (including dentin and enamel) has received rather limited attention. That is surprising when one considers the importance of human teeth to mastication and dietary intake.

Dentin is a hard tissue that occupies the majority of the human tooth. By volume it consists of approximately 45% mineral material, 33% organic material (collagen type I) and 22% water (Nanci, 2012). The thickness of dentin (i.e., from the pulp to the dentin enamel junction (DEJ)) is largely dependent on tooth type, but generally ranges from roughly 2 mm for mandibular incisors up to 3 mm in canines and molars. Furthermore, the thickness of dentin tends to increase with aging as a result of appositional growth (Gómez de Ferraris & Campos Munoz, 2009).

The microstructure of dentin is largely dominated by its tubules, which are responsible for housing the odontoblastic processes. The tubules extend from the pulp to the DEJ. A highly mineralized cuff of peritubular dentin surrounds the lumen of each tubule and contains mainly apatite crystals and a small proportion of organic proteins. The tissue located between the tubules is called intertubular dentin and contains a matrix of collagen fibers reinforced by apatite (Marshall, Marshall, Kinney, & Balooch, 1997). Based on its composition and structure, dentin is considered a hierarchical biological composite (Ziskind, Hasday, Cohen, & Wagner, 2011).

Dentinal tubules possess diameters ranging from approximately 1 to 3 μm, depending on patient age (Ingle, Bakland, & Baumgartner, 2008). Studies have shown that after the third decade of life there is a transition in the microstructure of dentin, in which the tubules become gradually filled with inorganic

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material (Kinney, Nalla, Pople, Breunig, & Ritchie, 2005). After a significant number of tubules have been filled, the tissue appears transparent, and is generally considered as "sclerotic". This process results in an increase in the mineral content of dentin, opposed to what occurs in bone where there is largely a decrease in mineral content with aging (Rosen, Glowacki, & Bilezikian, 1999). Furthermore, this increase in mineral content has been usually associated with increasing dentin fragility and, therefore, causes a variation in its mechanical properties (Koester, Ager, & Ritchie, 2008; Nazari, Bajaj, Zhang, Romberg, & Arola, 2009). Understanding the mechanical properties of dentin is important to comprehend the structural behavior of teeth with aging and in the development of new dental materials.

According to Kinney, Marshall, and Marshall (2003), the Young's Modulus of young dentin ranges from 20 GPa to 25 GPa, and the tensile strength ranges from 52 MPa to 105 MPa. The flexure strength ranges from roughly 130–180 MPa (Ryou et al., 2011). The results obtained for fracture toughness are similar to those found for bone. Values of roughly 1.7 MPa $m^{0.5}$ and 2.0 MPa $m^{0.5}$ have been obtained when tests were performed with crack extension parallel and perpendicular to the dentinal tubules, respectively (Arola et al., 2009). When measured by means of Vickers and microhardness tests, the average hardness of dentin is of about 0.5 GPa, with no significant dependence on indentation load or indentation time (Chuenarrom, Benjakul, & Daosodsai, 2009).

Within the field of dentistry, the importance of aging has become of greater interest in recent years due to its impact on the practice of restorative dentistry. Indeed, the tooth undergoes certain changes with age, including wear of enamel, the formation of transparent dentin, a decrease in the number of odontoblasts and an increase in dentin thickness as well as a production of reactionary dentin (Nanci, 2012). The changes in dentin microstructure produce variations in its mechanical properties, which are important for the introduction of restorative treatments and the greater potential for tooth fractures.

Several studies have been performed toward understanding the influence of aging on the mechanical behavior of dentin. For instance, Zheng, Nakajima, Higashi, Foxton, and Tagami (2005) analyzed the changes in hardness and Young's modulus of dentin with aging and reported that dentin does not undergo a significant change in hardness or Young's modulus with age in the middle and inner dentin. However, they found an increase of 16% in hardness and around 5% in Young's modulus within the outer dentin. Further studies have shown that the flexural strength of coronal dentin decreases almost 20 MPa per decade of life, beginning after adulthood. In addition, a reduction of 75% in the energy required to fracture dentin between young (age ≤ 30) and old patients (age > 55) was reported (Arola et al., 2009).

