THE MECHANICAL BEHAVIOR OF DENTIN: IMPORTANCE OF MICROSTRUCTURE, CHEMICAL COMPOSITION AND AGING

Carolina Montoya Mesa

Universidad Eafit

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Preface and Declaration

The work described in this dissertation was carried out at Universidad Eafit between January 2003 and February 2017.

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Financial support for this project was given to me by the Departamento Administrativo de Ciencia, Tecnología e Innovación, Colciencias. This dissertation is the result of my own work, except where specific reference has been made to the work of others. No part of the work has been, or is currently being, submitted for any degree, diploma or other qualification.

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Summary

Dental fracture is one of the three most common forms of failure of restored teeth and the most common cause of tooth loss or extraction in elderly patients. Previous investigations conducted on aging of hard tissues have identified that there is a considerable reduction in the mechanical properties (i.e. fracture toughness, fatigue and flexural resistance) of dentin with aging and that may predispose tooth fracture. These declines in properties have been attributed to microstructural and chemical composition changes over time. However, these aging processes have not been really quantified and related with the changes in mechanical properties. Accordingly, the aim of this work is to evaluate the aging process of coronal dentin in terms of the evolution of microstructure, changes in chemical composition and mechanical properties from selected age groups (young and old donors). The changes in these properties were evaluated in three different regions (outer, middle and inner) in order to identify spatial variations within the crown.

A brief description of the main literature on composition, microstructure and mechanical behavior of dentin is presented in chapter 2.

An extensive experimental study was carried out in chapter 3 to identify the changes in microstructure of dentin with aging by means of optical and electron microscopy; while changes in chemical composition were analyzed using Raman Spectroscopy to calculate the mineral-to-collagen ratio. Changes in mechanical properties were measured using Vickers micro-hardness.

Chapter 4 describes the importance of tubule density to the fracture toughness of dentin for young and old donor's groups. An approach previously proposed to study the mechanical behavior of porous materials was used to model the fracture toughness of coronal dentin in terms of the tubule characteristics. Results were then compared with published results from previous studies.

The time-dependent deformation response of dentin was analyzed via spherical indentation experiments at different indentation loads in Chapter 5. From the experimental observations was proposed a simple model to describe the time dependent deformation behavior of dentin. This model was based on previously proposed theories for indentation of time dependent materials, showing that the effective strain rate of dentin depends on its chemical composition (i.e. mineral-to-collagen ratio) and microstructure (i.e. lumen area fraction). The descriptions of the model were compared with the experimental results showing good agreement. The same model was validated with experimental results of aged dentin, finding a low change in the deformation response of dentin with aging, as presented in chapter 6.

Finally, preliminary results made on the mechanical properties of dentin have shown that the microstructure of aged human dentin can vary depending on the ethnic background of the donor and that this quality is critically important to the mechanical properties of the tissue. In chapter 7 preliminary results on the comparison between the microstructure, chemical composition and mechanical properties of Colombian, Chinese and American donors is presented. Finally, conclusions for the study are presented in chapter 8.

Products

As a result of this doctoral research the following products have been obtained:

Peer reviewed publications

Montoya, C., Arola, D., Ossa, E. A. (2017). Time Dependent Deformation Behavior of Dentin. *Archives of Oral Biology*, 76, 20-29.

Montoya, C., Arola, D., Ossa, E. A. (2016). Importance of tubule density to the fracture toughness of dentin. *Archives of Oral Biology*, *67*, 9-14.

Montoya, C., Arango-Santander, S., Peláez-Vargas, A., Arola, D., Ossa, E. A. (2015). Effect of aging on the microstructure, hardness and chemical composition of dentin. *Archives of oral biology*, *60* (12), 1811-1820.

Oral conference presentations

Montoya, C., Arola, D., Ossa, E. A. Prediction of fracture toughness of human dentin. 6th International Conference on Mechanics of Biomaterials and Tissues, 2015, Hawaii, USA.

Montoya, C., Arola, D., Ossa, E. A. Time dependent behavior of human dentin. TMS 2015, Orlando, USA.

Montoya, C. Propiedades mecánicas de la dentina. XX Jornada de Investigación-Facultad de Odontología UCC, 2014, Medellín, Colombia.

Montoya, C., Arola, D., Ossa, E. A. Effect of aging on the microstructure, hardness and chemical composition of human Dentin. TMS 2014, 2014, San Diego, USA

Montoya, C., Ossa, E. A. Composición química y microestructura de la dentina de pacientes colombianos. VII Congreso Internacional de Materiales, 2013, Medellín, Colombia.

Conference proceedings publications

Montoya, C., Ossa, E. A. Composición química y microestructura de la dentina de pacientes colombianos. Revista colombiana de Materiales, 5, 73-78.

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Chapter 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 *et al.,* 1996; Zioupos *et al.,* 1998; Zioupos *et al.,* 1999; Wang *et al.,* 2002; Wang *et al.,* 2004; Nalla *et al.,* 2004a; Ural and Vashishth 2006; Ural and Vashishth, 2007). 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.

Previous studies conducted on aging of dentin have identified that there is a considerable reduction in fatigue tolerance, fracture toughness and flexure resistance over time (Arola *et al*., 2010; Nazari *et al.,* 2009). These changes generated by aging predispose dental fractures, which along with the presence of caries and gum deterioration are the three most common causes of dental repair and failure; about a third of the teeth repaired daily correspond to any of these causes (White *et al.,* 1996). Further, some authors have linked tooth fracture with the completion of restoration processes (root canal treatments and tooth filling procedures), since cracks or stress concentrators may be generated, promoting tooth fracture (Lawrence Livermore National Laboratory, 2008). This behavior can be even seen in both young and old patients.

Microstructural changes occurring in dentin with aging have been associated with obliteration of dentinal tubules and variations on chemical composition (Kinney *et al.,* 2005; Rosen *et al.,* 1999). However, the aging process of human dentin have not been really quantified and related with the changes in mechanical properties. Therefore, the aim of this doctoral work is to quantitatively evaluate the aging process of coronal dentin in terms of the evolution of microstructure, changes in chemical composition and mechanical properties from selected age groups (young and old donors). The changes in these properties were evaluated in three different regions (outer, middle and inner dentin) in order to identify spatial variations within the crown.

The ***overall hypothesis*** of this project is that aging of dentin and the decrease in mechanical properties is directly correlated with the change in the microstructure and chemical composition. To achieve this objective, the following aims were developed:

Specific Aim 1: Test the hypothesis that human dentin undergoes a significant change in the microstructure and obliteration of dentinal tubules with increasing patient age.

Specific Aim 2: Test the hypothesis that human dentin undergoes significant changes in its chemical composition with increasing patient age.

Specific Aim 3: Test the hypothesis that human dentin undergoes a significant change in hardness and in its distribution within the crown with increasing patient age.

Specific Aim 4: Test the hypothesis that the viscoelastic or time dependent deformation response of dentin change along the tooth and undergo significant changes with increasing patient age.

With the results obtained in this investigation, it is expected to understand how the decrease in mechanical properties of aged dentin are related with the changes in microstructure and chemical composition, and therefore in the long-term development of dental restorative materials with differential properties as patient age, achieving thus a reduction in the recurrence of fractures and tooth extractions.

Chapter 2

Review of previous research on the mechanical behavior and aging of human dentin

2. 1 Introduction

A brief description of the main literature on microstructure, chemical composition and mechanical behavior of dentin and its changes with aging is presented in this chapter. Detailed reviews can be found elsewhere (e. g. Pashley *et al.,* 1989; Kinney *et al.,* 2003; Zhang *et al.,* 2014). Additionally, throughout the entire document additional information on previous research can be found where necessary to complement the discussion.

2. 2 Tissues of the human body

A tissue is a collection of cells having similar structure and function, usually having a common embryonic origin and working together to develop specialized activities (Gomez de Ferraris and Campos Munoz, 2009). According to the embryonic origin the tissues in the human body can be classified as (Henrikson and Kaye, 1986):

- Epithelium: Responsible of cover and protect organs, constituting the inner lining of the cavities, hollow organs and ducts of the body as well as form mucous and glands.
- Connective tissue: Protect, support and bind together different types of tissues and organs in the body.

- Muscular tissue: Produces movement and according to the location and function can be skeletal or striated muscle, smooth or non-striated muscle, and cardiac muscle.
- Nervous tissue: Initiates and transmits potentials that help coordinate activities.

These tissues, depending on their level of mineralization and therefore their hardness, may be classified as soft or hard tissues.

Soft tissues are responsible of connect, support, or surround other structures and organs of the body (i.e. ligaments, tendons and skin) (Derby and Akhta, 2015). While *hard tissues* are those that have been mineralized, and therefore serve as protective shield or structural support (Grumezescu, 2016). The hard tissues of humans are bone, tooth enamel, dentin, and cementum. Detailed reviews about each of these tissues and their chemical composition, formation and chemical properties can be found in: Kambic (1994), Rho *et al.* (1998), Meyes *et al.* (2008) and Lloyd *et al.* (2015).

2. 3 Microstructure and chemical composition of dentin

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). Although this chemical composition is assumed to dentin, it has been widely reported in literature that these percentages change within the tooth from the pulp to the Dentin Enamel Junction (DEJ) and along radicular dentin (Ryou *et al.*, 2011; Xu *et al.,* 2009; Tesch *et al.,* 2001).

The thickness of dentin (i.e. from the pulp to the 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 and deposition of secondary dentin (Gomez de Ferraris and Campos Munoz, 2009).

The microstructure of dentin is largely dominated by its tubules, which are responsible for housing the odontoblastic processes and maintain dentin vitality. The tubules extend from the pulp to the DEJ with a double "S" shape in coronal dentin and only one curvature in the dentin root (radicular dentin) (Nanci, 2012). These are called *primary curvatures* and are formed as the progressive stacking of dentinal tubules during the formation of dentin. As a result of this process there are a higher number of dentinal tubules near the pulp (~40.000 tubules/mm^2) and fewer near the DEJ (~17.000 tubules/mm^2) (Fehrenbach and Popowics, 2015).

Dentinal tubule diameters also vary along dentin, diameters of dentinal tubules range from approximately 1 to 3 μm, depending on patient age and its location, having larger diameters near the pulp (~2.00μm) and smaller near the DEJ (~1.20μm) (Ide Ingle *et al.,* 2008). Additionally, dentinal tubules have collateral ramifications that contribute to dentinal permeability and sensitivity; this branching have been reported as more evident in root dentin than in the crown. This condition promotes bacterial penetration and therefore periodontal disease in elderly patients (Pashley and Pashley, 1991).

A highly-mineralized cuff of *peritubular dentin (PTD)* containing mainly apatite crystals and a small proportion of organic proteins, surrounds the lumens of each tubule. The tissue located between the tubules is called *intertubular dentin (ITD)* and contains a matrix of collagen fibers reinforced by apatite (Marshall *et al.,* 1997). Peritubular dentin is characterized by the absence of collagen type I in its chemical composition and its high mineral content (Xu and Wang, 2012). Formation of PTD takes place after mineralization of intertubular dentin is complete and is slowly deposited centripetally after the development of the tooth (Kinney *et al.*, 1996). This process of formation makes that PTD show three clearly distinguishable areas: a hypo-calcified region near the outer edge, a highly-mineralized zone that is in continuous formation and a hypo-mineralized area, which is continuously forming and mineralizing throughout life (Gomez de Ferraris and Campos Munoz, 2009).

The process of formation and mineralization of dentin, known as dentinogenesis takes place during the stage of apposition of the tooth; where enamel, cementum and dentin are secreted in layers by ameloblasts, cementoblasts, and odontoblasts respectively (Goldberg *et al*., 2011). During dentinogenesis, odontoblasts release a non-mineralized matrix of collagen fibers that are later mineralized in the maturation stage by the formation of hydroxyapatite crystals (Fehrenbach and Popowics, 2015). This process takes place as long as the tooth is alive and that is why dentin is considered a live tissue (Nanci, 2012).

Since dentin is not a uniform tissue due to its variation in chemical composition, dentinal tubule density and diameter of dentinal tubules, various types of dentin have been identified (Nanci, 2012; Gomez de Ferraris and Campos Munoz, 2009; Spangberg, 1989):

- *Primary dentin:* This dentin forms the external shape of the tooth. Depending on its location can be named as mantle dentin (first formed dentin) and circumpulpar dentin (forms the remainder tissue). This tissue is formed during odontogenesis and until the tooth enters occlusion.
- *Secondary dentin:* Since dentin continues its formation through life, dentin formed since the tooth enters in occlusion is called secondary dentin. The formation pattern of this dentin is the responsible of forming the “S” shape of dentinal tubules, obliteration of dentinal tubules and decrease of the pulp chamber size with aging.

- *Tertiary dentin:* Also named reactionary or reparative dentin. This dentin is formed as a result of external stimuli (i.e. caries or trauma) in order to protect the sensitive pulp. This dentin formation occurs irregularly depending on the aggressiveness of the stimulus.

Based on its differences in chemical composition and microstructure, dentin is considered a hierarchical biological composite (Ziskind *et al*., 2011) and its mechanical properties change along different regions of dentin and with patient age.

2. 4 Mechanical Properties of dentin

The mechanical properties of dentin have been studied using techniques commonly used for the characterization of ceramic materials. Some of the properties that have been determined are hardness, Young's modulus, flexural strength, fracture toughness, fatigue strength, compressive strength and some viscoelastic properties. Some of the results obtained are described below:

2.3. 1 Hardness and Elastic Modulus

Hardness and elastic modulus of dentin have been determinated in different types and regions of dentin. Some of the techniques used include nanoindentation, Atomic Force Microscopy (AFM) and microindentation.

By means of Atomic Force Microscopy (AFM) have been found values of hardness between 2.23 GPa and 2.54 GPa for peritubular dentin and between 0.49 and 0.52 GPa for intertubular dentin. From these results was found that the values for peritubular dentin are independent of the location on the tooth, while hardness of intertubular dentine was found to be dependent on the position, being larger near the DEJ and lower near the pulp (Kinney *et al.,* 1996). The Young's modulus for peritubular dentin showed to have values of 25 GPa and between 18 GPa and 22 GPa for intertubular dentin. The higher value obtained for peritubular dentin was attributed to a higher mineralization degree of this tissue when comparing with intertubular dentin (Kinney *et al.,* 1996). By means of nanoindentation Ryou *et al.* (2012) found hardness values of 2.38 GPa and 1.31 GPa for peritubular and intertubular dentin, respectively; while the Young´s modulus had values of 29.8 GPa for peritubular dentin and 19.4 GPa for intertubular dentin. From the results, the authors found that intertubular dentin is almost isotropic and the anisotropic behavior of dentin is determined by the direction of dentinal tubules and the direction in which testing is performed.

An average hardness of 0.5 GPa has been reported using a Vickers microindentation techniques, with no significant dependence on indentation load or indentation time (Chuenarrom *et al.,* 2009). Additionally, Gutiérrez-Salazar and Reyes-Gasga (2003) used Vickers hardness to determinate how the tooth hardness change from outer enamel surface to inner dentin layer finding that enamel's hardness ranges from 2.65 GPa to 3.53 GPa and from 0.49 GPa to 0.58 GPa for dentin.

These properties have been measured not only in permanent teeth but also for deciduous teeth. For example, Angke *et al.* (2003) measured the hardness and elastic modulus of a deciduous molars using a Berkovich indenter. Measurements were made in coronal dentin from the cusps to the pulp. For both hardness and elastic modulus, an increase in the penetration depth was found closer to the pulp and a decrease near the DEJ. An average hardness value of 0.52 GPa was found for zones near de pulp and 0.91 GPa for the region near the DEJ. The elastic modulus near the pulp was 11.59 GPa while a value of 16.91 GPa was found near the DEJ. The statistical analysis showed that the values obtained for middle and near DEJ were not statistically significant. A summary of the reported results for the hardness and Young modulus of dentin are shown in the tables 2.1. and 2.2 respectively.

Dentin hardness has been widely studied and some of the differences obtained between authors can be attributed to the specimen preparation procedure, indentation load or type of tooth analyzed. Nonetheless, how dentin hardness changes spatially within the tooth and how this differences relates with the chemical composition and tubular characteristics (i.e. tubule density and diameter of dentinal tubules) have not been completely understood.

2.3. 2 Flexure Strength

Different types of bending tests have been performed to dentin in order to compare its properties with those obtained for restorative materials or adhesives used in restoration procedures. For example, Ryou *et al.* (2011) used specimens of two regions of coronal dentin to perform flexural test - the regions analyzed were named as "top" (region near DEJ) and "inner" dentin (region near pulp) -. All the samples showed a linear elastic behavior with a small zone of plastic deformation before failure. Further, it was found that the "top" dentin had the higher elastic modulus and strength when comparing with the inner regions. The flexure strength was found ranging from roughly 130 MPa to 180 MPa and the strain ranging from 0.008 mm/mm to 0.011 mm/mm. The fracture of the specimens was found to begin in the area subjected to tension stresses. Similar tests were performed by Plotino *et al.* (2007) on root dentin, in this particular case in order to evaluate the strength of different synthetic root materials like carbon and glass fiber composites, zirconia, gold, stainless steel and titanium. The flexure modulus for root dentin showed values of 17.5 ± 3.8 GPa and flexure strength of 212.9 ± 41.9 MPa. A comparison of results for natural and synthetic materials showed similar elastic moduli for the fiber-reinforced composite posts while other synthetic materials showed higher values. Finally, Staninec *et al.* (2008) also conducted tests on coronal dentin reporting an average flexure strength of 164.4 ± 9.1 MPa. A summary of the flexure strength of dentin results is shown in the table 2.3.

The changes in flexure strength for the different regions of dentin have been attributed to the chemical composition and microstructure of each region, where the higher values of flexural strength were found for outer dentin and radicular dentin where a lower number of dentinal tubules were found.

2.3. 3 Fracture Toughness

Various approaches have been used to measure the fracture toughness of dentin, including notched and compact tension specimens with different orientations of dentinal tubules with respect to the direction of crack propagation. Knowledge of the fracture toughness of dentin is important to establish the effects of a restoration procedures in the advent and propagation of cracks in dentin.

Triangular samples were used by Iwamoto *et al.* (2003) to study the fracture toughness of dentin. Samples were classified in three groups; *i)* those with load applied perpendicular to the plane of dentinal tubules, *ii)* parallel and aligned to the plane of dentinal tubules; and *iii)* the plane of crack propagation parallel and transverse to the plane of dentinal tubules. A scheme of the samples used is shown in Figure 2.1. From the results, they argued that the orientation of dentinal tubules with respect to the direction of the load had a statistically significant effect on fracture toughness of the tissue. Values of 1.13 MPa*m$^{0.5}$ were found for samples with the load applied perpendicular to the plane of dentinal tubules and an average of 2.00 MPa*m$^{0.5}$ was found when the load was applied parallel to the dentinal tubules with the crack propagating across the boundary between inter- and peritubular dentin.

Another type of test that has been used to study the fracture toughness of dentin involves notched specimens in three-point bending and two directions of crack propagation, in plane and anti-plane, with respect to dentinal tubules. The results obtained were used to calculate the stress intensity factor, showing that this factor is higher when the load is applied anti-plane due to a higher proportion of peritubular dentin with a higher mineralization degree (Yan *et al.,* 2009).

Compact tension specimens were used by Ivancik and Arola (2013) to examine how fracture toughness changes in outer, middle, and inner coronal dentin. They found that outer dentin required ~50% greater stress to propagate the crack as compared with inner dentin. This behavior was attributed to differences in tubule density and diameter of dentinal tubules along the tooth.

Additional test performed to measure the fracture toughness of dentin were reported by Imbeni *et al.* (2003), Nalla *et al.* (2003a) and Wang (2005); who found similar results to those mentioned above. A summary of the reported results are shown in table 2.4

2.3. 4 Compressive behavior

Human teeth are subjected to compressive loads and friction during mastication of food, whereas dentin works under compression stresses only. This makes that the understanding of the compressive behavior of dentin become very important for this tissue. Several studies have found compressive strength values that range between 275 MPa and 300 MPa (Craig and Peyton, 1958) and elastic modulus ~14 GPa (Kinney *et al.,* 2003). The elastic modulus obtained under compression loads is similar to the values obtained using indentation techniques like AFM and nanoindentation, as mentioned in the previous section (section 2.3.1).

2.3. 5 Fatigue strength

The fatigue strength of dentin is of great importance as teeth are continuously subjected to cyclic stresses during mastication. Likewise, after a restoration process is common to find cracks that can propagate even with low stresses but applied continuously. A detailed review on the fatigue behavior of dentin can be found in Kruzic and Ritchie (2008).

Studies on the fatigue behavior of human dentin have distinguished that dentin exhibits the traditional S–N response (Arola *et al.*, 2010; Arola and Reprogel, 2005; Nalla *et al.*, 2003b). From these results, has been established that dentin exhibits an apparent endurance limit that ranges from approximately 20–50 MPa, and that it is dependent on the frequency of loading (Nalla *et al.*, 2003b, Kruzic et al., 2003), the stress applied (Nalla *et al.,* 2004b), and tubule orientation with respect to the loading direction (Arola and Reprogel, 2006).

The dependence of these factors can be explained by the variations in the microstructure of dentin (i.e. Tubule density and diameter) and changes in chemical composition (i.e. collagen content) along the tooth.

2.3. 6 Viscoelastic Properties

Although the viscoelasticity of dentin is assumed due to its collagen content, little is known about its time-dependent behavior and its relation to the microstructure and mineral content. The mechanical properties of dentin involving stress-strain relations due to tension, bending and shear have been investigated under quasi-static conditions (see for instance sections 2.3.1 through 2.3.5). However, just a few studies have analyzed viscoelasticity and stress relaxation using various techniques and in different types and regions of dentin. One of the first tests conducted to determine the viscoelastic properties of dentin was performed by Duncanson and Korostoff (1975). They used radicular dentin cylinders under compressive stresses to analyze the relaxation behavior of the tissue, finding that dentin showed a linear dependence on the logarithm of time. They found that the relaxation modulus of dentin showed a linear dependence on the logarithm of time. The mathematical model of viscoelasticity previously presented by Alfrey and Doty (1945) was further compared with their experimental results finding that the stress relaxation response of dentin followed a linear viscoelastic behavior. Despite of these results, it is not clear if the same behavior can be extrapolated to coronal dentin, which supports different loads and shows distinct microstructure. On the other hand, Jantarat *et al.* (2002) also used radicular dentin cylinders manufactured from incisors and canines. Compressive loads of 100 N, 300 N, 500 N and 700 N were applied and held for time periods of 90 min. Strain recovery was measured for 60 min after removing the load. A typical viscoelastic behavior was found for the creep measurements with a strain increase over time while the stress was kept constant. Statistical differences were found between stress and creep rate when comparing for the different loads applied. Similar test made on radicular dentin have been performed by Trengrove *et al.* (1995) and Jafarzadeh *et al.* (2004) finding some viscoelastic behavior for the tissue when a load in constant for a period of time.

Viscoelastic test on coronal dentin performed by Pashley *et al.* (2003) in order to study the stress relaxation of demineralized dentin under tension showed that the dentin matrix exhibits both stress–relaxation and creep behavior. However, the stress–relaxation and tensile creep were independent of the initial strain applied.

Other techniques reported in the literature for measuring the viscoelasticity of dentin involved Atomic Force Microscopy (AFM) and nano-Dynamic Mechanical Analysis (DMA). For instance, Balooch *et al*. (1998) measured the viscoelastic properties of fully hydrated demineralized dentin using an AFM-based indentation method finding that the behavior of the demineralized material is nearly elastic, and that hardness and elastic modulus do not change with different maximum loads. According to these results, it was established that collagen in dentin does not contribute to the elastic modulus but it does to dentin strength and toughness. On the other hand, Ryou *et al*. (2012) studied the viscoelastic behavior of peritubular and intertubular dentin. Indentations were made using loads of 400, 700 and 1000 μN with a corresponding dynamic load of 20 μN and frequencies varying from 2 to 100 Hz. Complex modulus, loss moduli, storage moduli and *tan* δ showed an increase in magnitude with loading frequency. The complex and storage moduli of peritubular dentin were significantly larger than for intertubular dentin. No significant differences in the loss modulus and *tan* δ between intertubular and peritubular dentin were found, implying similar viscoelastic behavior in the two types of dentin. Recently, Chuang *et al.* (2015) performed nanoindentation creep tests to study the viscoelastic properties of dentin after de- and re-mineralization processes finding that the demineralization process increase the primary and secondary creep regimes, while the remineralization reduces the primary creep of dentin without increasing viscoelasticity.

Indentation techniques have been widely used to analyze the viscoelastic properties of tissues and biological materials (i.e. bone and teeth) due to its ability to obtain reliable results without damaging the samples when the loads used are low (Cheneler *et al.,* 2013; Staines *et al.,* 1981; Ahearne *et al.,* 2007). Various types of indenters have been used for these tests such as spherical, conical and pyramidal. Sharp indenters introduce a discontinuity at the tip followed by immediate inelasticity, while spherical indenters produce a uniform and axisymmetric distribution of stresses, allowing a soft transition between elastic and viscoelastic regimes during the indenter penetration, simplifying the viscoelastic behavior analysis of the material (Bower *et al.,* 1993). For instance, da Silva *et al.* (2008) used a

1.5 mm diameter tungsten-carbide ball to analyze the Hertzian response (i.e. contact modulus) of dentin with different loading rates and indentation directions as a function of dentinal tubules orientation (i.e. parallel and perpendicular), finding significant differences in the response of dentin with respect to loading rate but with no differences with tubules orientation.

2. 5 Aging process of dentin

Geriatric dentistry has attracted attention in recent years given the need to preserve and improve the quality of life of elder people. Additionally, maintaining the physiological function of the different organs in the aging population could help to reduce the burden on the existing medical systems as older individuals consume medical services (Sieck, 2003). The aging process begins since the day of birth and continues throughout life; however, the effects of the aging process are more evident in the third decade of life and are increasingly obvious after that (Vaughn, 2011).

The aging process of teeth includes wear of enamel, tooth loss due to alveolar resorption and periodontal disease and color change (darkening) (Nanci, 2012). On the other hand, in the case of dentin it has been found that the aging process can generate the following changes (Murray *et al.*, 2002):

- Increase in dentin thickness and decrease of the pulp chamber due to the deposition of secondary dentin throughout life: An approximate rate of secondary deposition of 43 μm per year, or 0.119 μm per day have been estimated (Solheim *et al.,* 1992).
- Reduction in the number of odontoblasts in the pulp chamber: Quantitative studies have reported a reduction in total pulp cell numbers by 50% between the ages of 20 and 70 years (Frolich *et al.,* 1970).

- Obliteration of dentinal tubules: The most obvious change in dentin with aging is the microstructural change due to the obliteration of dentinal tubules with *sclerotic or transparent dentin*. This process changes the chemical composition of dentin increasing its mineral content (Nanci, 2012).

As can be seen, all these changes are related and have an effect not only on the chemical composition as mentioned earlier, but on the mechanical behavior, permeability and sensitivity of the tissue (Arola and Reprogel, 2005). A few studies have been published aiming at identify the changes in mechanical properties of dentin with aging. An extensive review of the aged dentin properties was published by Arola *et al.* (2009). Some of the changes found for the mechanical properties of dentin include:

- A decrease in dentin tubule diameter due to deposition of secondary coronal dentin (Nanci, 2012). Nonetheless, these changes have not been quantified. This process of obliteration is known to begin at the root and move towards the crown of the tooth (Vasiliadis *et al.,* 1983).
- An increase in the mineral content of dentin has been found in aged dentin (Koester *et al.,* 2008a). Likewise, changes in crystallinity of the mineral material have been reported; intertubular dentin mineral crystallites are ~7–19% smaller in aged than in young dentin, while the dentin mineral crystals deposited - obliterating - in dentinal tubules are chemically similar to the intertubular mineral (Porter *et al.,* 2005). Increases in elastic modulus and hardness have been also found for radicular dentin, being more significant in the cervical portion of the root (Xu et *al.*, 2014).
- Increases in the elastic modulus of 5% and 9% in the hardness of the outer layer of dentin have been found by Senawongse *et al.* (2006) when comparing young and old

dentin. On the other hand, Zheng *et al.* (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 outer dentin.

- There is a decrease in flexural strength of dentin of almost 20 MPa per decade of life that begins shortly after reaching adulthood (~30 years) (Arola *et al*., 2009).
- A reduction in fatigue strength of dentin was found in aged dentin at higher levels of stress. However, at low stress levels transparent dentin appears to have the same behavior than young dentin (Kinney *et al.,* 2005).
- There is reduction in the fatigue crack growth resistance of dentin with increasing age. The average rate of fatigue crack growth in old dentin is greater by a factor of 10^2 in comparison to young dentin (Arola *et al.*, 1999).
- A reduction of 75% in the energy required to fracture dentin between young (age≤30) and old patients (age>55) was also reported (Arola *et al.,* 2009).
- Ryou *et al.* (2015) found a difference in the damping behavior of dentin with aging and therefore in its viscoelastic properties using nanoscopic dynamic mechanical analysis (nanoDMA). The results showed that either complex or storage modulus depend on age for peritubular and intertubular dentin while the loss modulus and tan δ are lower than the values obtained for young dentin.

2. 6 Conclusions

After reviewing the studies available on the mechanical properties of dentin and its aging process, the follow conclusions can be drawn to support the development of this doctoral project:

1. It has been widely reported in literature that aging causes a change in dentin microstructure, product of the obliteration of dentinal tubules. Most of these studies have been performed on root dentin, where aging begins, and the results obtained have been analyzed qualitatively using microscopy technics. However, these changes have not been quantified and there is no information available on how the obliteration of dentinal tubules changes spatially in coronal dentin. Obtaining this information for coronal dentin is important as the crown is responsible to support most of the stresses during mastication. Identifying these quantitative changes is essential to find relations between the decrease in mechanical properties due to the aging process.
2. It has been reported in literature that aging causes a change in the chemical composition of dentin. These changes have been related with an increase in mineral content due to the obliteration of dentinal tubules. However, the literature review shows no studies aimed at determine and quantify the changes in chemical composition of aged coronal dentin (spatial variations). These changes might be related with an increase in mineralization, crosslinking of collagen or changes in crystallinity.
3. Dentin hardness has been shown to have considerable local variations for young patients. For aged dentin, although it has been reported that hardness increases with aging, the changes obtained have been related to the amount of "free spaces" (i.e.

dentinal tubules density), but not with chemical composition changes that occur in the tooth. For this correlation, old dentin can include other additional changes that occur with aging such as obliteration of dentinal tubules. Due to differences in microstructural features of dentin it is expected to find a correlation between hardness and age.

4. The viscoelastic properties of coronal dentin have been measured in the bulk dentin. However, the fact that dentin is an anisotropic material, and that mechanical properties change spatially within the tooth suggests that the viscoelastic properties of dentin will too. Also, the existing literature shows only one study regarding the viscoelastic properties of aged dentin, considering intertubular and peritubular dentin, ignoring how these properties could change along different regions of dentin. The results obtained about the changes in viscoelastic properties of dentin may be related with the chemical composition and microstructure. The relative decrease of viscoelastic properties of the tissue can be assessed as a function of age.

2. 7 Tables

Table 2. 1. Results reported in the literature for the hardness of dentin.

Author	Technique	Region /Type of dentin	Hardness GPa)
Kinney *et al.* (1996)	AFM	PTD[1]	2.40
		ITD[2]	0.51
Ryou *et al.* (2012)	Nanoindentation	PTD	2.38
		ITD	1.31
Chuenarrom *et al.* (2009)	Microindentation/ Vickers	Bulk dentin	0.50
Gutiérrez-Salazar and Reyes-Gasga (2003)	Microindenttion/ Vickers	Outer	0.49
		Inner	0.58
Angke *et al.* (2003)	Berkovich indenter.	Primary dentin/ Outer	0.91
		Primary dentin/ Inner	0.52

1 PTD: Peritubular dentin

2 ITD: Intertubular dentin

Table 2. 2. Results reported in the literature for the Young Modulus of dentin.

Author	**Technique**	**Region /Type of dentin**	**Young modulus (GPa)**
Kinney *et al.* (1996)	AFM	PTD	25.0
		ITD	20.0
Ryou *et al.* (2012)	Nanoindentation	PTD	29.8
		ITD	19.4
Angke *et al.* (2003)	Berkovich indenter.	Primary dentin/ Outer	16.9
		Primary dentin/ Inner	11.6

Table 2. 3. Results reported in the literature for the flexural strength of dentin.

Author	Region /Type of dentin	Flexural Strength (MPa)
Ryou *et al.* (2011)	Outer	180.0
	Inner	130.0
Staninec *et al*. (2008)	Coronal	164.4
Plotino *et al*. (2007)	Radicular	212.9

Table 2. 4. Results reported in the literature for the fracture toughness of dentin.

Autor	Sample	Type /Region of dentin	Crack propagation direction	Fracture Toughness) (MPa*m$^{0.5}$)
Iwamoto *et al.* (2003)	Triangular sample	Coronal	⊥	1.13
			/ /	1.97
Ivancik and Arola (2013)	Compact tension	Outer	⊥	3.4
		Middle		2.7
		Inner		2.2
Yan *et al.* (2009)	Beam	Coronal	/ /	2.3
Imbeni *et al* (2003)	Notched Beam (pre-crack)	Coronal	⊥	1.79
	Notched Beam (without pre-crack)			2.72

2. 8 Figures

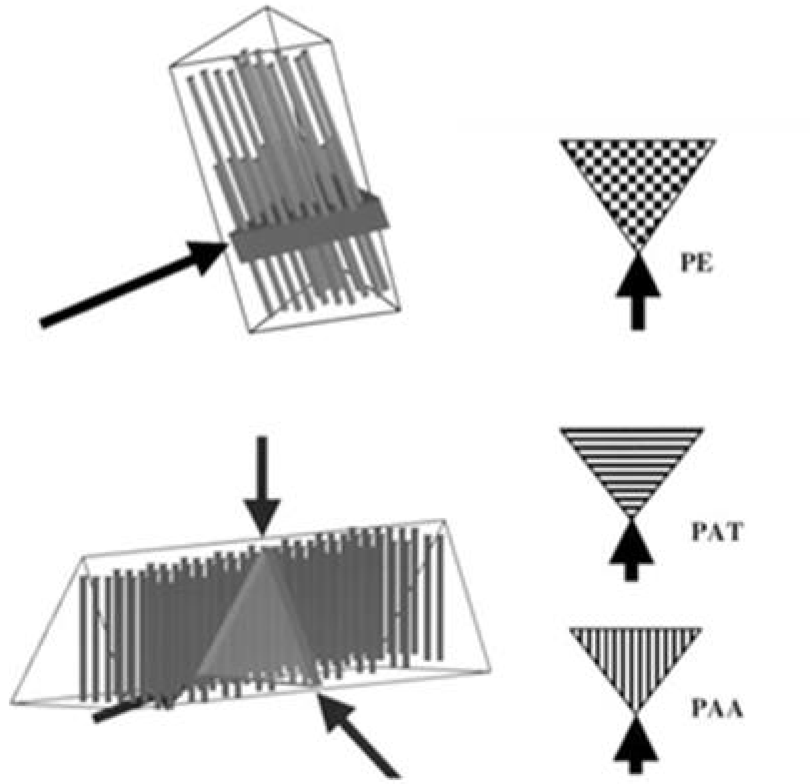


Figure 2. 1. Scheme samples used by Iwamonto and Ruse (2003) to measure the fracture toughness of dentin. Image taken from Iwamonto and Ruse (2003).

Chapter 3

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

3. 1 Introduction

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.

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 material (Kinney *et al.*, 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 *et al.,* 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 *et al.*, 2008b; Nazari *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 chapter is to identify the changes in microstructure, chemical composition and hardness of dentin with aging from selected age groups of Colombian patients.

3. 2 Experimental investigation

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 *et al.*, 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. 3.1a) using diamond abrasive slicing equipment with continuous water coolant. Secondary sections were cut transversely (Section A'-A') in order to expose the dentin as shown in Figure 3.1. For indentation analysis and microscopic evaluations, the specimens were embedded in cold-cured epoxy resin, following similar procedures used by other researchers (Park *et al.*, 2008; Rivera *et al*, 2013; Brauer *et al.*, 2011). The exposed dentin in the resin mount was polished using silicon carbide abrasive paper with successive smaller particle sizes until reaching 1200 grit. Further polishing by means of standard red felt polishing cloth wheels was then performed using diamond particle suspensions of 3 μm in size. After polishing, all samples were ultrasonically cleaned in an HBSS bath for 30 min before microscopic observation in order to eliminate particles of the diamond particle suspension or tissue resulting from the polishing process. The polished specimens were then kept in a HBSS bath solution prior to testing.

Vickers testing was used to study the variation in hardness as a function of dentin depth. Microindentation was performed using a micro-hardness tester (Wilson Instruments, Model 402 MVD, Norward, MA, USA) with a Vickers diamond indenter. Ten indentations were made on each surface, starting at the DEJ. Grinding and polishing was then performed to remove approximately 500 μm of material, after which another 10 indentations were performed. This procedure was repeated until the pulpal surface was reached. Indentations were made using an indentation load of 1.96 N and dwell times of 10 sec. These testing conditions generate an indentation large enough so that the hardness corresponds to the overall dentin hardness, which includes hardness of intertubular and peritubular dentin. Indentations were carefully made with a distance of at least 10 diagonals in length from each other in order to avoid any deformation from neighboring indentations.

The Vickers hardness number (HV) was estimated following the ASTM C1327 (2008) standard according to:

$$HV = \frac{0.1891 * F}{d^2}, \qquad (3.1)$$

where *F* is the indentation load and *d* is the indentation diagonal.

The same specimens were used to evaluate the dentin microstructure using an optical microscope (Axiovert 40 MAT, Carl Zeiss Microscopy, NY). Tubule density, diameter of the tubule lumens and diameter of the peritubular dentin were measured and calculated at each depth. A determination of such parameters was carried across the sample surface. Seven images, each with constant area, were randomly selected from every polished surface. In each image, the amount of tubules was calculated and expressed as tubules/mm^2. The tubule diameter and peritubular dentin diameter were also obtained. Values from the seven images were averaged to obtain information from each depth.

The results obtained for hardness and microstructure at each depth were normalized to the dentin thickness and then classified as outer (normalized depth between 0.76 to 1.00), middle (between 0.36 to 0.75) or inner (depth between 0.00 to 0.35) dentin. Differences in hardness between the outer, middle and inner dentin, as well as between young and old patients were evaluated using a two-way analysis of variance (ANOVA), defining significance of results by p-value ≤ 0.05, a Tukey post-hoc analysis was then performed.

For the analysis of chemical composition, selected molars from each of the two age groups were sectioned along the longitudinal axis (section A-A in Fig. 3.1a) and embedded in cold-cure epoxy resin with the sectioned surface facing outwards. The section surfaces were polished following the process previously explained. Chemical composition analysis of outer, middle and inner dentin was performed using Raman spectroscopy and the results were used to determine the mineral-to-collagen ratio for the three regions (i.e. inner, middle and outer). A confocal Raman spectrometer (Horiba Jobin Yvon LabRAM HR) was used and the spectrums were obtained over the spectral region of 400 to 1100 cm^{-1}. The Raman spectrometer had a laser diode with a wavelength of 785 nm and a spot diameter of approximately 1.1 μm. The mineral-to-collagen ratio was calculated from the ratio of area under the υ_4PO_4 peak at 589 cm^{-1}, which is associated with the phosphate bending of hydroxyapatite, and the area under the amide III peak at 1254 cm^{-1}, which is associated with movements of the peptide bond present in collagen (Goodyear *et al.*, 2009; Kazanci *et al.,* 2006). These bands were selected for analysis as they are reportedly less susceptible to orientation effects and the polarization direction of the incident light (Kazanci *et al.*, 2006).

Differences in the mineral-to-collagen ratio between the outer, middle and inner dentin, as well as between young and old patients were evaluated using a two-way analysis of variance (ANOVA), defining significance of results by p-value ≤ 0.05; a Tukey post-hoc analysis was then performed. Representative maps of hardness and mineral-to-collagen ratio were also obtained from selected teeth to convey the spatial variations present.

3. 3 Experimental Results

Microstructure

The microstructure of dentin from selected young and old donor teeth is shown in Figure 3.2. The microstructures shown for each group correspond to the three regions of coronal dentin evaluated, including the outer (Figs. 3.2b and 3.2c), middle (Figs. 3.2d and 3.2e) and inner (Figs. 3.2f and 3.2g) regions. As evident from the figures, there was an increase in tubule density and diameter of the tubules as the distance from the DEJ increased. Peritubular dentin can be seen surrounding each dentinal tubule (e.g. Fig. 3.2a). There was no differences in appearance between the peritubular dentin from young and old donor teeth. In comparing the two age groups, some of the tubules in the old donor group appeared to be obliterated, with greater number in the middle and outer dentin. Micrographs of tubules for young and old donor teeth are shown in Figure 3.3.

A quantitative comparison of the microstructural characteristics of dentin from the young and old groups is shown in Figure 3.4. Specifically, Figure 3.4(a) shows the tubule density in different regions of coronal dentin. A reduction in the tubule density was observed with increasing proximity to the DEJ in both age groups. For the young donor group, the average tubule density in the outer and inner regions was 25,000 tubules/mm^2 and 35,000 tubules/mm^2, respectively. A similar distribution was found for the old donor group (i.e. decrease in tubule density approaching the DEJ), but there was some difference in the tubule count. The most noticeable change was found in the outer dentin with lower tubule density (10% less) in comparison to that of young donors.

A comparison of the average tubule diameter within the different regions of dentin is shown in Figure 3.4(b). In the young donor group the average lumen diameter in the outer and inner regions was 1.36 ± 0.12 μm and 1.84 ± 0.10 μm, respectively. For the old group these values were 1.24 ± 0.08 μm and 1.81 ± 0.35 μm, respectively. Results obtained for the peritubular cuff diameter are shown in Figure 3.4(c). A decrease in the peritubular cuff diameter was found with increasing proximity to the DEJ for both age groups. In the young group the average diameters within the inner and outer regions were 3.51 ± 0.69 μm and 2.81 ± 0.40 μm, respectively. A similar result was found for old patients, where a larger diameter of tubules near the pulp was observed. Overall, there was a significant decrease in tubule density, tubule diameter and peritubular cuff diameter with depth ($p \leq 0.05$) with proximity to the DEJ for both age groups. In comparing results for the microstructure between young and old donor teeth, the differences found for tubule density, diameter, and peritubular dentin diameter were not statistically significant ($p>0.05$).

Hardness

The influence of indentation load on hardness was studied in order to identify the proper load for measuring the hardness of dentin. Results of that evaluation are shown in Figure 3.5. The measured hardness values decrease from approximately 1.5 GPa at 0.23 N indentation load to 0.7 GPa at 1.96 N load. A plateau in hardness is observed for loads of 1.96 N and greater. Consequently, all further hardness measures were conducted with a load of 1.96 N. The average hardness for the three regions of dentin are shown in Figure 3.6 for both the young and old donor teeth. These values correspond to hardness measured with load applied parallel to the dentinal tubules, as shown in Figs 3.5b and 3.5c.

Representative indentations made within the outer dentin of young and old donor teeth are shown in Fig. 3.7a and 3.7b, respectively. Indentations within the outer dentin covered an average of 36 tubules within the body of the indentation; for the inner dentin that number was 170 tubules. For the old donor teeth, an average of 27 and 156 tubules were included in the outer and inner dentin, respectively. It is worth noting that no cracks were observed emanating from the indentation corners for either group, even at the highest indentation load (9.8N). According to the distribution in measurements, an increase in hardness was found with proximity to the DEJ for both age groups. Average hardness values of 0.65 ± 0.03 GPa and 0.68 ± 0.01 GPa were found for the young and old donor groups, respectively. When comparing hardness results with respect to the specific regions of dentin between young and old donors, significant differences were found for the outer and inner dentin between young and old dentin (p-value ≤ 0.05).

Chemical composition

The distribution of mineral-to-collagen ratio as a function of distance across the coronal dentin for young and old donor teeth is shown in Figure 3.8. The dentin in both age groups showed a similar behavior, with increasing mineral-to-collagen ratio approaching the DEJ. A higher ratio indicates a lower proportion of organic material. The differences found among the areas evaluated in the young donors group were not statistically significant ($p>0.05$), as opposed to the old donors group where they were statistically significant ($p\leq0.05$).

When comparing the mineral-to-collagen ratios between the young and old patients, nearly a 4% difference was found nearest the pulp within the inner dentin. However, the difference in mineral-to-collagen ratio was approximately 40% in the middle dentin and 70% in the outer dentin. In comparing results for the mineral-to-collagen ratio between young and old donor teeth, the differences were statistically significant ($p\leq0.05$).

Comparisons of hardness and mineral-to-collagen ratio distributions in representative teeth from each age group after longitudinal sectioning can be seen in Figure 3.9. The results correspond to a young donor (18 years of age) and an old donor (65 years of age). For the young donor tooth there was an increase in hardness from the pulp up to the DEJ (Figs. 3.9a). As for the chemical composition of the young dentin (Fig. 3.9b), there was an increase in the mineral-to-collagen ratio with increasing proximity to the DEJ. In comparing Figures 3.9a and 3.9b it is seen that lower hardness values were obtained in regions of lower mineral-to-collagen ratio (inner dentin). Conversely, where a higher proportion of mineral was present, the hardness was greater.

Results of the hardness and mineral-to-collagen ratio measurements for an old donor tooth are shown in Figures 3.9(c) and 3.9(d). The chemical composition map for the old donor tooth shows a distorted pulp chamber due to deposition of secondary dentin within. This process can lead to complete pulp obliteration over sufficient time (Tronstad, 2003).

3. 4 Discussion

Microstructure

Consistent with the findings of earlier studies, there were differences in tubule density and dentinal tubule diameter as conveyed from the measures within the three regions of dentin evaluated (Fig. 3.2). The results obtained for tubule density in the young patients group are consistent with those of Marshall *et al*. (1997), who reported values of 20,000 tubules/mm^2 (outer) to 43,000 tubules/mm^2 (inner).

For the dentin of old donor teeth only full or partially opened tubules were considered in the tubule density measurements. Obliterated tubules were excluded from the measurements to assess how dentinal tubule density changes with aging as caused by the filling process. Clearly a reduction in the number of open tubules contributes to changes in dentin permeability and potentially other aspects of its physical behavior. For example, the changes with aging could be important to dentinal sensitivity and the resistance to tooth fracture.

There was a decrease in tubule diameter from the pulp to the DEJ for both age groups evaluated. The measures of tubule diameters are consistent with those reported by Ivancik *et al.* (2014) for the dentin from young U.S. patients. Interestingly, larger tubule diameters have been reported for the dentin of Brazilian donor teeth (diameters of 2.99 μm and 2.42 μm for inner and outer dentin, respectively) and no significant differences with depth (Lopes *et al.*, 2009). When comparing the measured values from young and old donor teeth of the present study, similar results were obtained for the two groups in the inner and middle regions. There was some difference in the measures for diameter in the outer dentin, with 9% lower values in the old dentin. Undoubtedly this difference is attributed to the onset of sclerosis.

In general, the measures of peritubular cuff diameter were approximately twice the diameter of the tubule lumens. The formation of peritubular dentin occurs after the mineralization of intertubular dentin has completed (Gomez de Ferraris and Campos Munoz, 2009). Deposition of dentin is continued by odontoblasts following a circular pattern surrounding the dentinal tubules. During this process, and as the odontoblasts produce several layers of dentin, the pulpal chamber reduces its size, odontoblasts move to the interior of the pulp, and odontoblastic processes remain inside the dentinal tubules (Gomez de Ferraris and Campos Munoz, 2009).

To identify the change in number of obliterated dentinal tubules relative to the total number of open tubules for each region of dentin, the occlusion ratio was calculated according to:

$$Occlusion\ ratio\ (\%) = \frac{Nr.Obliterated\ dentinal\ tubules}{Nr.Open\ tubules} * 100, \qquad (3.2)$$

A higher occlusion ratio indicates a higher fraction of obliterated dentinal tubules. Measured estimates for the occlusion ratio in the three regions of dentin evaluated are shown in Figure 3.10. As expected, the occlusion ratio in young donor teeth is approximately zero in all three regions. Indeed, the visible changes in microstructure of dentin with aging begin in the third decade of life (Gomez de Ferraris and Campos Munoz, 2009; Nazari *et al.,* 2009; Curtis and Watson, 2014). On the other hand, an increase in occlusion ratio was noted as one approaches the DEJ. Hence, the highest proportion of obliterated tubules in the tooth crown is located in the outer dentin and decreases with depth. The occlusion ratio measurements correspond to an average of 1170 obliterated tubules/mm^2 in outer dentin and 120 obliterated tubules/mm^2 in inner dentin. Thus, in the tooth crown filling of the lumens begins at the ends of the tubules.