Changes in mechanical properties of dentin with aging have largely been attributed to the increase in mineralization due to filling of the dentinal tubules. However, it remains unclear whether these changes can be attributed to the mineral occupying the dentinal tubules, a complimentary change of the mineral of the intertubular dentin, or crosslinking of collagen by non-enzymatic processes (Miura et al., 2014). In fact, little information is available on the relationship between the changes in microstructure of dentin with age and spatial variations in chemical composition. Thus, the aim of this work was to identify the changes in microstructure, chemical composition and hardness of dentin with aging from selected age groups of Colombian patients.

2. Materials and methods

Human third molars were obtained from selected patients after written consent and following all the protocols required by the Dental Clinic at Universidad Cooperativa de Colombia (UCC). Exclusion criteria included presence of caries and previous restorations. The teeth were obtained from donors residing in Medellín, Colombia, and were divided into two age groups, namely a "young" group with donors between 18 and 25 years of age ($N = 12$), and an "old" group with donors between 47 and 65 years of age ($N = 8$). There were an equal number of male and female samples in both groups. Immediately after extraction, all the specimens were kept in Hank's Balanced Salt Solution (HBSS) at 2 °C to avoid dehydration and loss of mineral (Habelitz, Marshall & Balooch, 2001). In addition, the specimens were tested within two weeks of extraction to limit the loss of mineral and organic materials.

Each molar was sectioned along its longitudinal axis (section A–A in Fig. 1a) using diamond abrasive slicing equipment with

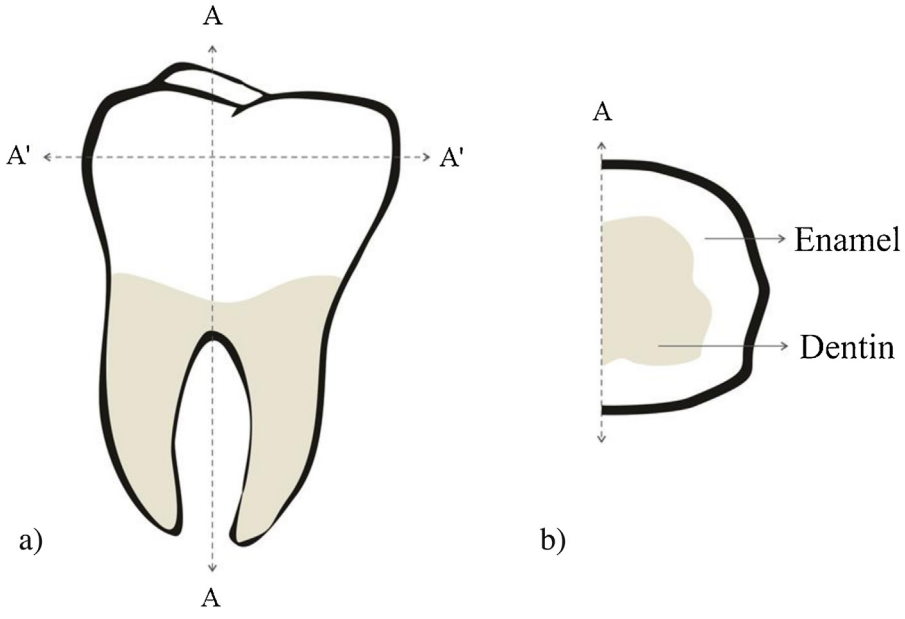

Fig. 1. Schematic diagram of a sectioned molar after (a) longitudinal (A–A), and (b) transverse (A′–A′) cutting. The specimen is then embedded in cold-cure epoxy resin with the sectioned surface facing outwards.