In comparing the results obtained for microstructure between young and old patients (e.g. tubule density, diameter, and peritubular dentin diameter) there was no significant difference ($p > 0.05$). However, there was a significant difference in the occlusion ratio between the young and old groups, and between the outer and inner dentin ($p \leq 0.05$) as shown in Table 3.1.

Hardness

There was load-dependency in the measured hardness of dentin as shown in Figure 3.5a. This behavior is at least partly explained by the number of tubules involved within the indentation area. The difference in number of tubules involved in the indentation response is evident from a comparison of Figures 3.5b and 3.5c. Load-dependent behavior has been previously reported in the indentation response of dental enamel (Rivera *et al.*, 2013; Park *et al.*, 2008). In enamel, which is absent of tubules, the load dependence was described in terms of the change in mechanisms of deformation with increasing load. That could also play a role in the response of dentin through the relative viscous behavior and degree of water movement.

Results of the hardness measurements show that there are limited differences in the hardness between the three regions of dentin evaluated. An overall average hardness of 0.65± 0.03 GPa was obtained for the young dentin, which is in agreement with results from previous studies (Fuentes *et al.,* 2003). Fuentes *et al.* (2003) found that the hardness of dentin ranged between 0.60 GPa and 0.61 GPa, with only little variation along the tooth. Nevertheless, the spatial variations in hardness identified in the present study are not as significant as those found by other authors, where values between 0.25 GPa to 0.8 GPa have been reported (Pashley *et al.,* 1985). It is important to note that previous studies have generally involved data derived from the teeth of patients living in North America. Differences in the microstructure and mechanical behavior of dentin have been noted in a previous comparison of donor groups from North and South America (Ivancik *et al*., 2014). That may be relevant here.

An increase in hardness occurred with increasing proximity of the DEJ for both age groups. The lower hardness of inner dentin is at least partly associated with the higher number (and area) of dentinal tubules (Pashley *et al.*, 1985). The area occupied by dentinal tubules in the inner dentin is approximately 20% higher than the outer dentin (Pashley *et al.*, 1985; Ivancik and Arola, 2013). This behavior might be attributed to differences in chemical composition and variations between the amount of organic and inorganic material within dentin. However, according to the results obtained for the mineral-to-collagen ratio, these differences were not statistically significant in the young patients group.

An analysis of the hardness results for the young and old donor groups showed that there were significant differences for outer and inner dentin between young and old groups ($p \leq 0.05$). These results are in agreement with those found for the occlusion ratio and obliteration of dentinal tubules. Nevertheless, the results are not consistent with some previous results concerning the hardness of dentin and age. For instance, Senawongse *et al.* (2006) used nano-indentation techniques to determine changes in hardness of different types of dentin with age and did identify significant differences. However, the secondary and mantle dentin did show an increase in hardness. Zheng *et al.* (2005), used nano-indentation to investigate the changes in hardness of dentin with age. In that investigation there were no significant differences in dentin hardness between age and location. However, the transparent regions of dentin from old donor teeth did exhibit significantly greater hardness. The discrepancy between the earlier studies and present results may be explained by the use of nano-indentation techniques, and differences in the representation of peritubular or intertubular dentin with single indents. A load-dependency may also contribute to unique responses for the intertubular and peritubular components. More work is needed in this area.

Chemical composition

An increase in mineral-to-collagen ratio was found with increasing proximity to the DEJ for both young and old donors, but no significant differences were found along the tooth. These results contradict the findings by Ryou *et al* (2011), who found a reduction in the mineral-to-collagen ratio from the pulp to the DEJ in an evaluation of coronal dentin from US donors using FTIR. Ryou *et al* (2011) used the bands associated with the υ_3PO4 peak and the Amide I peak to find the mineral-to-collagen ratio of dentin, contrary to the bands used in this study. Authors like Kazanci *et al.* (2006) have argued that it is important to consider the effect of orientation and polarization of different bands for Raman Spectroscopy analysis. They have also found that Amide III band (used in this study) is less susceptible to polarization effects than other amide bands. However, Märten *et al* (2010) measured the volume fraction of mineral within the coronal dentin using Small-angle X-ray scattering (SAXS) and found that the mineral volume fraction in dentin is uniform and only found statistically significant differences near the DEJ.

A lower mineral content within the inner dentin could be attributed to differences in the mineralization of the intertubular dentin at different regions. For example, Kinney *et al.* (1996) measured the hardness of intertubular and peritubular dentin using AFM; the results obtained established a lower hardness in intertubular dentin near the pulp and an increase towards the

DEJ. A correlation made by Pashley *et al.* (1985) between the change in hardness and tubule density demonstrated that the decrease in hardness of dentin towards the pulp can be accounted for by the decreased hardness of the intertubular dentin and that correlation with tubule density may be coincidental.

It is important to note that most of the results found in the literature were obtained from studies using teeth of mostly anglo-saxons living in the USA. Therefore, the differences in dentin features from Colombian donors (i.e. the variation in the mineral-to-collagen ratio along the tooth) might be attributed to individual characteristics, such as oral health and nutritional status, among others. In addition, several authors have suggested that a relation between ethnicity and some dental features, such as tooth size (Merz *et al*., 1991; Bishara *et al*., 1989), enamel thickness (Hall *et al*., 2007) and tooth formation rate (Olze *et al.,* 2007), might exist. Merz *et al.* (1991) found that dental arches in black patients are significantly wider and deeper than the ones in white patients; while Hall *et al.* (2007) found thicker enamel on the distal aspect of the black donor tooth. Differences between tooth dimensions of permanent teeth in three populations: Egyptians, Mexicans, and the North Americans were found by Bishara *et al.* (1989). However, only a few studies on dentin characteristics and how they might be determined by ethnicity have been carried out (Bajaj *et al*., 2008).

The results obtained for the mineral-to-collagen ratio are in agreement with those found for hardness distribution, where higher hardness values were associated with areas where there was a higher amount of mineral.

The observed distribution in hardness and chemical composition of the old donors group corresponds to the progression of aging. It has been well established that aging of dentin starts from root dentin and continues in the coronal direction Drusini *et al.* (1991). It has also been reported that the total obliteration of tubules for coronal dentin might occur near the age of 70 (Tronstad, 2003).

It is important to note that the results shown in the hardness maps (Figs 3.9a and 3.9c) were obtained after longitudinal sectioning of the tooth (section A-A in Fig. 3.1a). Consequently, the indentation load was applied perpendicular to the dentinal tubules. A comparison of hardness measurements from the parallel and perpendicular loading orientation is shown in Figure 3.11. In comparing the results for these two directions, significant differences in hardness were found between the middle and inner dentin ($p \leq 0.05$) of young donor teeth. For the old dentin there were no significant differences ($p > 0.05$). The unique behavior of the inner and middle dentin may be attributed to a greater number of tubules in these regions, which yields a higher proportion of voids and lower hardness. For old dentin there is a consistent increase in hardness approaching the DEJ with both orientations of indentation. In this case, the increase is more significant than the one discussed earlier (load applied parallel to dentinal tubules) due to the presence of large areas of peritubular dentin within the indentation as a result of obliteration of the dentinal tubules.

3. 5 Conclusions

According to the results obtained, the following conclusions were drawn:

1. The tubule density in the dentin of young and old donor teeth ranged from approximately 22,000 to 35,000 tubules/mm^2. The tubule diameter ranged from approximately 1.2 μm to 1.8 μm. There was a significant decrease in tubule density from the pulp to the DEJ in both age groups studied ($p \leq 0.05$).
2. There was a significant difference ($p \leq 0.05$) in the measures of occlusion ratio between the outer (4.71%) and middle dentin (2.50%) of the old donor teeth. The

largest proportion of obliterated dentinal tubules was near the DEJ and decreased towards the pulp.

3. A significant decrease in hardness was found with increasing distance from the DEJ for both young and old donor teeth ($p \leq 0.05$). A larger hardness was found in the old dentin when compared to young dentin. There were significant differences ($p \leq 0.05$) in the hardness in the outer and middle dentin between the young and old donor teeth.
4. The mineral-to-collagen ratio was found to increase with proximity to the DEJ for both young and old patients. This behavior is opposed to previous results reported in the literature for dentin from US donors, which in turn raises questions whether the chemical composition of dentin and its percentages of organic material and collagen might be affected by ethnicity.
5. The outer dentin exhibited the highest mineral-to-collagen ratio, while lower values were found in the areas near the pulp. A greater mineral-to-collagen ratio was found for the old dentin, with the highest values found in the outer dentin.
6. A comparison of the hardness and mineral-to-collagen ratio distributions showed that the regions with the highest mineral-to-collagen ratio also exhibited the highest hardness.

3. 6 Tables

Table 3. 1. Results from the ANOVA (p-values) in comparing the microstructure of dentin from young and old donor teeth. Note the statistically significant differences for the occlusion ratio for the middle and outer dentin.

	Young vs. Old			
Region	**Tubule Density**	**Occlusion Ratio**	**Tubule Diameter**	**Peritubular Diameter**
Outer	0.1425	***0.0002***	0.2123	0.6466
Middle	0.4333	***0.0001***	0.7358	0.4379
Inner	0.9703	0.3739	0.8497	0.6694

3. 7 Figures

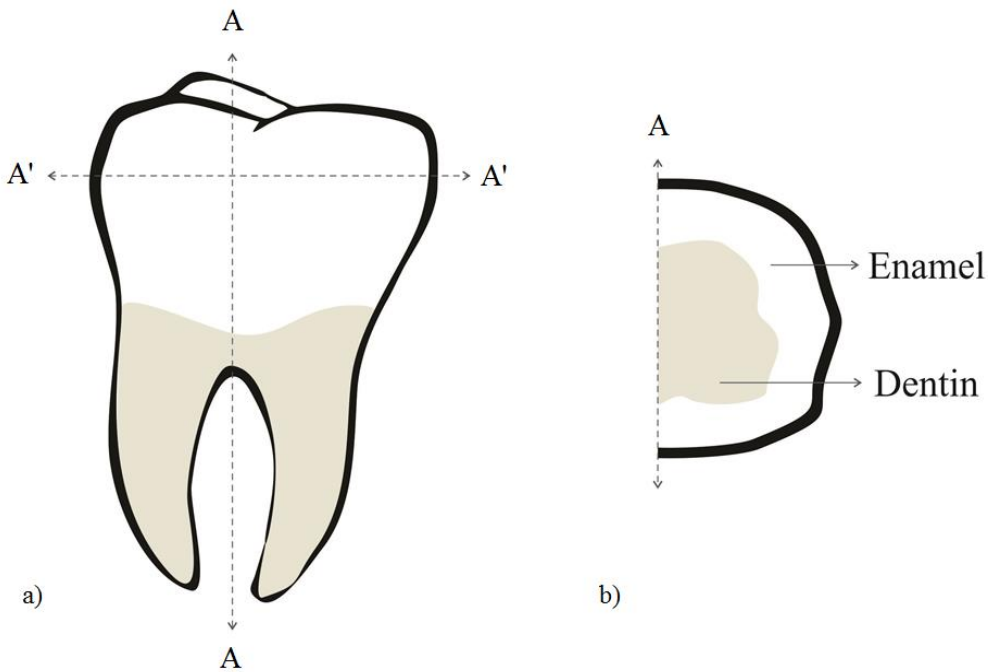


Figure 3. 1. Schematic diagram of a sectioned molar after (a) longitudinal (A-A), and (b) transverse (A’-A’) cutting. The specimen is then embedded in cold-cure epoxy resin with the sectioned surface facing outwards.

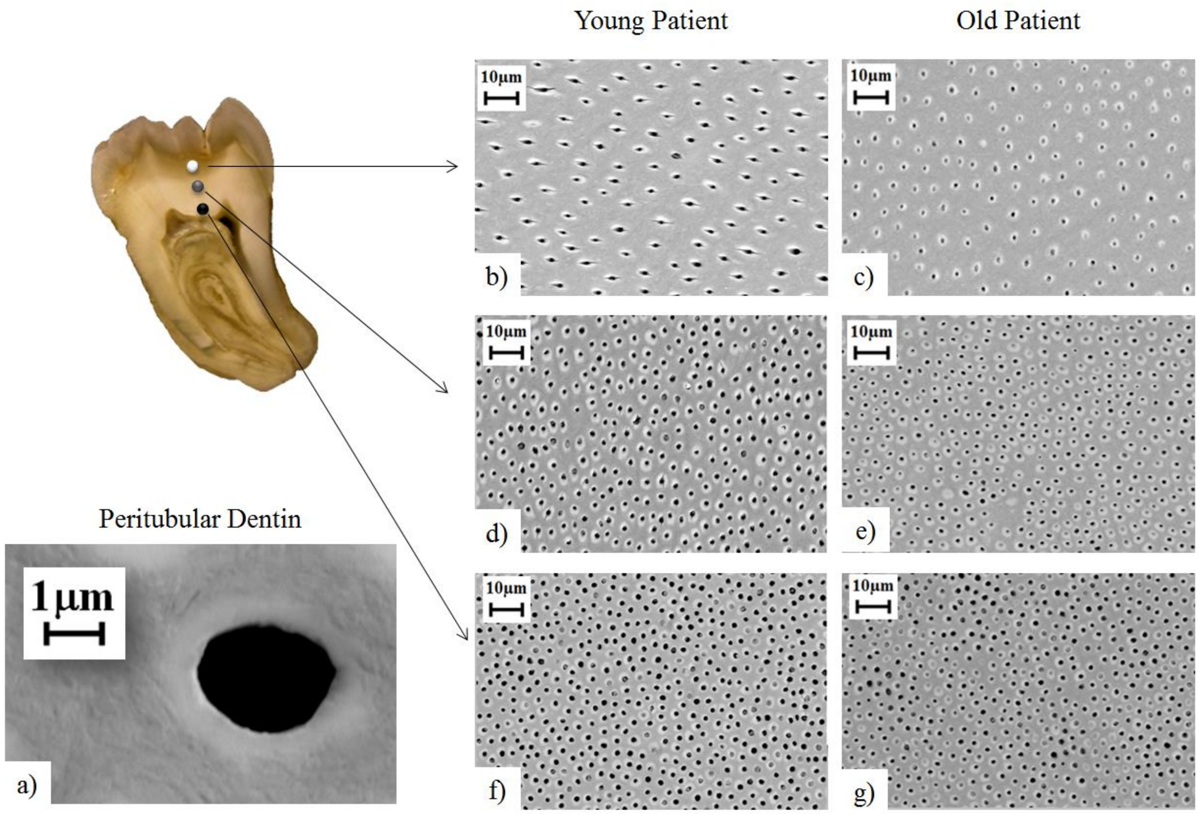


Figure 3. 2. Micrographs of the dentin microstructure. a) single tubule; b-c) Outer dentin; d-e) Middle dentin; f-g) Inner dentin. Note the obliterated dentinal tubules for the old donor teeth in the middle and outer regions (evident in (e) and (g)).

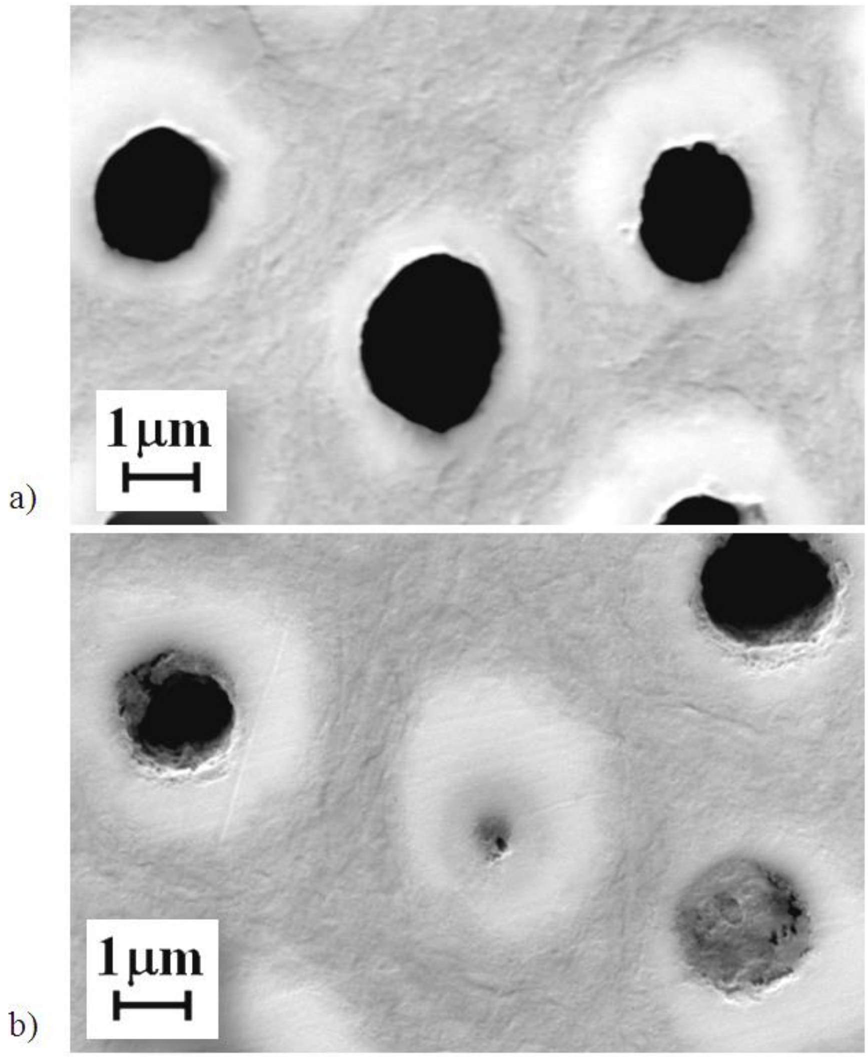
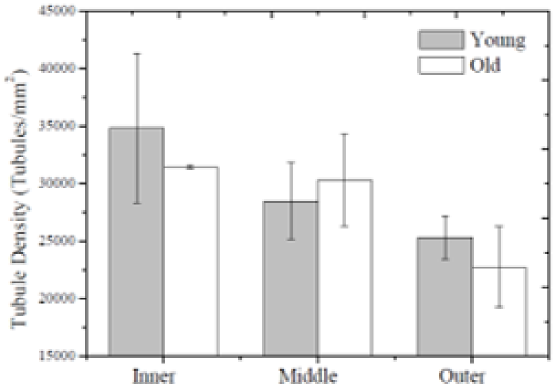
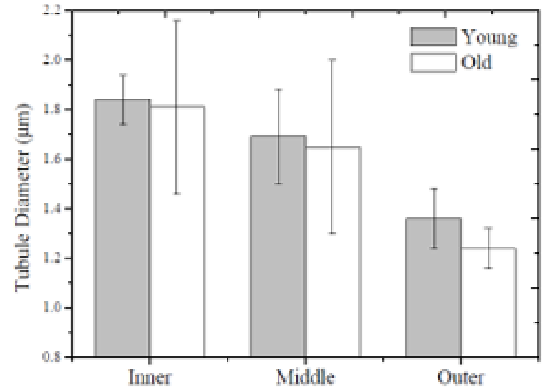


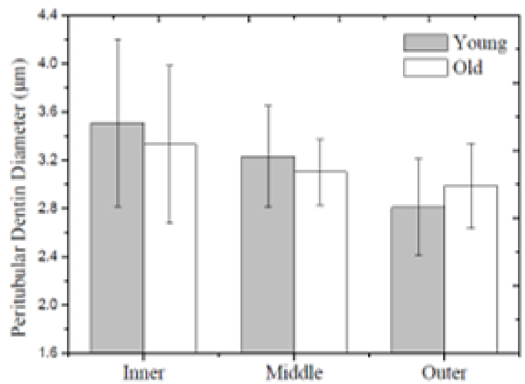
Figure 3. 3. Micrographs of dentinal tubules from outer dentin. a) young donor; b) old donor.



a)



b)



c)

Figure 3. 4. Comparison of microstructure as a function of depth in the coronal dentin. a) tubule density; b) tubule diameter; c) peritubular dentin diameter.

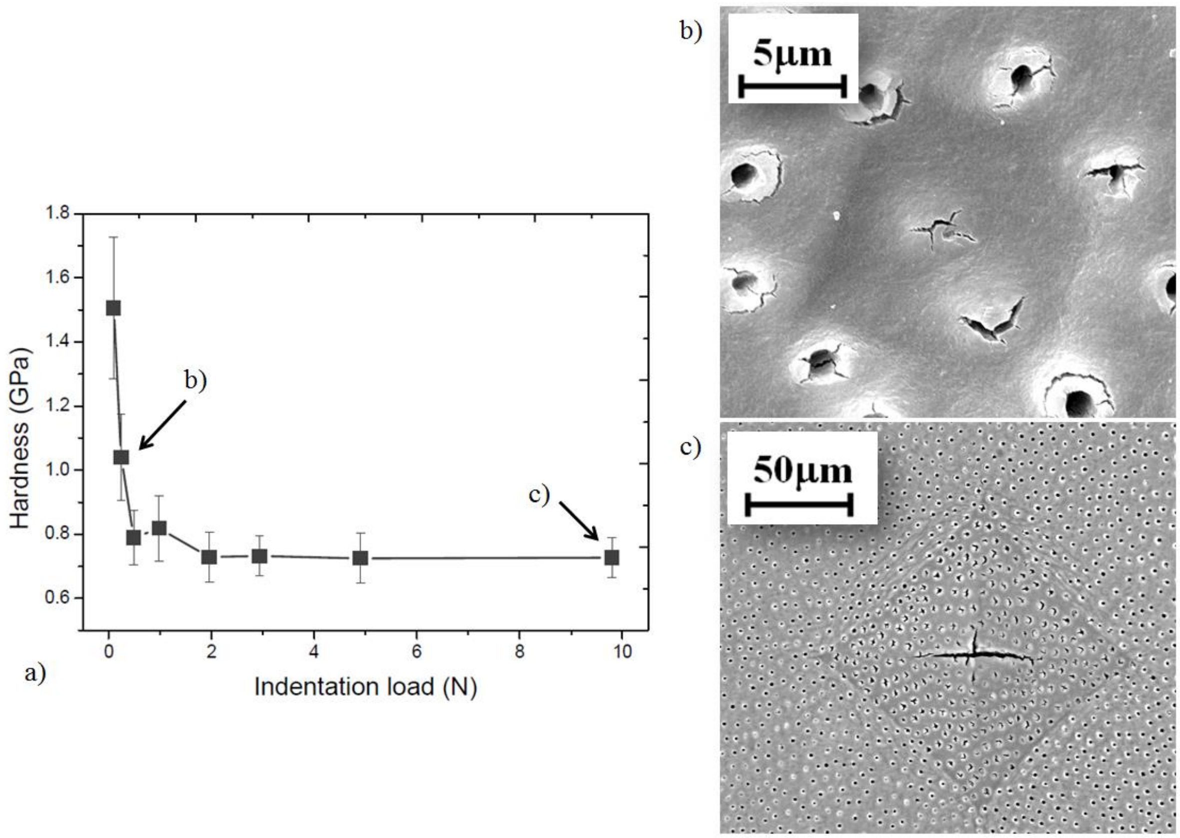


Figure 3. 5. Effect of indentation load on the Vickers hardness of dentin from a young donor tooth. a) change in hardness with indentation load. (b) indentation at 0.23 N; c) indentation at 9.80 N.

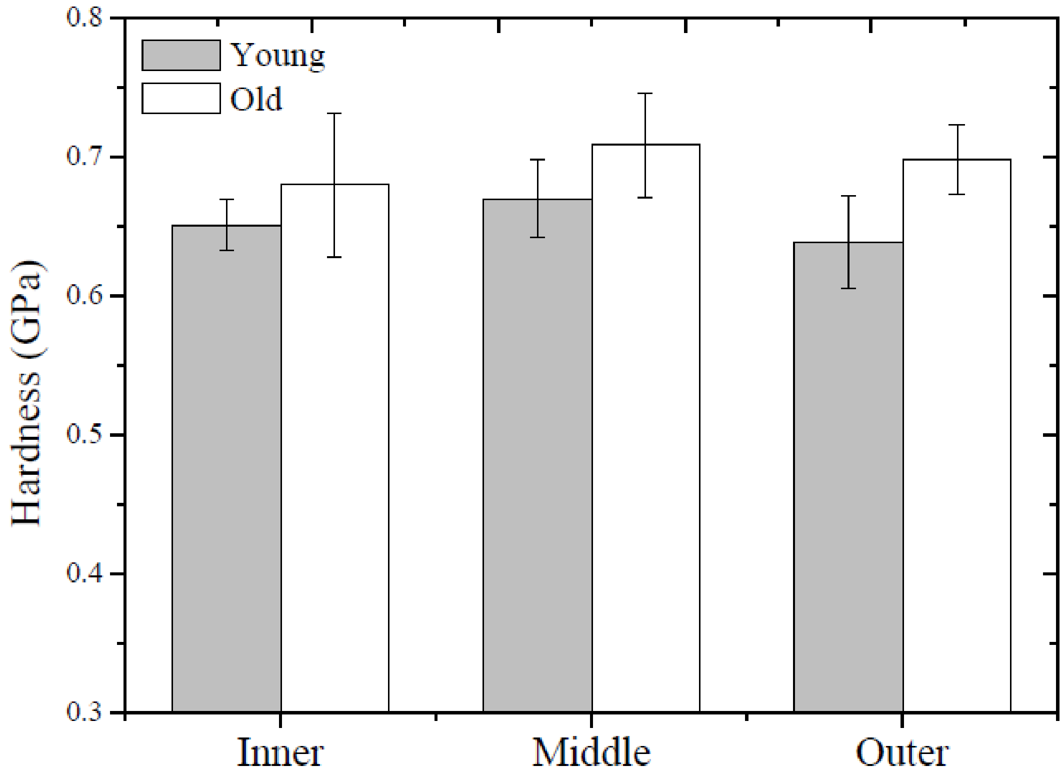


Figure 3. 6. Vickers hardness obtained for dentin from young and old donor teeth according to depth. The direction of applied load is parallel to the dentinal tubules.

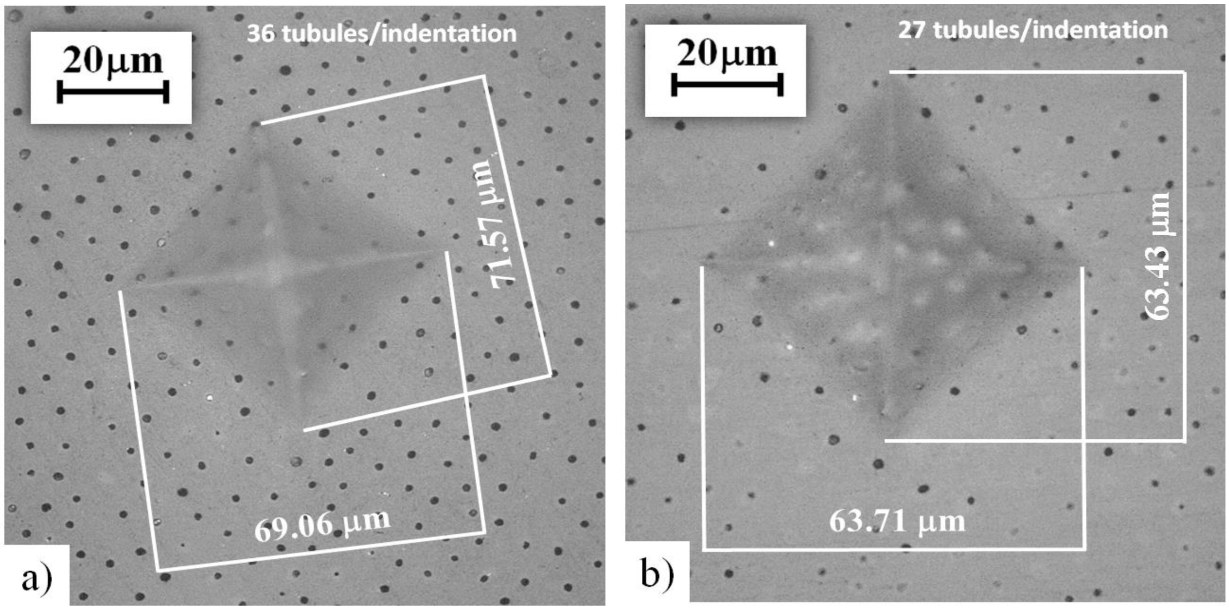


Figure 3. 7. Indentation of dentin for determination of Vickers hardness. The indentation locations are within the outer dentin of a young (a) and old (b) donor tooth.

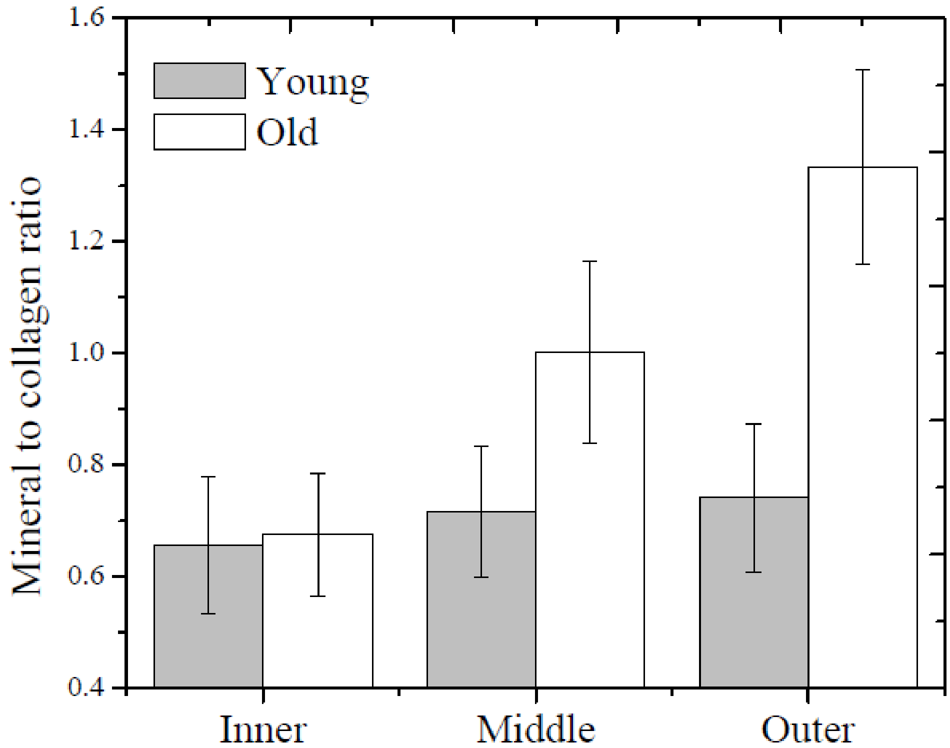


Figure 3. 8. Distribution of mineral-to-collagen ratio of dentin from young and old donor teeth according to depth.

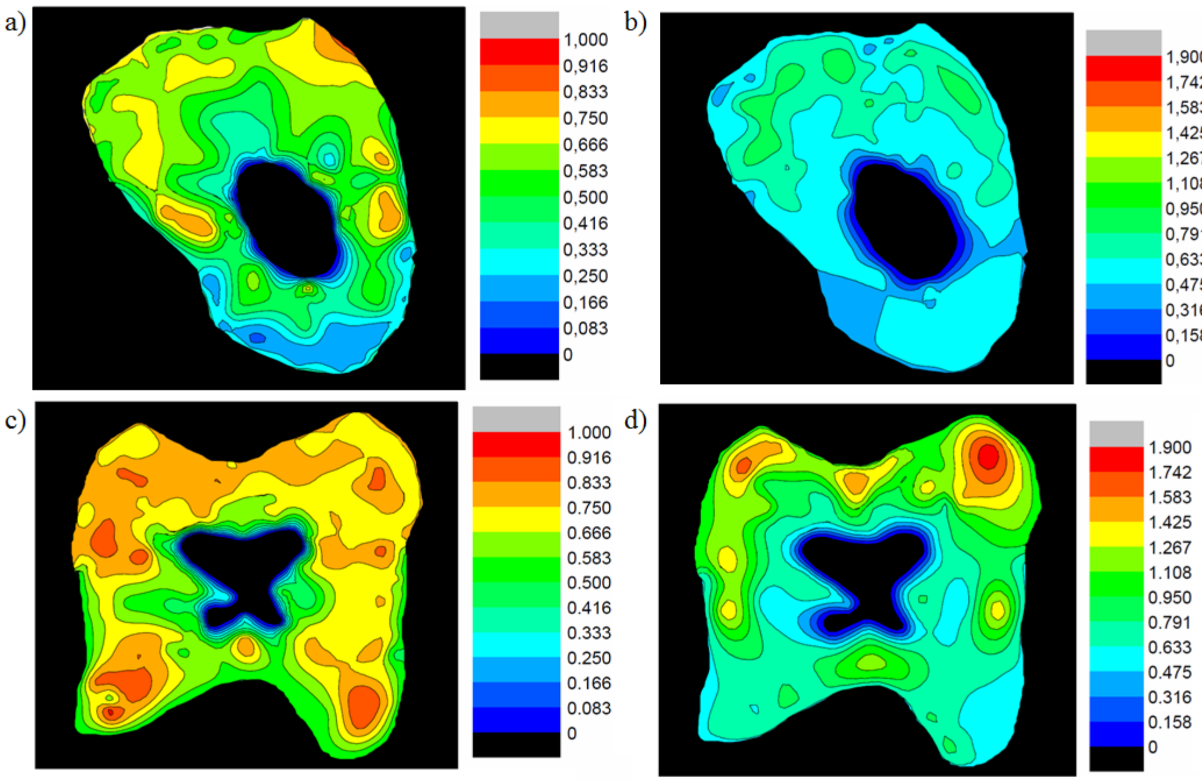


Figure 3. 9. Comparison of the hardness and chemical composition distributions in a tooth as evident from longitudinal sectioning. a) hardness and b) chemical composition (mineral to collagen ratio) from the tooth of a 18 year old donor, c) hardness and d) chemical composition from the tooth of a 65 year old donor.

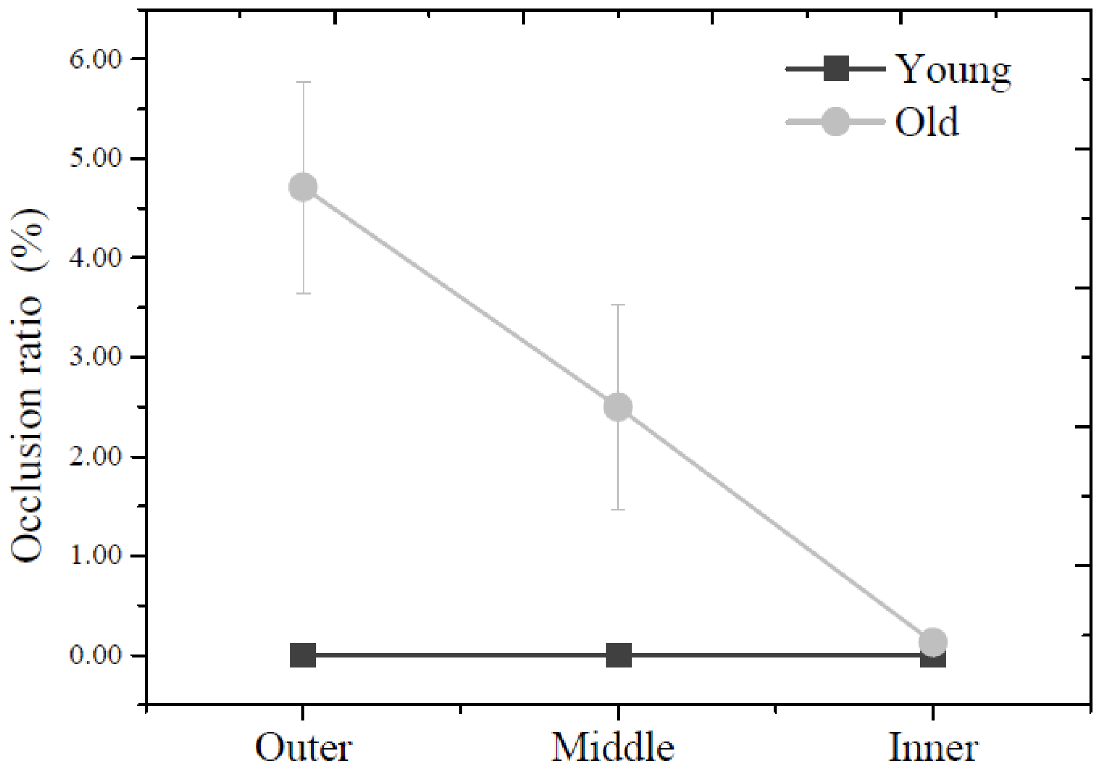


Figure 3. 10. Occlusion ratio for the three different regions of coronal dentin and a comparison of results for the young and old donor teeth.

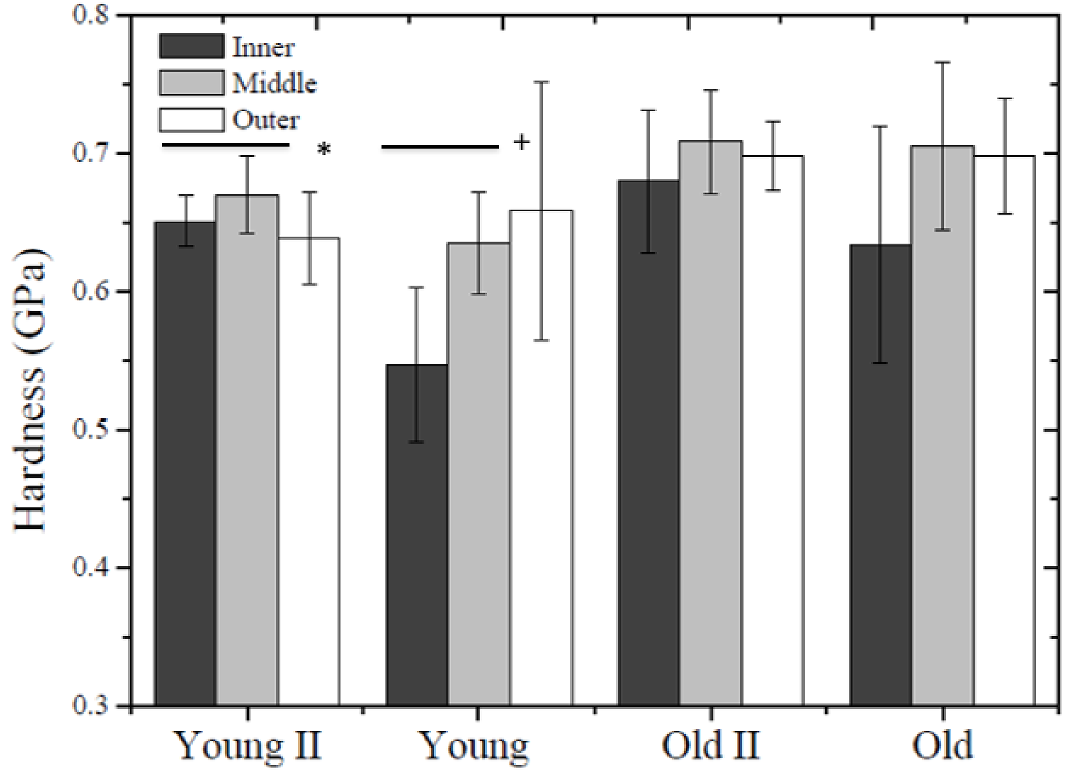


Figure 3. 11. Vickers hardness obtained for dentin of young and old donor teeth according to depth. The applied load is parallel (//) and perpendicular (⊥) to the dentinal tubules. Columns with significant differences ($p \leq 0.05$) are grouped in a line and marked with a cross (+) and a asterisk (*).

Chapter 4

Importance of Tubule Density to the Fracture Toughness of Dentin

4. 1 Introduction

The "cracked tooth syndrome" was coined by Cameron (1964) over a half-century ago to describe the prominence of cusp fractures observed in restored teeth. Fracture is no less important to the field of restorative dentistry today (e.g. Opdam *et al.,* 2007*;* Barreto *et al.,* 2015).

The human dentition is subjected to a variety of cyclic stresses, including those of mastication and other para-functional activities. Acute stresses can promote the formation of micro cracks in the tooth (Homewood, 1998), in addition to other events including trauma and restorative procedures (Bastone *et al*., 2000; Majd *et al*., 2012; Lee *et al.*, 2014). Damage and microcracks in dentin may not cause fracture, but can facilitate the failure of restored teeth through fatigue crack growth (Arola *et al*., 1999; Nalla *et al*., 2003b; Bajaj *et al.,* 2008). Spatial variations in the resistance to fracture of dentin can contribute to this process and is an important factor to consider.

Due to its importance, the fracture toughness of coronal dentin has been evaluated, with primary emphasis on the importance of tubule orientation (see section 2.3.3). With the increase in senior dentate, aging has become of greater importance to the field of restorative dentistry ((McNally *et al.,* 2014; Yellowitz and Schneiderman, 2014). Human teeth undergo changes with increasing age, including a decrease in the number of odontoblasts, an increase in dentin thickness and the formation of transparent dentin (Toto *et al.,* 1971; Bernick and Nedelman, 1975; Murray *et al*., 2002; Nanci, 2012; Timiras, 2007). In addition, there are changes in mechanical properties, such as an increase in elastic modulus and hardness, a decrease in strength and decrease in fatigue crack growth resistance (see section 2.5). There is also a reduction in fracture toughness with increasing age (Kinney *et al.*, 2005; Koester *et al.,* 2008a; Nazari *et al*., 2009). Changes in the fracture toughness with aging may be related to dehydration (Toto *et al.,* 1971), increases in the degree of mineralization (Porter *et al.,* 2005), or an increase in the degree of crosslinking of the collagen fibrils (Miura *et al.,* 2014). However, as mentioned earlier the most visible change is the decrease in diameter and density of the tubules, which results from an increase in mineral deposited within the tubules.

To the authors' knowledge, no investigation has examined the fracture toughness of coronal dentin in terms of tubule characteristics and number of obliterated tubules that occur with increasing age. Hence, the objective of this investigation was to examine the effect of tubule density on the fracture toughness of dentin from young and old donors and utilize an approach previously proposed for porous materials to quantify the spatial variations in fracture resistance.

4. 2 Experimental investigation

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 prevent dehydration (Habelitz *et al.,* 2001). In addition, the specimens were tested within two weeks of extraction to limit the loss of mineral and degradation of organic materials. All species were prepared according to the procedure explained in section 3.2.

Measurement of the tubule density (ρ_t) and tubule lumen diameter (ϕ_l) was performed using commercial image analysis software (AxioVision LE). Seven randomly selected images with a constant area (approximate size of each image 80 µm x 100 µm) were obtained over the polished surface. The mean tubule diameter and number of tubules were calculated for each image. Values from the seven images were averaged and used to estimate the lumen area fraction (ξ) within the three regions of evaluation as:

$$\xi = \frac{A_l}{A_T}, \qquad (4.1)$$

where A_l is the area (mm^2) occupied by lumens and A_T is the total area of dentin measured (mm^2) in each image. The average lumen area was calculated using the measures of tubule diameter and density.

4. 3 Experimental results

The microstructure of dentin from selected young and old donor teeth is shown in Figure 4.1. Representative images are presented from the three regions of evaluation including the outer (Figs. 4.1a and 4.1b), middle (Figs. 4.1c and 4.1d) and inner (Figs. 4.1e and 4.1f) dentin. For both age groups the peritubular dentin can be seen surrounding each dentinal tubule. Several obliterated tubules are evident in the images for the old donor group, with greater number of filled tubules in the middle and outer regions. Micrographs of tubules within the outer dentin of young and old donor teeth obtained at higher magnification are shown in Figures 3.3(a) and 3.3(b), respectively. An example of a tubule that has become filled with mineral is shown in Figure 3.3(b). No obliterated tubules were evident in the dentin of the young donor group regardless of the region of evaluation.

Figure 4.2 shows the estimates for the lumen area fractions (ξ) in the three regions of dentin evaluated. Overall, there was a significant decrease in ξ with proximity to the DEJ for both age groups ($p \leq 0.05$). In the young donor group the average ξ in the outer and inner regions was 3.7±0.6% and 9.3±1.0%, respectively. For the old group these values were 2.9±0.4% and 9.6±1.1%, respectively. The primary difference between the two age groups was the lower value of ξ (37% less) in the outer region of dentin for the old group. This difference is attributed to the reduction in diameter of the dentin tubules due to deposition of mineral within the lumens as a result of sclerosis. When comparing the results for ξ in each region between the young and old donors, significant differences were found only for outer dentin ($p \leq 0.05$).

4. 4 Discussion

While the dentin tubules play an important role in tooth sensitivity and pain stimuli (Magloire *et al.*, 2010), they also serve as a form of porosity that contributes to the mechanical behavior of dentin. Several models have been proposed to describe the mechanical behavior of porous materials (Ryshkewitch, 1953; Schiller, 1985; Hasselman, 1963; Ji *et al.*, 2006). According to these models, it is possible to define relationships between porosity and physical properties such as Young's modulus, hardness, and strength (Ji *et al.*, 2006; Salomon and Kosmač, 2013; Luo and Stevens, 1999; Kováčik, 1999). However, the lack of uniformity in the distribution and shape of pores has been considered a limitation of these approaches in their general application to materials.

Balshin (1949) developed an empirical model for the prediction of mechanical properties for materials with microstructures similar to dentin. According to this approach, the variation in fracture toughness of dentin can be described as a function of the porosity or the lumen area fraction (ξ) by

$$Kic = Kic_o(1-\xi)^m \,, \qquad (4.2)$$

where Kic and Kic_o are the Mode I fracture toughness of dentin with specific porosity and of solid dentin (i.e. with no lumens), respectively, and m is a constant that depends on the degree of stress concentration developed around the lumens (i.e. lumen geometry). According to Bocaccini *et al.* (1996) the value of m varies from 1, for long cylindrical pores orientated parallel to the stress direction (z/x $\sim\infty$), up to about 7 for oblate spheroids (axial ratio z/x < 1). The axial ratio z/x is related to the shape of the pores, where z and x are associated with the length and width of the pore, respectively.

The reported range in fracture toughness for human dentin is roughly 1.1 $MPa \cdot m^{0.5} \leq Kic \leq 3.5\ MPa \cdot m^{0.5}$. The lowest values have been obtained for cracks oriented perpendicular to the tubules (Iwamoto and Ruse, 2003), whereas higher values are reported for cracks extending in-plane with the tubules (Yan *et al.,* 2009) and for outer dentin, which exhibits a low tubule density (Pashley *et al.,* 1985). Nonetheless, estimates for the fracture toughness of dentin without the presence of dentinal tubules (solid dentin) have not been reported.

Recently, Ivancik and Arola (2013) evaluated the fracture behavior of coronal dentin obtained from the teeth of young donors residing in the US. They obtained estimates of *Kic* for the outer, middle, and inner dentin. The average fracture toughness for the outer coronal dentin (with lowest density of tubules) was 3.40 MPa·m$^{0.5}$. Their results are shown in Figure 4.3 along with a prediction from the Balshin model (Eq. 4.2) using Kic_o = 3.40 MPa·m$^{0.5}$ (outer dentin) and m=3, which is appropriate for long cylindrical pores (e.g. dentinal tubules) oriented perpendicular to the direction of stress (Bocaccini *et al.,* 1996). As evident in Figure 4.3, the model predicts a decrease in fracture toughness of dentin with increasing tubule area fraction (porosity). That distribution correctly captures the decrease in fracture toughness approaching the pulp due to a higher proportion of dentin tubules and larger average diameter. A reasonable correlation was found between the model and experimental results (R^2= 0.89).

An alternate approach was also used to obtain the parameters of Eq. (4.2) by performing a best-fit to the experimental results (Fig. 4.3), which resulted in Kic_o = 3.76 MPa·m$^{0.5}$ and m=4.5. A comparison of the model to experimental data with these "best-fit" parameters results in R^2=0.998, which indicates very good agreement. The value obtained for Kic_o is similar to the maximum value obtained by Ivancik and Arola (2013) of 3.74 MPa·m$^{0.5}$, which was obtained for the outer dentin with a lumen area of just under 2%. Therefore, the value of Kic_o obtained in this study is effectively related to dentin without tubules. According to Boccaccini *et al.* (1996), a value of m=4.5 corresponds to a porous microstructure composed of oblate spheroids with z/x = 0.3 and an orientation of α = 30º, which is a reasonable approximation to the mean shape and orientation of the S-shape lumens in dentin. Therefore, the model provides a reasonable description for the effect of ξ on the fracture toughness of coronal dentin.

Estimates for the fracture toughness of dentin from the teeth of young and old Colombian donors were obtained using Eqn. (4.2) and are shown in Figure 4.4. These predictions were obtained using the best-fit estimates for Kic_o and m, along with the lumen area fraction measurements (ξ) from the Colombian donor teeth in Figure 4.2. As evident in this figure, there is a significant increase ($p \leq 0.05$) in fracture toughness with increasing proximity to the DEJ for the middle and outer regions of young dentin, which agrees with the reported results (Ivancik and Arola, 2013). Furthermore, in comparing the estimated *Kic* between the young and old dentin of the three regions there is a significant difference ($p \leq 0.05$) in values for the outer dentin only; the *Kic* for the old dentin is 4.0% greater. These results contrast those previously reported for the fracture toughness of dentin from senior patients (Kinney *et al.,* 2005; Nazari *et al.,* 2009) where a substantial reduction (20%) in fracture toughness was noted with aging, regardless of the direction of crack growth (Koester *et al*., 2008a; Ivancik and Arola, 2013).

According to the adopted model (Eq. 4.2), the microstructural changes in dentin that occur with aging would cause an increase in fracture toughness. This behavior is related to the reduction in number and diameter of stress concentrators in the material (as represented by lower ξ). But if the tubules are obliterated by deposited mineral with low fracture toughness, the overall toughness should be higher than that of a porous material (with empty tubules) if the mechanisms of fracture do not change with aging. An obvious limitation of the proposed model is that only geometrical features are considered (i.e: lumen area). The question that arises is “If the dentin of old patients undergoes a decrease in the number of stress concentrators, why is there a reduction in the fracture toughness with aging?”.

The primary mechanisms of crack growth toughening in dentin involve crack deflection, uncracked-ligament bridging, crack branching and collagen fibril bridging (Nalla *et al*., 2003; Nalla *et al.,* 2006). *In situ* observations of crack growth in dentin have shown that the obliteration of tubules in old dentin causes changes to the path of crack growth (Koester *et* *al.,* 2008a) and the mechanisms of toughening. Specifically, tubules that are filled with mineral resist penetration of the crack. Consequently, crack extension occurs about the interface of the peritubular cuff and intertubular dentin. In young dentin, the peritubular cuffs located within the K-dominant region undergo fracture. As a consequence, crack growth occurs by the "linking" of these spurious microcracked tubules or so-called daughter cracks (Nalla *et al*., 2006; Ivancik *et al.,* 2012). Due to the suppression of peritubular cuff fracture in old dentin, crack deflection and uncracked-ligament bridging (two of the dominant mechanisms of toughening) are essentially inactivated. Koester *et al*. (2008a) commented that the most commonly observed change in fracture characteristics with aging was a decrease in crack branching. That process occurs by crack extension from tubule to tubule and the propensity for branching is determined by the number of unfilled tubules. Therefore, aging causes a decrease in fracture toughness of dentin due to a reduction in the number of contributing toughening mechanisms as well as a potential reduction in the potency of remaining mechanisms. A definitive description for the changes in toughening mechanisms that occurs in dentin with aging has not been presented. A quantitative description for the reduction in toughness related to the individual mechanisms, including the increase in mineralization of intertubular dentin, cross linking or degradation of the collagen matrix, would be valuable.

The changes in toughening mechanisms with aging necessitates that new values of Kic_o and m are obtained for the model (Eq. 4.2) when applied to old dentin. Some modifications in the Balshin model parameters may also be required due to differences in the fracture resistance of dentin between the teeth of US and Colombian donors. Ivancik *et al*., (2014) compared the microstructure and fracture resistance of dentin from US and Colombian donor teeth. Results showed that there were significant differences in the tubule lumen diameters between the two groups in the inner and outer dentin. Furthermore, it was found that the fatigue crack growth resistance for the dentin of teeth from the Colombian donors was independent of location, which contradicts observed trends in the dentin of US donors. Thus, the value of Kic_o should be tuned to the degree of mineralization that is related to age, and the other unique physical characteristics of the tissue.

4. 5 Conclusions

According to the results obtained, the following conclusions were drawn:

1. A quantitative analysis of the changes in tubules occurring in coronal dentin with aging showed that the primary difference between young and old dentin was a reduction in the lumen area fraction. In the outer dentin there was nearly a 40% reduction in the lumen area with aging. This difference is attributed to obliteration of dentinal tubules with formation of sclerotic dentin.
2. An approach previously proposed to study the mechanical behavior of porous materials was employed to model the fracture toughness of dentin and the influence of spatial variations in dentinal tubules. The model showed a strong correlation to experimental results previously reported in the literature for the dentin of young donor teeth. However, results for the old dentin were not in agreement due to differences in the mechanisms of fracture.

4. 6 Figures

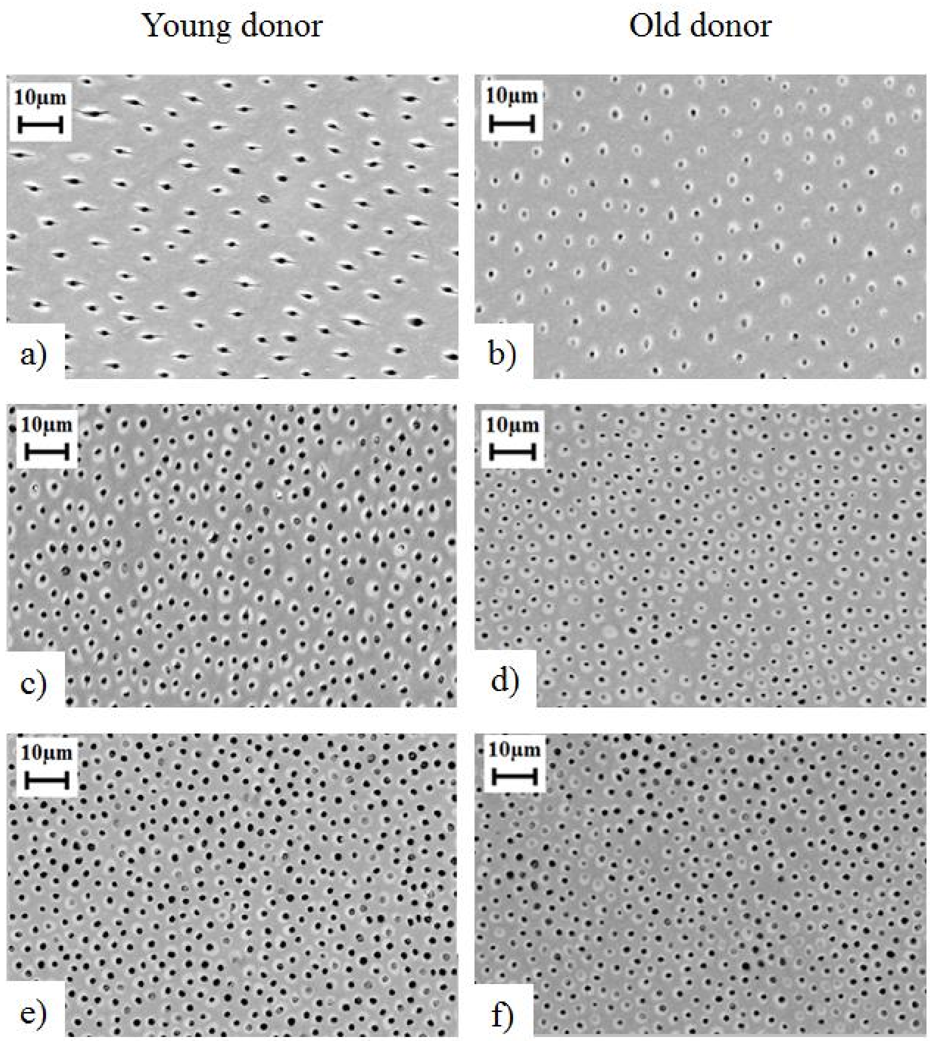


Figure 4. 1. Micrographs of the microstructure for the young and old dentin as a function of location. a-b) Outer dentin; c-d) Middle dentin; e-f) Inner dentin. Note the obliterated dentinal tubules in micrographs for the middle and outer dentin of the old donor teeth.

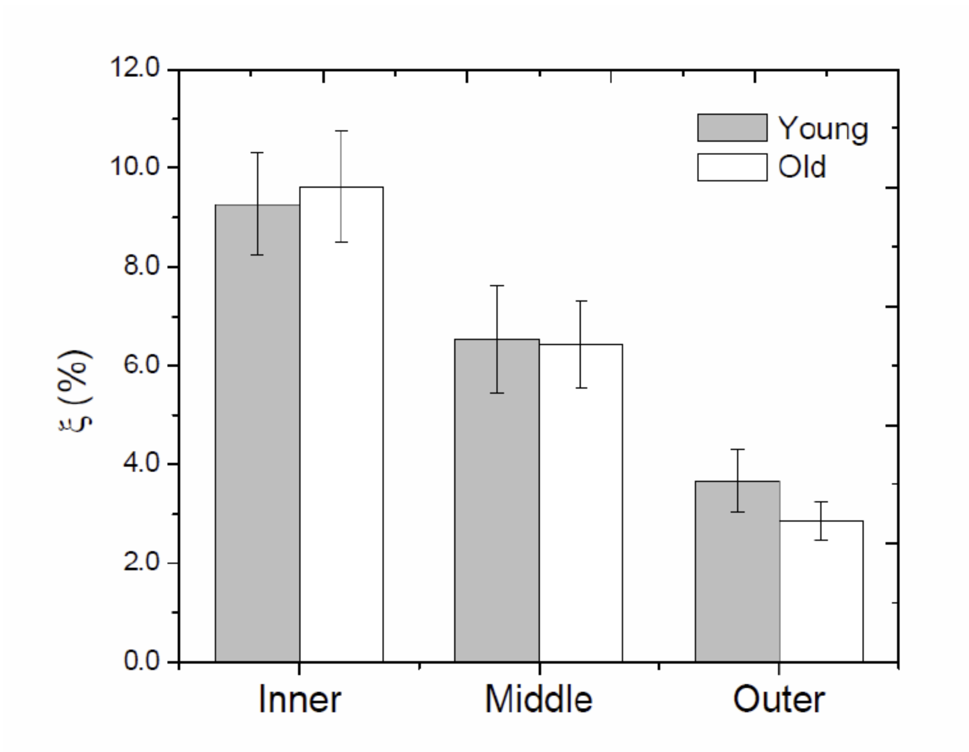


Figure 4. 2. Lumen area fraction (ˣ) for the three different regions of coronal dentin and a comparison of results for the young and old donor teeth.

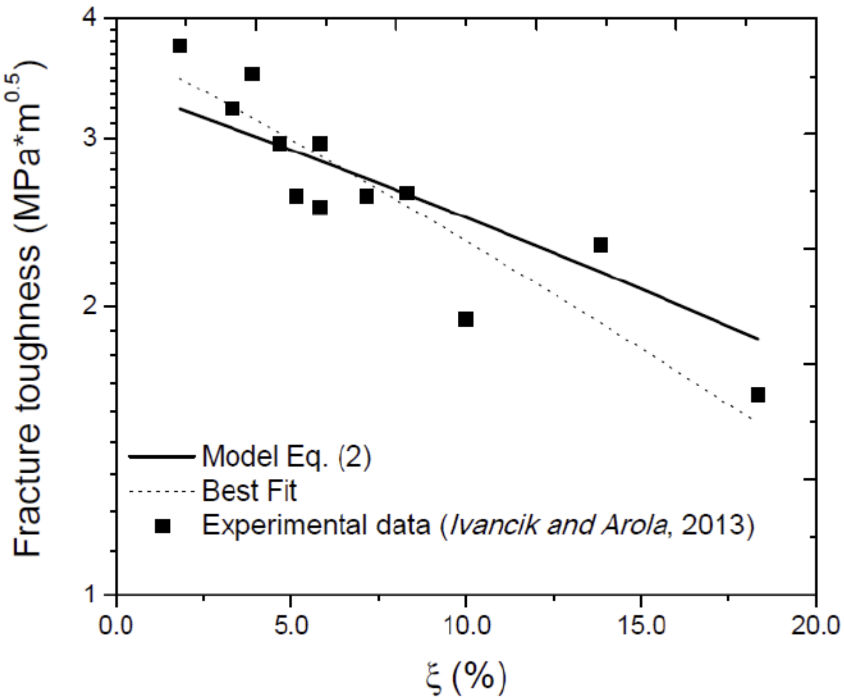


Figure 4. 3. Comparison of experimental and predicted fracture toughness of dentin as a function of the lumen area fraction (x). The experimental data corresponds to Ivancik and Arola (2013).

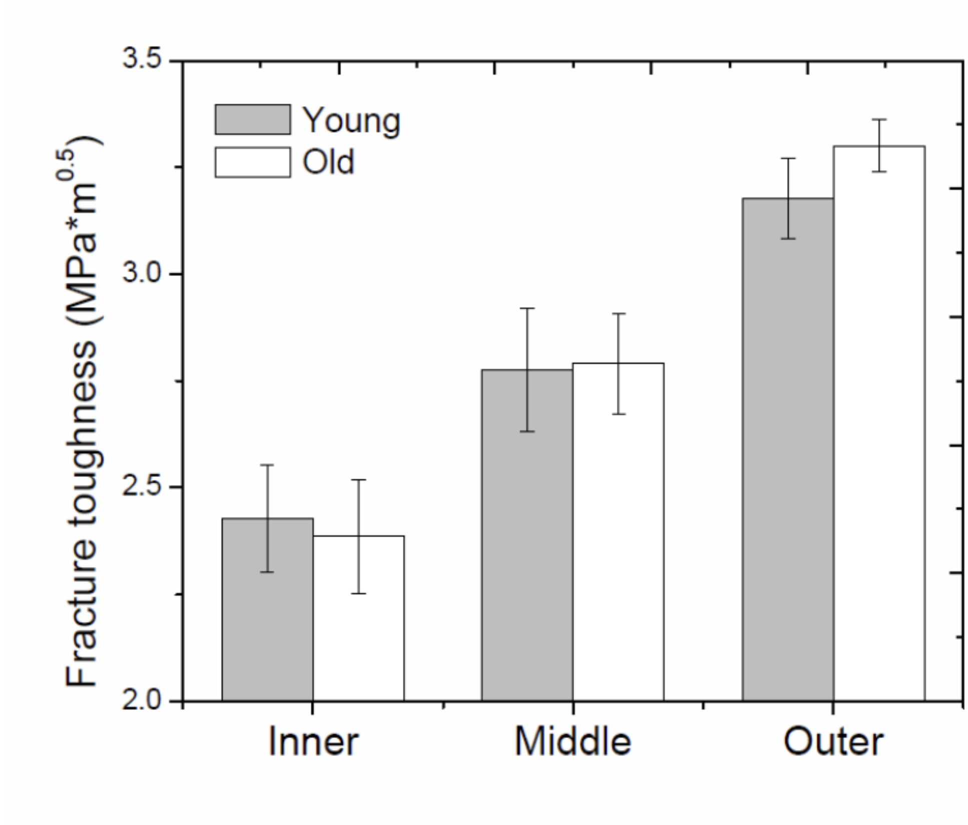


Figure 4. 4. Estimated fracture toughness for different regions of coronal dentin for the young and old donor teeth. These estimates are obtained from the lumen area fraction (x) measurements and the use of the Balshin equation (Balshin, 1949).

Chapter 5

Time dependent deformation behavior of human dentin

5. 1 Introduction

Most biological tissues exhibit viscoelastic behavior, which is considered to be important to their function. For instance, the time dependent deformation behavior of dentin potentially contributes to the distribution of loads within the tooth and the resistance to fracture (Kinney *et al*., 2003; Trengrove *et al.,* 1995; Duncanson and Korostoff, 1975).

The viscoelastic behavior of dentin and its ability to undergo time dependent deformation are considered to be important to oral functions and its capacity to resist fracture. There are spatial variations in the microstructure of dentin within the crown, which could be important to the viscous behavior. Despite previous studies for the viscoelastic properties of dentin, little is known about its time-dependent deformation and the effect of spatial variations within the crown. Understanding the time-dependent loading deformation behavior of dentin is important to comprehend the structural behavior of teeth and in the development of new dental materials with mechanical properties consistent with those of the hard tissue. Therefore, the aim of this work was to develop a simple model for the time dependent loading deformation behavior of coronal dentin, considering the spatial variations in microstructure and composition within dentin. First, an experimental study of the spherical indentation behavior of coronal dentin is presented. Based on the experimental results, a model based on previously proposed theories for indentation of time dependent materials is proposed and validated.

5. 2 Background

Several models have been proposed to determine the displacement and stress fields produced in a time dependent material under a rigid indenter (e.g. Bower *et al.,* 1993; Graham, 1965; Lee and Radok, 1960; Hunter, 1960). Bower *et al.* (1993) solved the problem of axisymmetric indentation of a half-space comprised of power-law creep of the form:

$$\dot{\varepsilon} = \dot{\varepsilon}_o \left(\frac{\sigma}{\sigma_o} \right)^n , \qquad (5.1)$$

using the similarity transformations suggested by Hill *et al*. (1989), where σ_o and $\dot{\varepsilon}_o$ are the reference stress and strain rates, and n the power law creep exponent of the material. These transformations are based on the observation that at any given instant, the velocity, strain rate and stress fields in the half-space only depend on the size of the contact a and the indentation rate $\dot{h}$, and are independent of the loading history (see Fig. 5.1). Thus, the general indentation problem is reduced to calculating stresses and displacements in a non-linear elastic solid, indented to a unit depth by a rigid flat punch of unit radius (in the axisymmetric problem). For indentation by a frictionless spherical indenter, the similarity solutions dictate that the contact radius a is related to the indentation depth h by:

$$a = c\sqrt{2Rh} , \qquad (5.2)$$

where a is the contact radius and R is the radius of the indenter. The constant c is only a function of the material constant n and may be thought of as the ratio of the true to nominal contact radius, where the nominal contact radius is $\sqrt{2Rh}$. Similarly, the applied load F is related to the indentation rate $\dot{h}$ via:

$$\frac{F}{\pi a^2 \sigma_o} = \alpha \left(\frac{\dot{h}}{a\dot{\varepsilon}_o} \right)^{1/n} = \alpha \left(\frac{\dot{a}}{\dot{\varepsilon} c^2 R} \right)^{1/n} , \qquad (5.3)$$

where the constant α is again only a function of the power-law exponent n. Values of c and α for selected values of n were deduced by Bower *et al*. (1993) from a series of finite element calculations and are listed in Table 5.1. Eqs. (5.2) and (5.3) can be written in terms of an effective stress and effective strain under the indenter. The effective stress s^{eff} under the indenter is defined as

$$\sigma^{eff} = \frac{F}{\pi a^2}, \qquad (5.4)$$

while the effective strain and strain rate under the indenter are specified as

$$\varepsilon^{eff} = c\sqrt{\frac{h}{2R}}, \qquad (5.5)$$

and

$$\dot{\varepsilon}^{eff} = \frac{\dot{a}}{2R} = \frac{c\dot{h}}{2\sqrt{2hR}}, \qquad (5.6)$$

respectively. Substituting these definitions in (5.2) and (5.3) gives the empirical results of Mulhearn and Tabor (1960):

$$\dot{\varepsilon}^{eff} = \left(\frac{\sigma^{eff}}{\alpha\sigma_o}\right)^n \frac{\dot{\varepsilon}_o c^2}{2}, \qquad (5.7)$$

for the strain rate under an indenter in a solid that undergoes power-law creep.

Dentin is a porous solid. Several models have been proposed in the literature to describe the mechanical behavior of porous and composite materials (see for instance Ji *et al*., 2006; Jones, 1975). Perhaps the most convenient approach utilizes the rule of mixtures, where the property of interest is estimated according to the sum of contributions from each individual component of the mixture. Following this approach, the effective stress supported by dentin as a function of the lumen area fraction (ξ) (i.e. porosity) can be specified as:

$$s^{eff} = s\left(1 - x\right), \qquad (5.8)$$

where σ^{eff} is the effective stress supported by dentin for a given lumen area fraction (ξ) and σ is the applied stress. With the use of this expression it is assumed that the stress applied in dentin is supported only by dentin and the lumens are considered to have null strength.

5. 3 Experimental investigation

Human third molars were obtained from selected patients with signed written consent and following all the protocols required by the Dentistry Clinic at Universidad Cooperativa de Colombia (UCC). The teeth were obtained from donors living within Medellín, Colombia, between 18 and 25 years of age, and with nearly equal number of males and females. Exclusion criteria included presence of caries and previous restorations. 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 *et al.,* 2002). Furthermore, the specimens were tested within two weeks of extraction to limit the loss of mineral and degradation of the organic materials.

The teeth were sectioned using diamond abrasive slicing equipment with continuous water coolant to obtain specimens from outer (N=30), middle (N=35) and inner (N=27) regions of dentin, which were located approximately at 0.5 mm, 2.0 mm and 3.5 mm away from the DEJ, respectively. The specimens were embedded in cold-cured epoxy resin with the sectioned surface facing outwards as shown in Figure 5.2. The exposed dentin was then polished using silicon carbide abrasive paper with successive smaller particle sizes from #600 to #1200 grit. Further polishing was then performed by means of standard red felt polishing cloth wheel and diamond particle suspensions until reaching 3 μm particle size.

Spherical indentation tests

Indentation tests were performed using a dedicated fixture with tungsten carbide sphere of 12.67 mm of diameter, allowing low depth of penetration at the low loads used. This indentation diameter allows low depth of penetration at low loads, but generates a contact area large enough so that the response of the test corresponds to the overall dentin and not only regions of peritubular or intertubular dentin, and as such the variability in microstructure with depth is limited. Also, the use of spherical indenter generates a more homogeneous stress distribution.

Loading was performed using a universal testing machine (Instron Model 3366) equipped with a 500 N load cell and accuracy of 0.1% of full scale. Indentations were made using five different loads (e.g. 1, 5, 10, 30 and 50 N) applied instantaneously (1N/s) and then held constant until reaching steady state deformation behavior (corresponding to approximately 12 h, 2 h, 1 h, 30 min and 15 min, respectively, for each load). Displacement versus time curves were recorded for the duration of the tests. Between 5 and 8 tests were performed for each load and region. The specimens were kept in the HBSS solution during testing to avoid dehydration and minimize friction between the indenter and dentin. A schematic diagram of the test is shown in Figure 5.1. The results were evaluated using a one-way ANOVA with a Tukey post-hoc analysis. Significance was defined by $p \leq 0.05$ to establish differences between the groups.

Chemical Composition Analysis

A chemical composition analysis of dentin was performed by means of Raman spectroscopy. The measurements were performed at the same locations of the indentation tests in order to find the mineral-to-collagen ratio of dentin at these specific locations. A confocal

Raman spectrometer (Horiba Jobin Yvon LabRAM HR) was used and the spectra were obtained over the spectral region of 400 to 1400 cm^{-1}. The Raman spectrometer had a laser diode with a wavelength of 785 nm, with a spot diameter of approximately 1.1 μm. The mineral-to-collagen ratio (χ) was calculated from the ratio of area under the υ_4PO_4 peak at a wavelength of 589 cm^{-1}, which is associated with the phosphate bending of hydroxyapatite, and the area under the amide III peak at 1254 cm^{-1}, which is associated with the collagen amide III band (Kazanci *et al.,* 2006; Goodyear *et al*., 2009).

Microstructural Analysis

Previous to each indentation test the specimens were used to evaluate the dentin microstructure using optical microscopy (Axiovert 40 MAT, Carl Zeiss Microscopy, NY). The tubule density and diameter were determined from measures obtained at seven unique locations with an approximate window size of 80 μm x 100 μm. In each image, the number of tubules was calculated and expressed as tubules/mm^2. The values from the seven images were averaged and used to determinate the tubule density for each dentin region.

5. 4 Results

Spherical indentation tests

Figure 5. 3 shows the evolution of indentation depth (*h*) within inner dentin as a function of time for selected applied loads. The curves displayed an initial "instantaneous" elastic def

ormation followed by a decreasing indentation rate (primary or transient creep regime). Thereafter, the response exhibited an approximately constant indentation rate corresponding to the steady-state creep regime. A difference in the elastic deformation was obtained for all indentation loads and in different regions of dentin. The higher values of elastic deformation were found at inner dentin with a decrease as approaching the DEJ. Approximate values of 25 μm were found for middle dentin using an indentation load of 1 N and 125 μm using an indentation load of 50 N. It is worth mentioning that the maximum depth reached from the tests was of around 500μm, allowing to consider all the tests as being performed on a "half-space", without any substrate effect underneath the indentation.

The indentation rate ($\dot{h}$) was extracted from the steady-state region of each test performed. The distribution in the indentation rate as a function of the load is shown in Figure 5.4. There was a significant increase in the indentation rate with load for the three different regions evaluated ($p \leq 0.05$). Furthermore, the indentation rates were higher for inner dentin in comparison to results for the middle and outer dentin.

At each of the three depths evaluated the results for indentation creep rate exhibited a power law response that could be described in the form:

$$\dot{h} = \dot{h}_o \left(\frac{F}{F_o} \right)^{n_o}, \qquad (5.9)$$

where $\dot{h}_o$ is a reference indentation rate, F_o is a reference load, F is the applied indentation load and n_o is the power law exponent for dentin. The power law exponent was determined to be constant ($n_o=1.44$) for all three depths evaluated as estimated through least-squares error evaluation. This response shows that dentin exhibit a creep behavior in which deformation occurs at constant loads for long periods of time; therefore, eqs. 5.2 to 5.6 and the model proposed by Bower *et al.* (1993) can be used to describe the mechanical behavior of dentin.

Results for the effective stress versus effective strain rate calculated from the indentation test results and obtained using equations (5.4) and (5.6) are presented in Figure 5.5. These results follow a power law behavior with a creep rate exponent of $n=3.38$. The individual values of the parameters found using equation (5.7) are listed in Table 5.2. A strong correlation was found between effective stress and effective strain rate for all regions of dentin ($r^2=0.90$ for inner, $r^2=0.94$ for middle and $r^2=0.89$ for outer dentin). The values of σ_o where found to be constant (σ_o = 0.5 MPa) and not dependent on the region of dentin analyzed. On the other hand, the reference strain rate $\dot{\varepsilon}_o$ was found to exhibit a strong exponential dependence on the mineral to collagen ratio (χ), and an increase with proximity to the pulp (Figure 5.6), which corresponds to decreasing mineral content.

A comparison of the values for effective strain rate obtained over the range of applied indentation loads and for the different regions of dentin analyzed is shown in Figure 5.7. An increase in effective strain rate occurred with increase in indentation load within each of the three regions. Furthermore, there was a decrease in effective strain rate from inner to outer dentin regions, regardless of the indentation load. Significant differences ($p \leq 0.05$) were observed with increasing distance for most indentation loads as shown in Figure 5.7.

Chemical Composition Analysis

The mineral-to-collagen ratio (χ) of dentin is shown as a function of the normalized distance from the pulp to the DEJ (ranging from 0 to 1) in Figure 5.8. There is an approximate linear increase in mineral-to-collagen ratio approaching the DEJ. A higher mineral to collagen ratio indicates a higher proportion of inorganic material.

Microstructural Analysis

Micrographs detailing the microstructure of outer (Fig. 4.1a), middle (Fig. 4.1c), and inner (Fig. 4.1e) dentin are shown for a representative donor tooth in Figure 4.1. A highly mineralized peritubular cuff is evident surrounding each dentinal tubule, which is most evident in Figures 4.1(a) and 4.1(c). As clear from comparison of the micrographs, there is an increase in tubule density and diameter of dentinal tubules as the distance from the DEJ increases. The average density ranged from ~25,000 tubules/mm^2 within the outer dentin to ~35,000 tubules/mm^2 within the inner dentin. Similarly, the average diameter of the tubules ranged from ~1.36 μm at the outer dentin to ~1.81 μm at the inner dentin. Based on these measurements there was a significant increase in tubule density and tubule diameter with increasing distance from the DEJ ($p \leq 0.05$).

The results obtained for tubule density and diameter in the three regions of evaluation were used to calculate the lumen area fraction (ξ), which is defined by the ratio between the area occupied by lumens and the total area of dentin measured. The average lumen area fraction corresponding to the outer and inner regions was 3.68 ± 0.63% and 9.28 ± 1.04%, respectively. Based on the statistical analysis, there was significant increase ($p \leq 0.05$) in the lumen area fraction as the distance from the DEJ increased.

5. 5 Time dependent deformation model for dentin

A time dependent model is proposed here to capture the steady-state deformation behavior of dentin in response to monotonic loads. The model is motivated by the following experimental observations:

- The spherical indentation response of dentin was adequately described by a power law model, following the formulation of Bower *et al.* (1993).

- The deformation response within dentin was found to be a function of the distance from the pulp to DEJ. Furthermore, this response was found to be affected by spatial variations in the chemical composition (i.e. mineral to collagen ratio) and geometrical factors (i.e. lumen area fraction).

The total strain rate $\dot{\varepsilon}$ of dentin is written as the sum of the elastic $\dot{\varepsilon}^e$ and time dependent (viscous) strain rates $\dot{\varepsilon}^v$. Thus, for an arbitrary loading history, the total strain rate is described by:

$$\dot{\varepsilon} = \dot{\varepsilon}^e + \dot{\varepsilon}^v, \qquad (5.10)$$

The elastic response of dentin to an applied load is described by

$$\dot{\varepsilon}^e = \frac{\dot{\sigma}}{E}, \qquad (5.11)$$

where E is the Young's modulus of dentin. The time dependent response can be described using an extension of equation (5.7) as:

$$\dot{\varepsilon}^v = \dot{\varepsilon}^{eff} = \left[\frac{\sigma(1-\xi_i)}{\alpha\sigma_o}\right]^n \frac{\dot{\varepsilon}_{oi}c^2}{2}, \qquad (5.12)$$

where $\dot{\varepsilon}^{eff}$ is the effective strain rate at the normalized position δ_i, σ is the stress resulting from applied load, ξ_i is the lumen area fraction of dentin at position δ_i, n is the creep stress exponent of dentin, c and α are parameters of dentin that depend uniquely on n, $\dot{\varepsilon}_{oi}$ is a reference effective strain rate at position δ_i and σ_o is the reference stress for dentin.

The effective strain rate $\dot{\varepsilon}_{oi}$ at position δ_i is dependent on the chemical composition and can be calculated according to:

$$\dot{\varepsilon}_{oi} = \dot{\varepsilon}_o e^{-M\delta_i\varpi}, \qquad (5.13)$$

where $\dot{\varepsilon}_o$ is the reference effective strain rate of a "reference" dentin with the lowest degree of mineral content (e.g. with no mineral), ϖ is the spatial trend in chemical composition of dentin, which is described by the slope of the change of mineral-to-collagen ratio (χ) with the normalized distance from pulp to DEJ (δ), as shown in Figure 5.8; δ is the normalized position and M is the mineralization rate decay of dentin. This parameter describes the spatial variations in mineralization of dentin. The importance of M to the decay in reference strain rate is shown schematically in Figure 5.9. Note that a large value of M corresponds to a dentin with larger gradient in $\dot{\varepsilon}_o$ from the pulp to the DEJ, and vice versa.

To obtain the strain resulting from an applied stress history it is then necessary to integrate Eqs. (5.10) to (5.12) with respect to time. The model was then used to describe the time dependent behavior of dentin using the parameters listed in Table 5.2 in the three normalized positions of $\delta=0.25$ (for inner dentin), $\delta=0.55$ (for middle dentin) and $\delta=0.85$ (for outer dentin). These results for the model are added in Figure 5.5. As evident from the comparison of model and experiment, there is good agreement for all three regions evaluated.

5. 6 Approximate calibration of the model

Six parameters - ξ, n, $\dot{\varepsilon}_{oi}$, σ_o, ϖ and M - are required in the proposed model to fully describe the effective strain rate of coronal dentin at a given spatial location. A four-step approximate calibration procedure requiring a minimum of four spherical indentations and two dentin depths is described below.

Step 1: The first step is to characterize the steady-state spherical indentation behavior of dentin at a given depth δ_1. Experience indicates that it is adequate to perform two spherical indentation tests at two given constant loads (e.g.: F_1=10 N, F_2 = 40N). From those tests measure the steady-state indentation rates $\dot{h}_1$ and $\dot{h}_2$, respectively. Equation (5.9) can be used to obtain:

$$n_o = \frac{\log(\dot{h}_1 / \dot{h}_2)}{\log(F_1 / F_2)}, \quad (5.14)$$

α and c can be found from Table 5.1. Now use equations (5.4) and (5.6) to calculate $\dot{\varepsilon}_i^{eff}$ and s_i^{eff} for each of the tests performed and then find n, using:

$$n = \frac{\log\left(\dot{\varepsilon}_1^{eff} / \dot{\varepsilon}_2^{eff}\right)}{\log\left(\sigma_1^{eff} / \sigma_2^{eff}\right)}, \qquad (5.15)$$

and calculate the new values of α and c. The reference stress σ_o can be assumed constant with a value of 0.5MPa. With this, $\dot{\varepsilon}_{o1}$ can be found for depth 1 as:

$$\dot{\varepsilon}_{o1} = \dot{\varepsilon}_1^{eff} \left(\frac{\sigma_1^{eff}}{\sigma_o}\right)^{1/n}, \qquad (5.16)$$

Step 2: Repeat step 1 for a different depth to obtain $\dot{\varepsilon}_{o2}$.

Step 3: On each of the depths tested measure the lumen area fractions (ξ_1 and ξ_2) and mineral-collagen ratios (χ_1 and χ_2). The spatial trend in chemical composition ϖ can be found as:

$$v = \frac{c_2 - c_1}{d_2 - d_1}, \qquad (5.17)$$

Step 4: The mineral rate decay M can be found as:

$$M = \frac{1}{\varpi(\delta_2 - \delta_1)} \ln\left(\frac{\dot{\varepsilon}_{o2}}{\dot{\varepsilon}_{o1}}\right), \qquad (5.18)$$

Thus, a minimum of two depths with four spherical indentation tests are necessary to calibrate the model proposed. More tests and depths can be conducted to improve the statistical accuracy of the fitted parameters.

5. 7 Discussion

The creep behavior of coronal dentin was studied using spherical indentation, and the results supported the development of a model describing the time dependent response as a function of spatial variations of the dentinal tubules density (lumen area fraction) and chemical composition (mineral-to-collagen ratio). The relationship between effective stress and effective strain rate showed that dentin exhibits a non-linear creep behavior with a stress exponent of $n=3.38$, which is indicative of a non-linear viscous solid.

The power-law behavior of hard tissues has been previously reported in the literature. Figure 5.10 shows a comparison of the results obtained for the steady state creep rate for coronal dentin and published data for compact bovine bone (in uniaxial tension, Rimnac *et al.,* 1993) and radicular dentin (in uniaxial compression Jantarat *et al*., 2002). The results reported by these authors were used to calculate the parameters of the power law creep model (Eq. 5.1) shown in Table 5.3.

The creep stress exponents reported for compact bovine bone ($n=5.2$) and radicular dentin ($n=1.5$) are in general agreement with that estimated here for coronal dentin. Based on the creep exponents, coronal dentin (e.g. this study) and compact bovine bone exhibit more extensive non-linear behavior than radicular dentin. This behavior could be attributed to the creep characteristics of the type I collagen present in both materials. While type I collagen is the primary organic component of both tissues, the hydroxylysine content is higher in dentin than in bone (Kumar, 2010). Hydroxylysine content has been connected with covalent cross-linking of the collagen chains and manifests as an increase in the stiffness and strength of collagen (Cardinale, 2011). This difference in collagen conformation could explain, in part, why compact bone exhibits higher non-linear behavior than dentin. Conversely, radicular dentin displays a value of stress exponent closer to true linear viscoelastic behavior (n=1). The difference in viscous response between coronal and radicular dentin could be associated with differences in the dentinal tubule orientation relative to the applied stress. In compression of radicular dentin the tubules are oriented perpendicular to the stress direction. In addition, radicular dentin has higher tubule density and smaller lumens in comparison to those qualities of the crown (Arola *et al*., 2009), which could contribute to larger volume fraction of peritubular dentin without collagen fibrils (Xu and Wang, 2012). These qualities could be responsible for the lower degree of time dependent deformation in radicular dentin.

From the results obtained from both the model and experimental data, the inner dentin exhibited the highest effective strain rate ($\dot{\varepsilon}^{eff}$), and there was a decrease approaching the DEJ. This behavior is related to the composition and mineralization pattern during dentinogenesis. In the course of odontogenesis, odontoblasts produce primary dentin until eruption of the tooth (Schour, 1948). As soon as the tooth erupts, the formation of secondary dentin begins and continues throughout life (Goldberg *et al.,* 2011). Prior to mineralization of dentin, an extracellular organic matrix is produced (Almushayt *et al.,* 2006), making the dentin located nearest the pulp (newly produced by odontoblasts) to display the lowest degree of mineralization, leading to higher strain rates than for the subsequent dentin layers. Furthermore, the larger tubule diameters of inner dentin provide a larger volume of "free spaces" occupied by dentinal fluid during normal function of the tooth. This fluid has been reported to have a similar composition to plasma (e. g.: 90-92% water and the remaining corresponding to solids) (Driessens and Verbeeck, 1990; Chatterjea and Shinde, 2011). The water has been suggested to have a plasticizing effect in dentin, participating in its viscoelastic behavior (Jameson *et al.,* 1993; Kishen and Asundi, 2005).

Although results from the current study provide further understanding of the time dependent deformation behavior of human dentin, there are recognized limitations that should be considered. There has been a good deal of discrepancy concerning the distribution in mineral to collagen ratio of coronal dentin. Some researchers have found that the amount of mineral content increases from the pulp to the DEJ (Hayakawa *et al.,* 2000; Angker *et al.,* 2004; Clementino-Luedemann and Kunzelmann, 2006), while others have reported the opposite trend, namely a reduction in this direction (e.g. Xu *et al*., 2009; Ryou *et al*., 2011). This contradiction would not require changes to the proposed model since a higher mineral-to-collagen ratio (higher mineral content) in any position of dentin will be accounted for directly by the term $\delta_i\varpi$ in eq. (5.13). However, if the model is applied to the dentin of seniors, then the model may need adjustment to account for the changes in microstructure, as well as for the potential effects of collagen cross-linking (Ivancik *et al.,* 2012, Miura *et al.,* 2014).

It is worth noting here that the model in its present form can be applied only to coronal dentin and wherein the indentation load is applied parallel to the dentinal tubules. If the direction of the indentation load is changed, a correction factor must be considered, corresponding to the stress concentration posed by the dentinal tubules. These factors are commonly used in the study of mechanical properties of porous materials (Balshin, 1949; Boccaccini *et al*., 1996) and even to predict the fracture toughness of dentin (Chapter 4). A correction of the stress concentration factor is expected to be important if grinding or clenching problems are investigated, where shear forces are deemed to be critical.

Also of concern, it is often reported that dentinal tubules possess sigmoid ("S") shape distribution in coronal dentin, as shown schematically in Figure 5.11a (Nanci, 2008; Gómez de Ferraris and Campos Munoz, 2009). This sigmoid shape it is well known have a significant effect on the mechanical response of dentin, when a load is applied, dentinal tubules have different orientations (i.e. angles) relative to the direction of the load; making the tissue behavior even more anisotropic; this behavior was analyzed by Han *et al.* (2012) who developed a model to evaluate the mechanical and material properties of dentin tubules with various orientation angles.

However, the time dependent model proposed assumes that dentinal tubules extend straight from the pulp up to the DEJ and that the three regions of dentin analyzed (outer, middle, and inner) are homogenous and isotropic. It is then expected that the sigmoid shape might have an effect on the model results depending on the specific orientation of the tubules. For instance, if the dentinal area described by the model corresponds to straight areas of the tubules (Fig. 5.11b), the model results will be unaffected. On the other hand, if the description corresponds to wavy regions of the lumens (Fig. 5.11c), some discrepancy between model and reality could be expected.

An analysis of the error level of the model due to these geometrical factors an others well-known characteristics of dentin like the branching of dentinal tubules, change of the diameter of peritubular dentin with depth are beyond the scope of this study and is a topic for future study.

5. 8 Conclusion

The time dependent deformation behavior of coronal dentin was studied using instrumented spherical indentation. The experimental results showed that the deformation behavior followed a power law behavior, which was appropriately captured by an extension to previously proposed models for the time dependent deformation behavior of structural materials. The model accounts for the spatial variations of chemical composition (e.g. mineral to collagen ratio) and microstructure (e.g. tubule density) of coronal dentin. Regions with higher mineral to collagen ratio exhibited a stiffening effect, with reduced strain rate for indentation loads. Similarly, a reduction in tubule density also caused a decrease in the deformation behavior. The power law creep exponent for dentin was found to have a value of $n=3.38$ which was independent of the spatial variations in composition and microstructure. Furthermore, this parameter was found to be dependent on the specific composition of the collagen present in the tissue and not on its amount.

5. 9 Tables

Table 5. 1. Indentation model parameters α and c as a function of the power-law exponent n (reproduced from Bower *et al.* (1993)).

n	*α*	*c*
1.00	0.849	0.707
1.11	1.085	0.747
1.25	1.332	0.788
1.43	1.602	0.831
1.66	1.886	0.875
2.00	2.176	0.920
2.50	2.465	0.966
3.33	2.734	1.013
5.00	2.973	1.065
10.00	3.110	1.128
100.00	3.051	1.201

Table 5. 2. Dentin parameters obtained from the proposed model.

Parameter	Value
n	3.38
α	2.75
c	1.01
s_o (MPa)	0.50
$\dot{\varepsilon}_o$ (s^{-1})	0.013
M	17.02
ϖ	0.48
ξ outer (%)	3.68
ξ middle (%)	6.00
ξ middle (%)	9.28

Table 5. 3. Parameters describing the basic power-law creep behavior for different hard tissues.

Parameter	***Compact bovine bone*** (Rimnac *et al.* 1993)	***Radicular dentin*** (Jantarat *et al.* 2002)	***Coronal Dentin***
n	5.20	1.47	3.38
s_o (MPa)	0.50	0.50	0.50
$\dot{\varepsilon}_o$ (s^{-1})	4.21×10^{-15}	7.00×10^{-9}	Outer: 7.07×10^{-9} Middle: 9.63×10^{-8} Inner: 7.31×10^{-7}

5. 10 Figures

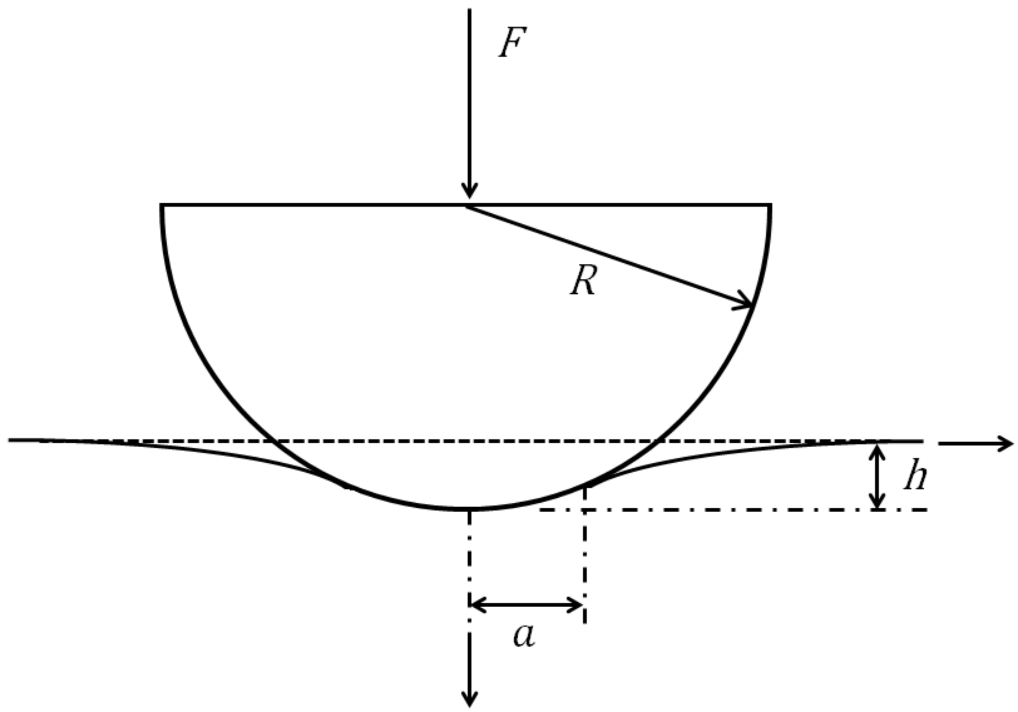


Figure 5. 1. Schematic diagram of a half-space under indentation by a rigid sphere. The variables F, R, h and a represent the indentation force, indentor radius of curvature, depth of indentation and the radius of permanent indentation, respectively.

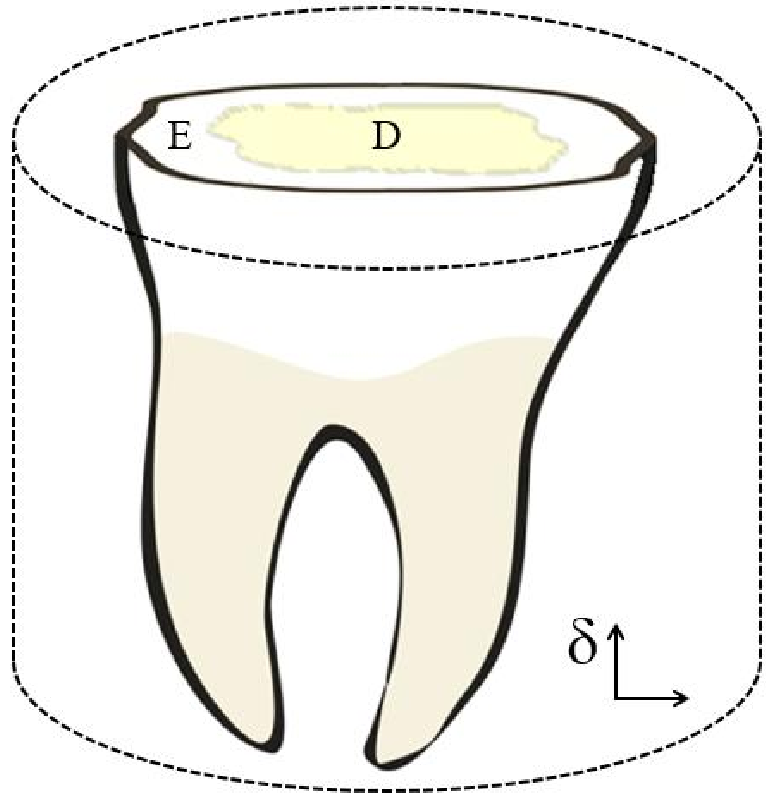


Figure 5. 2. Schematic diagram of a sectioned molar with the exposed dentin embedded in cold cured epoxy ready for the indentation test. The letters D and E refer to dentin and enamel, respectively.

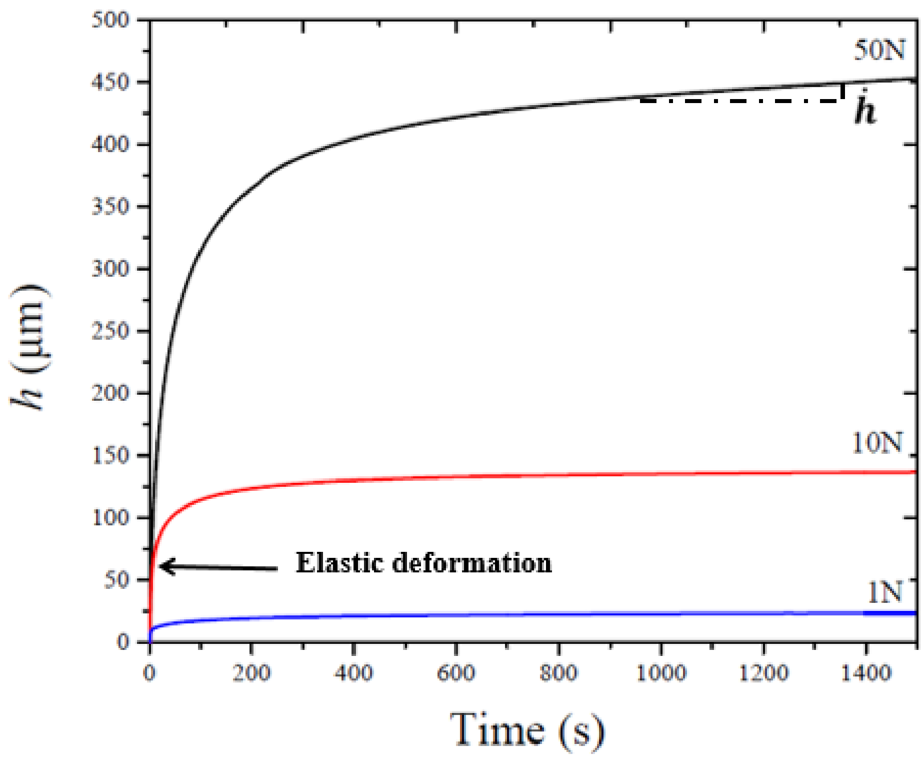


Figure 5. 3. Selected indentation depth versus time results for inner dentin at applied loads of 1, 10 and 50 Newtons.

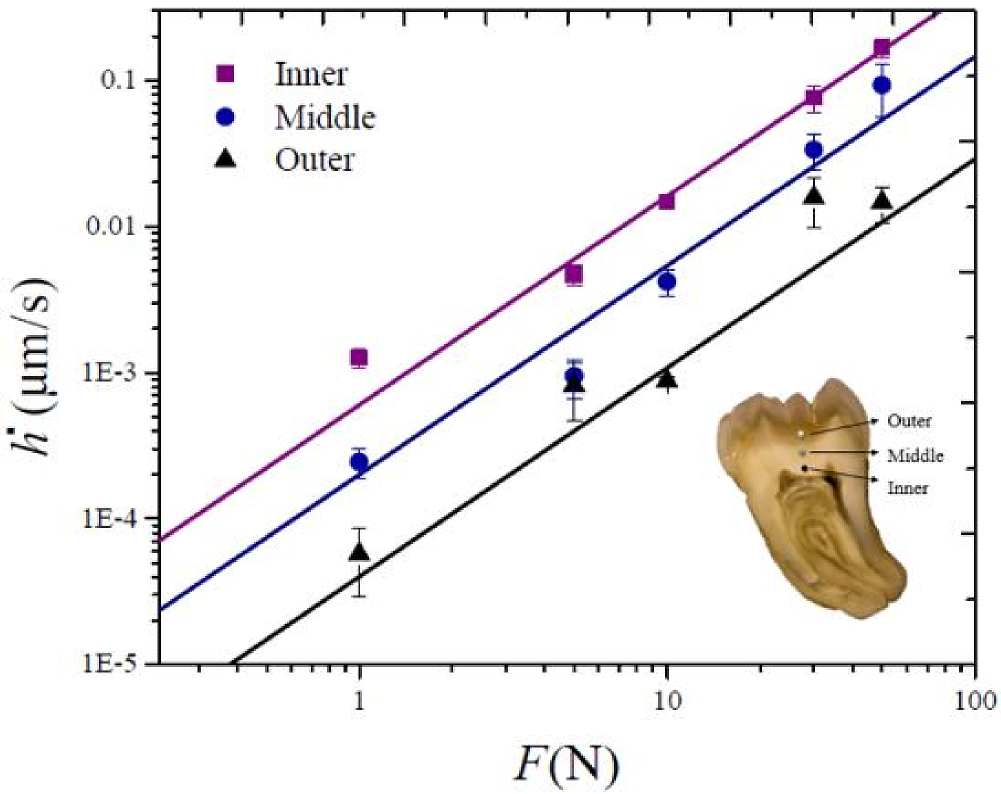


Figure 5. 4. Steady state indentation rate ($\dot{h}$) versus indentation load response for the three regions of dentin evaluated.

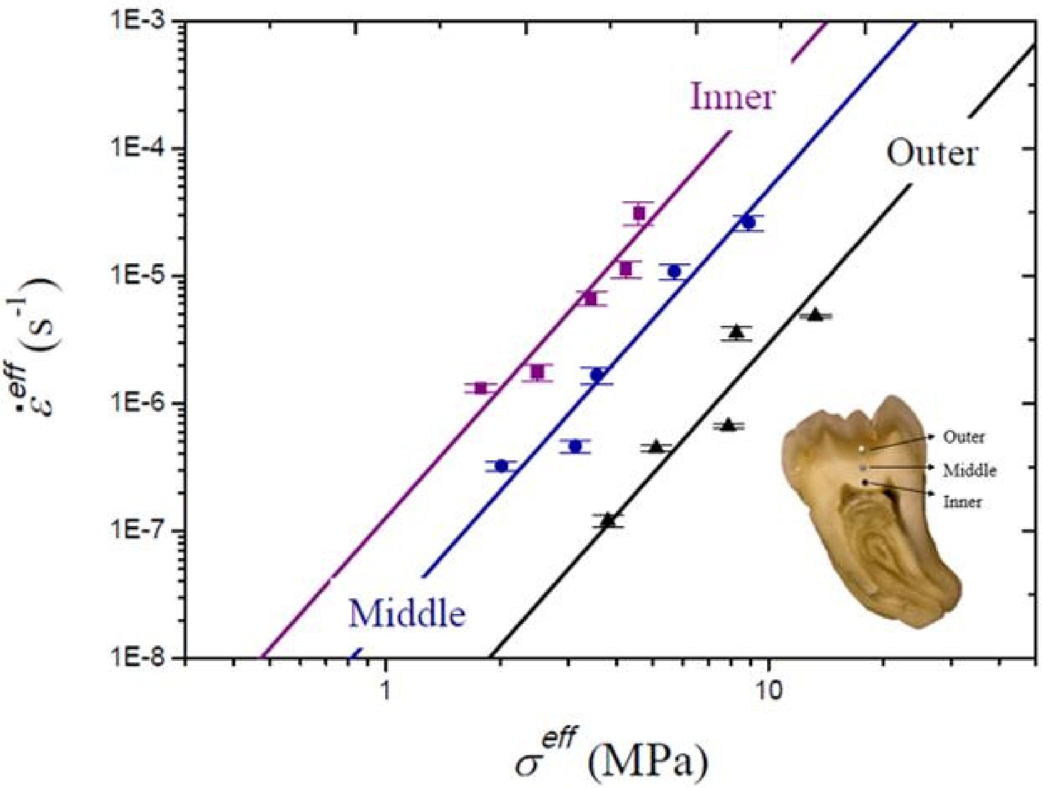


Figure 5. 5. Comparison of the experimental steady-state effective stress and effective strain rate of coronal dentin (markers) with predicted responses (lines).

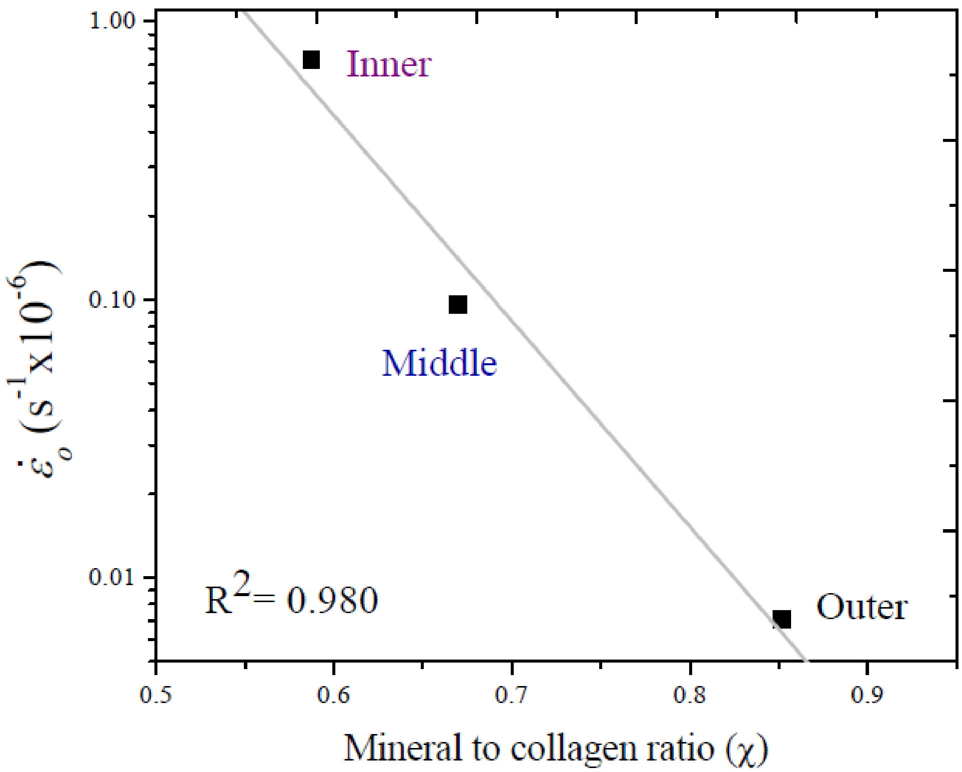


Figure 5. 6. Dependence of the reference effective strain rate ($\dot{\varepsilon}_o$) on the mineral-to collagen ratio (χ) within the three different regions of coronal dentin.

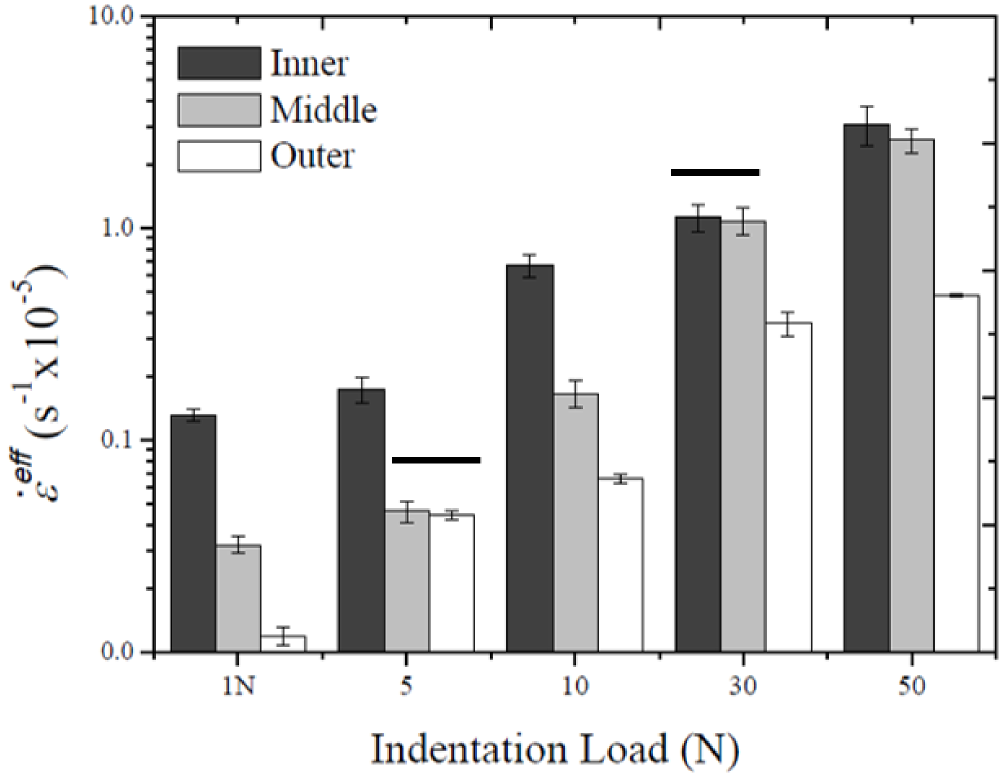


Figure 5. 7. Effect of indentation load on the effective strain rate for different regions of coronal dentin. Columns without significant differences (p >0.05) are grouped with a line.

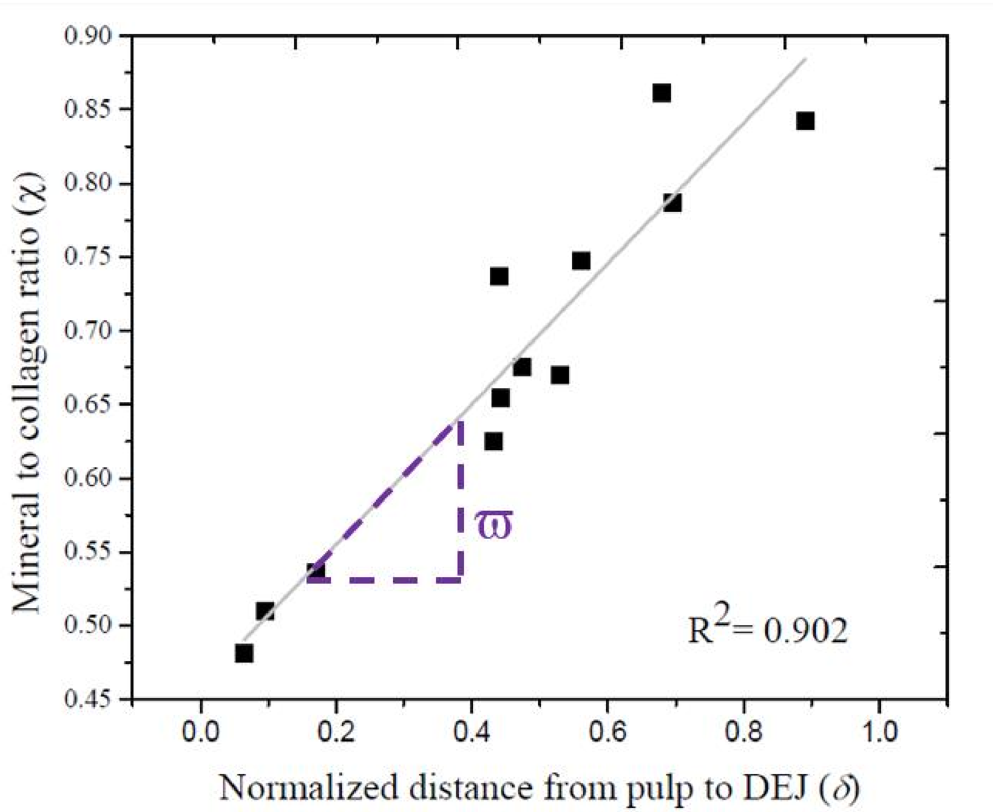


Figure 5. 8. Distribution of the mineral-to-collagen ratio of dentin according to the normalized distance from the pulp.

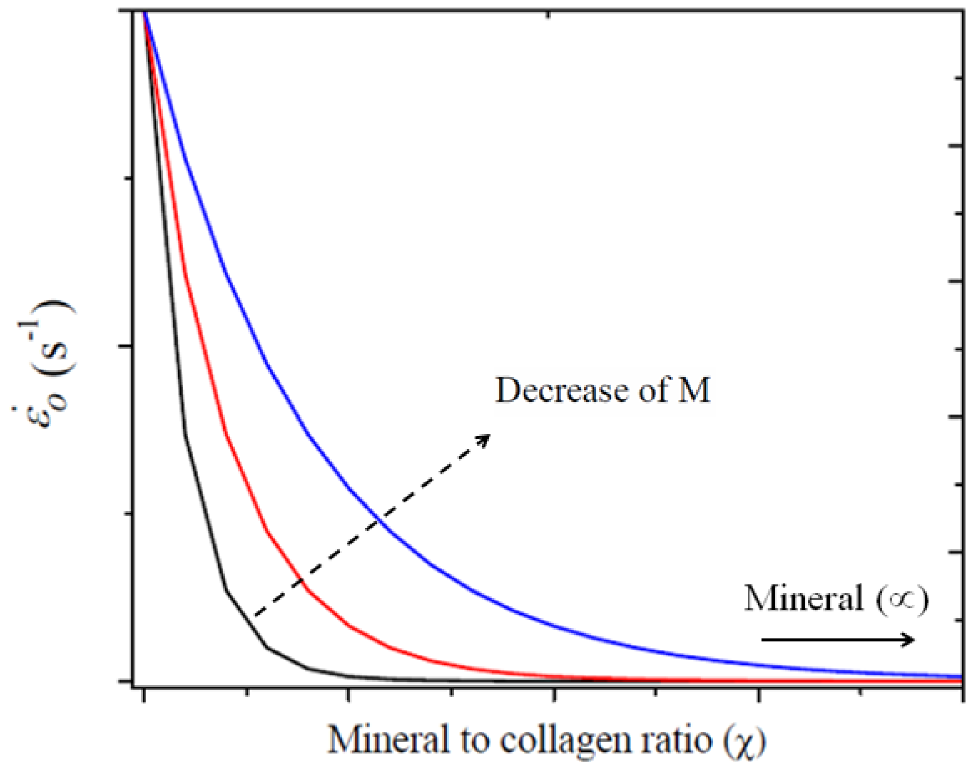


Figure 5. 9. Schematic diagram showing how changes in the degree of mineralization affect the response of the reference strain rate $\dot{\varepsilon}_o$.

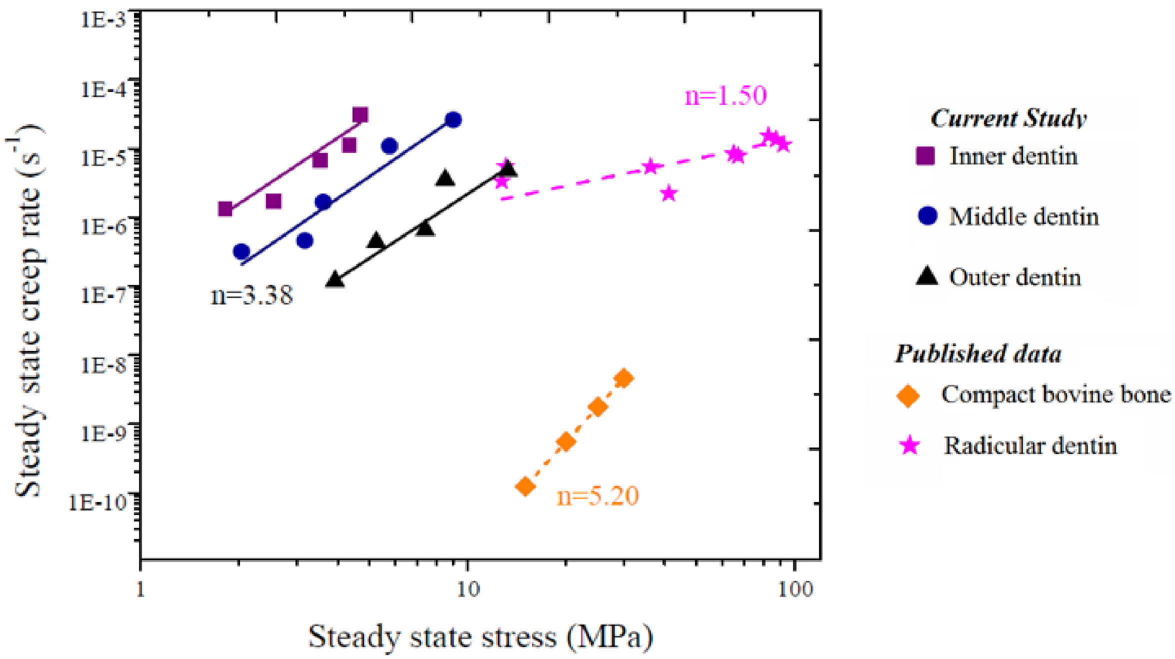


Figure 5. 10. Experimental results reported for the steady state creep rate for bone (Rimnac *et al*. 1993) and radicular dentin (Jantarat *et al*. 2002) and comparison with results of the current study.

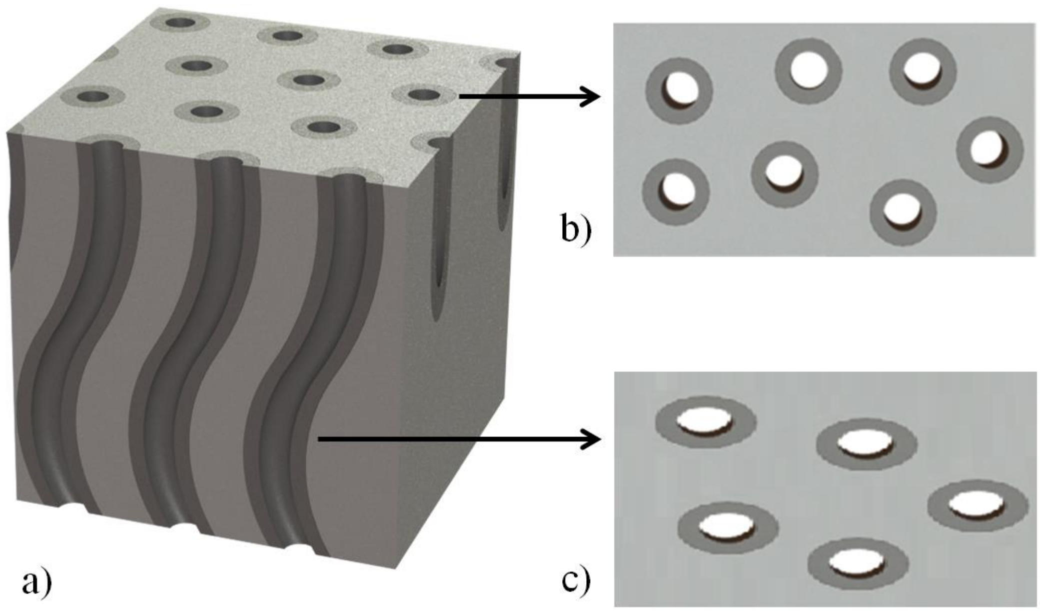


Figure 5. 11. Schematic representation of how the dentinal tubules are distributed in dentin. a) and b) top views of an indentation test on the regions indicated.

Chapter 6

Contributions of aging to the time dependent deformation of dentin

6. 1 Introduction

Previous chapters have described the mechanical response of dentin and its dependence on changes in the density and diameter of its dentinal tubules (i.e porosity), and changes in chemical composition along the tooth. Several studies have previously reported the viscoelastic properties of coronal, intertubular and peritubular dentin. However, little is known about the time-dependent behavior of aged dentin and the importance of its spatial structure and composition variations.

The time dependent deformation behavior of young coronal dentin and its spatial variations along the tooth have been analyzed in chapter 5 using instrumented spherical indentation. The experimental results indicated that dentin follows a power law deformation behavior and that the strain rate of dentin changes along its thickness depending on the chemical composition (e.g. mineral to collagen ratio) and microstructure (e.g. tubule density). From these results a model to predict the time dependent deformation of dentin was proposed and validated. Based on this model, in this chapter the spherical indentation response of aged coronal dentin was analyzed for outer, middle and inner dentin, and its time dependent deformation response was modeled in terms of its microstructure (i.e. tubules) and chemical composition. Likewise, the changes that suffer the mineral and organic materials with aging were analyzed using Raman spectroscopy.

6. 2 Experimental investigation

Sample collection and preparation

Human incisors and canines (N=40) and third molars (N= 10) were obtained from selected patients after 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 local patients (Medellín, Colombia), and were classified in two age groups named “young” and “old” with donors between 18 and 25 years of age (molars) and 54 and 85 years of age (incisors and canines), respectively. Before two weeks after extraction, teeth were sectioned and polished using the same procedure explained in section 5.3.

Spherical Indentation Tests

Indentation tests of the “old” samples group were performed using the same fixture reported in section 5.3. Indentations were made using six different loads (e.g. 1, 5, 10, 30, 50 and 60 N) applied instantaneously and then held constant until reaching a steady state deformation regime. Between 5 and 9 tests were performed for each load and dentin depth. A schematic of the test is shown in figure 5.1.

Chemical composition analysis

Sectioned and polished samples of outer, middle and inner regions of dentin from both age groups were analyzed using Raman spectroscopy in order to determinate changes in the mineral or collagen matrix with aging. Ten measurements covering the entire sample surface were made using the same equipment previously reported (Section 5.3).

The spectrums were normalized and the baseline was corrected using data processing software. Spectral decompositions and curve fitting were used in the amide I peak present in collagen (between 1590 and 1720 cm^{-1}) (Lefèvre *et al.*, 2007) and in the $\upsilon_1\upsilon_3PO_4$ bands present in hydroxyapatite (between 990 and 1100 cm^{-1}) (Farlay *et al.*, 2010). The spectrums were decomposed with mixtures of Lorentzian and Gaussian functions and the following parameters were calculated:

- Crystallinity of hydroxyapatite: Calculated as the full width at half maximum (FWHM) of υ_1 peak at 960 cm^{-1} (Xu *et al.*, 2012), associated to the symmetric stretches of the phosphate group (Wopenka *et al.*, 2008).
- Carbonate-to-phosphate ratio: Calculated as the ratio of the area under the 1070 cm^{-1} peak associated to the symmetric stretching of the CO_3 groups (B-type carbonate apatite) (de Castro *et al.*, 2014) and under the 960 cm^{-1} peak.
- Collagen cross-linking: Found as the area under the 1664 cm^{-1} peak, corresponding to the stretching vibration of the C=O bond of the amide I band of collagen (Rabotyagova, 2008) and under the 1684 cm^{-1} peak associated to type I Beta (β) turns in collagen (Gąsior-Głogowska, *et al.,* 2013).
- Mineral to collagen ratio: Calculated as the ratio of area under the υ_4PO_4 peak at a wavelength of 589 cm^{-1}, which is associated with the phosphate bending of hydroxyapatite, and the area under the amide III peak at 1254 cm^{-1}, which is associated with the collagen amide III band (Goodyear, *et al.,* 2009).

Microstructural Analysis

Samples of the "old" group were used to evaluate the dentin microstructure using optical microscopy (Axiovert 40 MAT, Carl Zeiss Microscopy, NY). Tubule density and diameter of the tubules were measured for each region of dentin, and the results were used to calculate the lumen area fraction (ξ) as the ratio between the area occupied by lumens and the total area of dentin measured.

Statistical Analysis

Differences in the results obtained for the individual tests were evaluated using a one-way ANOVA with a significance of 0.05, and a Tukey post-hoc analysis were used to locate differences between the groups (young vs. old donors).

6. 3 Experimental results

Spherical Indentation

Figure 6.1 shows the evolution of indentation depth (h) as a function of time for selected samples of the old donors group at different regions of dentin and at a constant load of 60 N for a period of 900 s. All the samples showed an initial "instantaneous" elastic deformation followed by a decreasing indentation rate (primary or transient regime). Thereafter the response exhibited an approximately constant indentation rate corresponding to the steady-state regime of deformation.

From each of the tests performed, the indentation rate ($\dot{h}$) was extracted as the slope in the steady-state region. From the results, a significant difference in the indentation rate was obtained only for some regions and some indentation loads, implying that the indentation depth does not change spatially significantly with indentation load.

Using the data obtained from the spherical indentation tests performed on aged dentin, the effective stress (σ^{eff}) and the effective strain rate ($\dot{\varepsilon}^{eff}$) were calculated using Eqs. (5.4) and (5.6), respectively to get the results shown in figure 6.2. The results show that the relation between s^{eff} and $\dot{\varepsilon}^{eff}$ of aged dentin follow an approximate linear behavior in the log-log plot that can be fitted to a power law response with a creep exponent *n=2.1*.

Figure 6.3 shows a comparison between the values of strain rate obtained for the different regions of dentin with the applied indentation loads. An increase in strain rate was found as the indentation load increases for the same region of dentin. Further, the different regions of dentin show a decrease in the strain rate from inner to outer dentin using the same indentation load. These results show significant differences in some cases (p-value ≤ 0.05). Columns without significant differences ($p \leq 0.05$) are grouped with a line. The low statistical significance of the data shows that the old dentin does not have significant changes along different regions of dentin on its time dependent behavior. These little differences between the three regions of dentin may also be seen in figure 6.2 were the data of the three different regions are close to each other.

Chemical composition analysis

The results obtained for some of the dentin characteristics using Raman Spectroscopy are showed in figure 6.4. The results obtained for the crystallinity of hydroxyapatite (FWHM of the ν_1 peak) as a function of distance across the coronal dentin for young and old donor teeth are shown in Figure 6.4a. Dentin in the young donor group showed a similar value for the three regions of study; meanwhile, for the old donor group was found an increase in the FWHM as approaching the DEJ. A higher FWHM (wider peak) indicates lower crystallinity. A decrease in crystallinity denotes that a greater proportion of crystals have less perfect lattices, with higher substitutions (Farlay *et al.*, 2010). The differences found among dentin regions in the young donors group were not statistically significant ($p>0.05$), as opposed to the old donors group where there was a statistically significant increase in the FWHM for the outer dentin when comparing with the middle and inner regions of the aged tissue. On the other hand, when analyzing the results with respect to the specific regions of dentin between young and old donors, significant differences were only found for the outer dentin between young and old donors.

Carbonate-to-phosphate ratio results for the three different regions of coronal dentin are presented in Figure 6.4b. The carbonate-to-phosphate ratio is related with the substitution of the PO_4^{3-} group by CO_3^{2-} (B-type carbonate apatite) groups (Penel *et al.,* 1998). An increase in this ratio (an increase in the substitution) is normally associated with a mineral material with increased amorphous regions (Sahar, 2009). The results obtained for the young donor group showed a similar value for the three regions of dentin with no statistically significant differences ($p>0.05$). On the other hand, for the old donor group, an increase in the carbonate-to-phosphate ratio was found from inner to outer dentin with statistically significant differences between the three different regions ($p\leq0.05$). When comparing the results between young and old donors (for specific regions), significant differences were found for the young and old outer dentin.

Figure 6.4c shows the results obtained for collagen cross-linking. An increase in collagen cross-linking has shown to be proportional to the relative amounts of the trivalent cross-links in the collagen matrix (Mandair and Morris, 2015). The fibril structure of collagen tends to form intermolecular and interfibrillar cross-links, in this process the divalent cross-links present in the structure react with another telopeptide aldehyde group to form a trivalent mature bond linking three tropocollagen molecules (Eyre and Wu, 2005; Depalle *et al.*, 2015). The results for the young donors group are not statistically significant ($p>0.05$) finding the same values for the three regions of dentin; but when comparing the collagen cross-linking between young and old patients, nearly a 29% difference was found near the pulp at inner dentin. However, the difference in the collagen cross-linking was approximately 4% at middle and 30% at outer dentin.

Figure 6.4d shows the distribution of mineral-to-collagen ratio (χ) of dentin as a function of the normalized distance from the pulp to DEJ - measured in the δ direction shown in fig. 5.2 -. For both age groups an increase in mineral-to-collagen ratio approaching the DEJ was found. A higher mineral-to-collagen ratio indicates a higher proportion of inorganic material. On the young donor group the differences found were not statistically significant along the crown. While for the old donor group, an increase in the mineral to collagen ratio is observed when compared to young dentin. This increase is more evident as approaching the DEJ (outer dentin). An increase of 15% was found for inner dentin while for the middle and inner regions an increase of ~62% was found.

When comparing the results between the young and old donor groups, significant differences were found for the outer and middle dentin when comparing specific regions.

Microstructure

The microstructure of dentin from a selected old donor incisor evaluated is shown in Figure 6.5. Representative micrographs correspond to the three regions of evaluation; outer (Fig. 6.5b), middle (Fig. 6.5c) and inner (Fig. 6.5d) dentin. For each region, a peritubular dentin cuff can be seen surrounding each dentinal tubule, being more evident this cuff in the middle and outer regions. Several obliterated tubules are evident in the images corresponding to outer and middle dentin, an example of some obliterated dentinal tubules found in outer dentin is also shown in fig. 6.5a.

From the microstructural analysis was found a reduction in the tubule density with increasing proximity to the DEJ. The average tubule density in the outer and inner regions was of 14,000 tubules/mm^2 and 29,800 tubules/mm^2, respectively. As for the diameter of dentinal tubules, an average of 1.84 ± 0.18 µm was found for inner dentin and 1.16 ± 0.17 µm for outer dentin. For both characteristics, the differences obtained along different regions of dentin were statistically significant ($p \leq 0.05$). From these results the lumen area fraction (ξ) at each depth was calculated. The average ξ for outer and inner regions being of 1.15±0.30% and 7.82±1.00%, respectively ($p \leq 0.05$).

6. 4 Discussion

The time dependent deformation behavior of aged coronal dentin was studied using spherical indentation. The experimental results obtained for the indentation depth versus time were used to calculate the steady-state strain rate of dentin in three different regions - inner, middle and outer dentin-. The relationship between effective stress and effective strain rate showed that old dentin exhibits a non-linear behavior with a creep rate exponent of $n=2.1$ and was found to be independent of the region of dentin analyzed, finding similar results for inner, middle and outer regions. The creep exponent n governs the non-linear behavior of a material with a creeping solid behavior and can range from $n=1$, associated with a linear viscous solid, to $n=\propto$ representing a perfectly viscous solid.

According to the model proposed in chapter 5 (section 5.5), the time dependent loading behavior of dentin can be described using different tissue parameters depending not only on the creep exponent of the tissue, but on its chemical composition and microstructural features. With the results obtained for the microstructure and chemical composition of aged dentin, the model parameters (Eqs. 5.12 and 5.13) were calculated and the results are listed in table 6.1. These parameters were then used to describe the time dependent behavior of aged dentin in the normalized positions $\delta=0.25$ (for inner dentin), $\delta=0.55$ (for middle dentin) and $\delta=0.85$ (for outer dentin). The results are also shown in figure 6.2 (solid lines). From the results, there can be seen a good agreement between experimental results and model predictions for the inner and outer dentin, finding $r^2 = 0.967$ for inner and 0.950 for outer dentin, while for middle dentin the r^2 was 0.761.

In order to determinate how the aging process affects the time dependent behavior of dentin, the results obtained for the old dentin donors were compared with those obtained previously for young dentin, as shown in figure 6.6. From these results, a decrease in $\dot{\varepsilon}^{eff}$ - for the same σ^{eff} - can be seen for the inner and middle dentin. While for outer dentin an increase in $\dot{\varepsilon}^{eff}$ was observed for the old donors group. A decrease on $\dot{\varepsilon}^{eff}$ is related with a lower ability to undergo time-dependent deformation of the tissue. This behavior for the inner and middle dentin can be attributed not only to the change in tubule density due to obliteration of dentinal tubules, but to the changes in chemical composition - increase in mineral content as shown in fig. 6.4d - and organization and interaction between the mineral and organic phases. While the results obtained for outer dentin are contrary to what would be expected for this region, where it is well known that the aging process of coronal dentin begins (obliteration of dentinal tubules begins near the DEJ and progress towards the pulp) (Drusini *et al.,* 1991); this pattern of aging will imply a higher change in the viscoelastic properties of outer dentin with aging, which in this case was not found.

The most obvious change observed when comparing the time dependent deformation of young and old donors' dentin is the change in the creep rate exponent (n). The creep rate exponent for young dentin has been previously reported to have a value of *3.38*, while the value obtained for the old donor group was found to be of *2.1*. Previous studies on the time dependent deformation of young dentin argued that the viscoelastic behavior of dentin is mainly attributed to the creep characteristics of the collagen type I present in the tissue (Kinney *et al.,* 2003; Chuang *et al.,* 2015; Bertassoni *et al.*, 2015). Therefore, this decrease in the creep exponent with aging implies that the collagen of the tissue is suffering significant changes in its deformation capacity over time. The change in collagen characteristics can be seen in Figure 6.4c, where the collagen crosslinking for different regions of dentin are shown. Although no statistically significant differences were found between different regions of dentin, an increase in crosslinking is observed when comparing young and old dentin groups. Moreover, an increase in the amount of mineral material (increase in mineral-to-collagen ratio showed in figure 6.4d) may also have an effect on the deformation capability of collagen when subjected to stresses. Since the collagen present in intertubular dentin form a three-dimensional network reinforced by apatite crystals (Bertassoni *et al.,* 2012), an increase in the mineral material or more imperfect crystals (as showed in fig. 6.4a and 6.4b with a decrease in crystallinity and an increase in the carbonate-to-phosphate ratio respectively) could have an effect on the interaction between the two phases and a change in the mobility of the softer organic phase.

From the results obtained in the chemical composition analysis was found an increase with aging in the Carbonate-to-phosphate ratio, product of the filling of dentinal tubules with carbonated apatite (fig. 6.4b), being more significant at outer dentin where the obliteration was higher. This change has a direct effect on the crystallinity and perfection of the HA lattice which is in agreement with the results reported in fig. 6.4a. A decrease in crystallinity and an increase in the Carbonate-to-phosphate ratio have been linked to a detriment in mechanical properties such as hardness and elastic modulus in other hard tissues (i.e. enamel) (Xu *et al.,* 2012); which could explain in part the low change in the time dependent behavior of outer dentin. However, the results obtained for the decrease in crystallinity of dentin by Raman spectroscopy could be the result of two phenomena reported in literature that are contrary to each other. For intertubular dentin a decrease in the crystal width of the hydroxyapatite has been reported (which increases crystallinity), while on the other hand the dentinal tubules are occluded by larger crystals when comparing to normal dentin (decreases crystallinity); this phenomenon was reported by Porter *et al*. (2005) who argued that smaller crystals on intertubular dentin could be the result of a dissolution process of the mineral from intertubular dentin, which is then deposited into the lumens resulting in the obliteration process. This dissolution and re-precipitation of minerals in the peritubular cuff can reduce the poroelastic processes related to water movement in dentin, as the precipitated particles can fill the transverse secondary tubules and restrict water and dentinal fluid movement, which can have an impact on the deformation capability of the tissue.

In this study the differences found in the time dependent behavior between young and old dentin specimens is not limited to age, some differences can be attributed to the intrinsic differences between a molar tooth (young samples) and incisors and canines (old samples) and the use to which the samples have been subjected. In the case of third molars (young donors group) these samples can be regarded as "new" teeth that have not been subjected to mastication stresses. On the other hand, the incisors and canines used for testing the elder donor's groups are samples that can be considered as "used", with a possible cumulative damage generated not only by mastication but by brushing (Addy, 2005) that can produce enamel wear (Liu *et al.,* 2014). These changes can be denominated "dental erosion" which is a non-carious deterioration process that can include: abrasion, demastication, attrition, abfraction, resorption and erosion (Imfeld, 1996). Dental erosion does not only affect enamel, but can also affect dentin, causing hypersensitivity, or in extreme cases, pulp exposure and even tooth fracture (Addy *et al.*, 1987). The surface of erosive lesions is hypo-mineralized and its incidence can increase by consuming soft drinks, sugar and bleaching procedures; and although it can be found at any age, in the elderly is a normal feature. Some previous studies have reported that aging teeth tend to develop more dental erosion on its occlusal and incisal surfaces. However, these studies also suggest that more evidence is required to ensure that age is a determining factor in dental erosion (Bartlett and Dugmore, 2008). Since the indentation tests performed in this study were superficial and the indentation depth was only of a few microns, if the tested tissue has a significant dentinal erosion which penetrates dentin, it seems normal to think that this dentin will display higher indentation depths and strain rates. Likewise, differences would also be expected between the two groups of samples (incisors/canines and molars) in terms of the orientation of the incremental lines -that show the deposition pattern of secondary dentin-, collagen fibril (Arola *et al.*, 2009), and between tubule density, diameter of dentinal tubules and chemical composition. The differences between old molars, incisors and canines in terms of microstructural characteristics are presented in figure 6.7. The results correspond to the distribution of their lumen area fraction (ξ) across the three regions of aged coronal dentin. Overall, there was a significant decrease in ξ with proximity to the DEJ for both groups ($p \leq 0.05$). In the molar group the average ξ is higher when comparing with the incisors and canines group. Values of ξ for outer dentin of 2.86% and 1.15% were found for molars and for the canines and incisors group, respectively.

The mechanisms contributing to the changes in the time dependent deformation of dentin and its spatial changes with aging are not clearly understood and there is no consensus on how each mechanism affect the mechanical behavior of the tissue, which one is the primary mechanism or if there is a balance between them. Decrease in crystallinity and increase in the carbonate-to-phosphate ratio could promote deformation of dentin. While increase in mineral content, obliteration of dentinal tubules with sclerotic dentin with lower crystal size and collagen cross-linking have the opposite effect. Further work is necessary to isolate the mechanisms responsible for the changes on the mechanical behavior of dentin with aging and in order to understand what happen with these properties between the ages of 30 and 50 years, where there is little available information.

6. 5 Conclusions

According to the results obtained, the following conclusions can be drawn:

1. The time dependent deformation and chemical composition of aged dentin was evaluated in outer, middle and inner coronal dentin. The experimental results showed to follow a power law deformation behavior with a decrease in the stress exponent when comparing with young dentin.
2. Significant changes in the mineral-to-collagen ratio (increase), crystallinity (decrease) and carbonate to phosphate ratio (increase) were found with increasing donor age. Most of these changes were found to be more significant in the middle and outer dentin.
3. A model previously proposed to predict the time dependent deformation response of dentin was employed to model the creep behavior of aged dentin. The model showed a strong correlation between the experimental and the predicted results. A decrease in the time-dependent behavior of middle and inner aged dentin was found when compared with young dentin.

6.6 Tables

Table 6. 1. Dentin parameters obtained to describe the time dependent behavior of aged dentin.

Parameter	Value
n	2.10
α	2.22
c	0.93
s_o (MPa)	0.50
$\dot{\varepsilon}_o$ (s^{-1})	8.0×10^{-7}
M	1.1
ϖ	0.99

6. 7 Figures

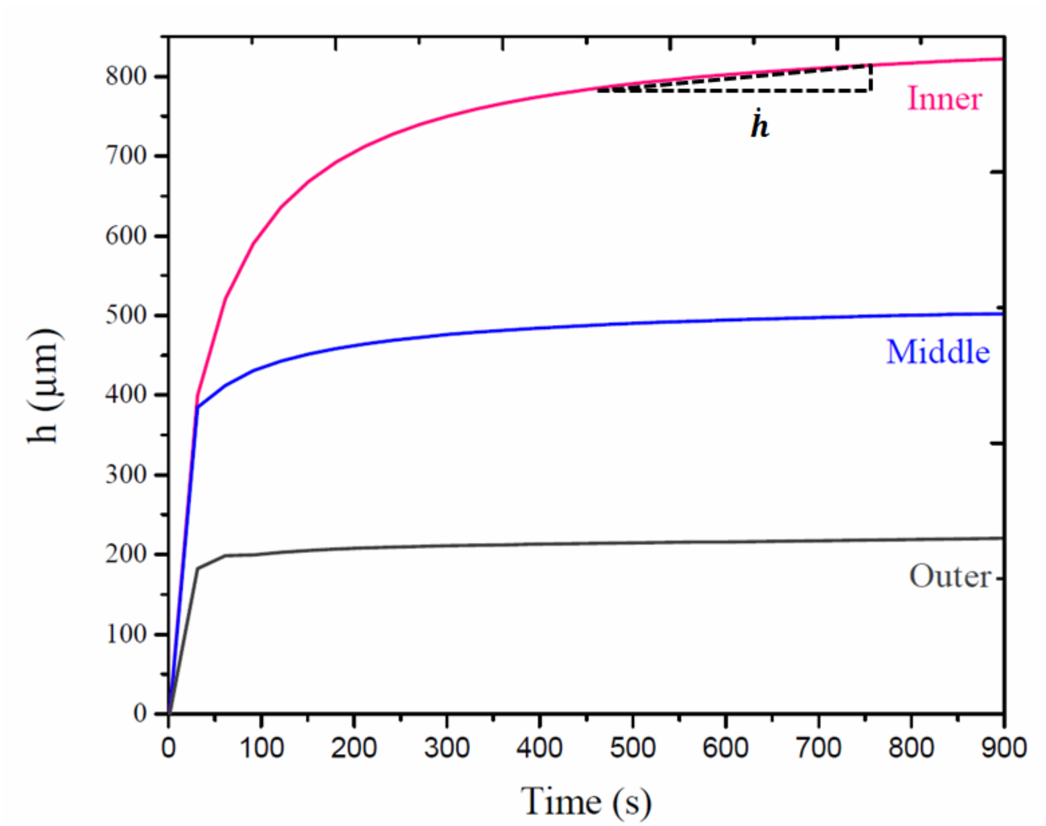


Figure 6. 1. Selected indentation depth versus time results for different regions of dentin at a constant applied load of 60N. The results correspond from an incisor of a 70-year-old donor.

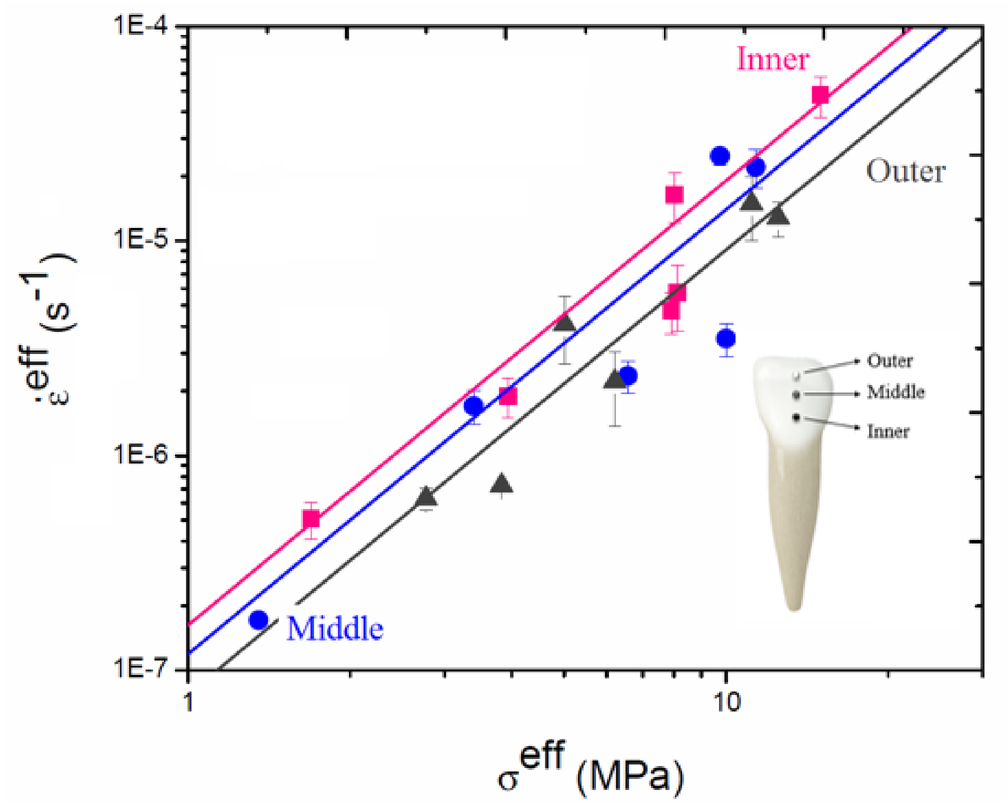


Figure 6. 2. Comparison of the experimental steady-state effective stress and effective strain rate of coronal aged dentin (markers) with predicted responses (lines).

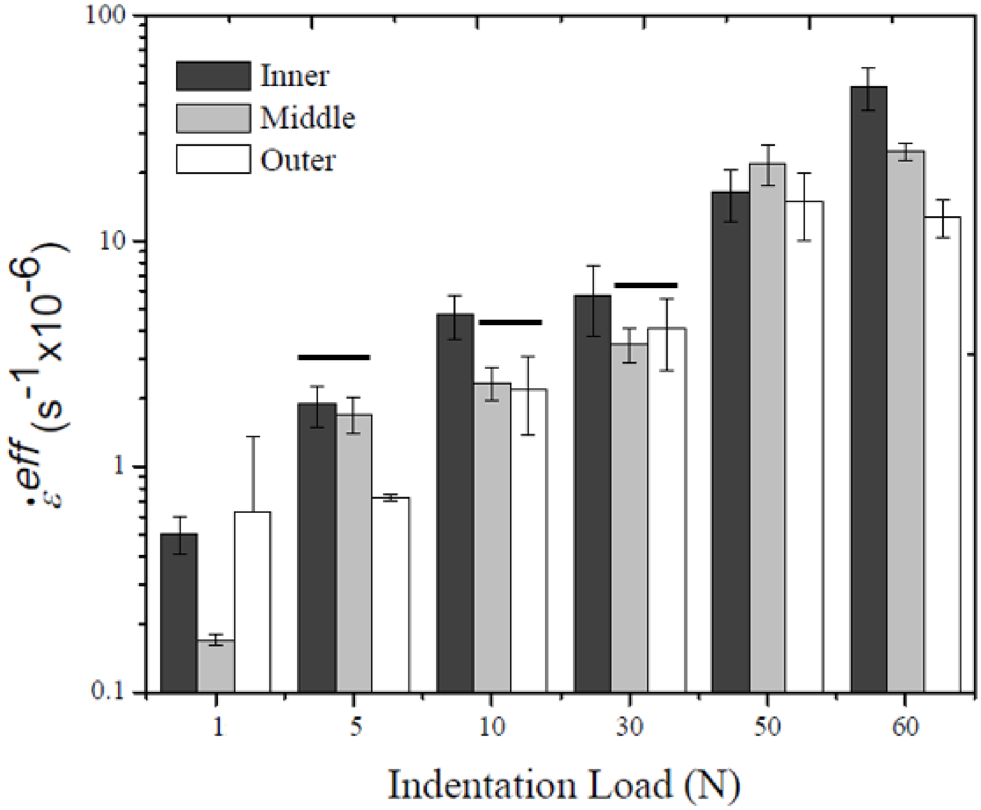


Figure 6. 3. Effect of indentation load on the effective strain rate of different regions of coronal dentin. Columns without significant differences ($p \leq 0.05$) are grouped with a line.

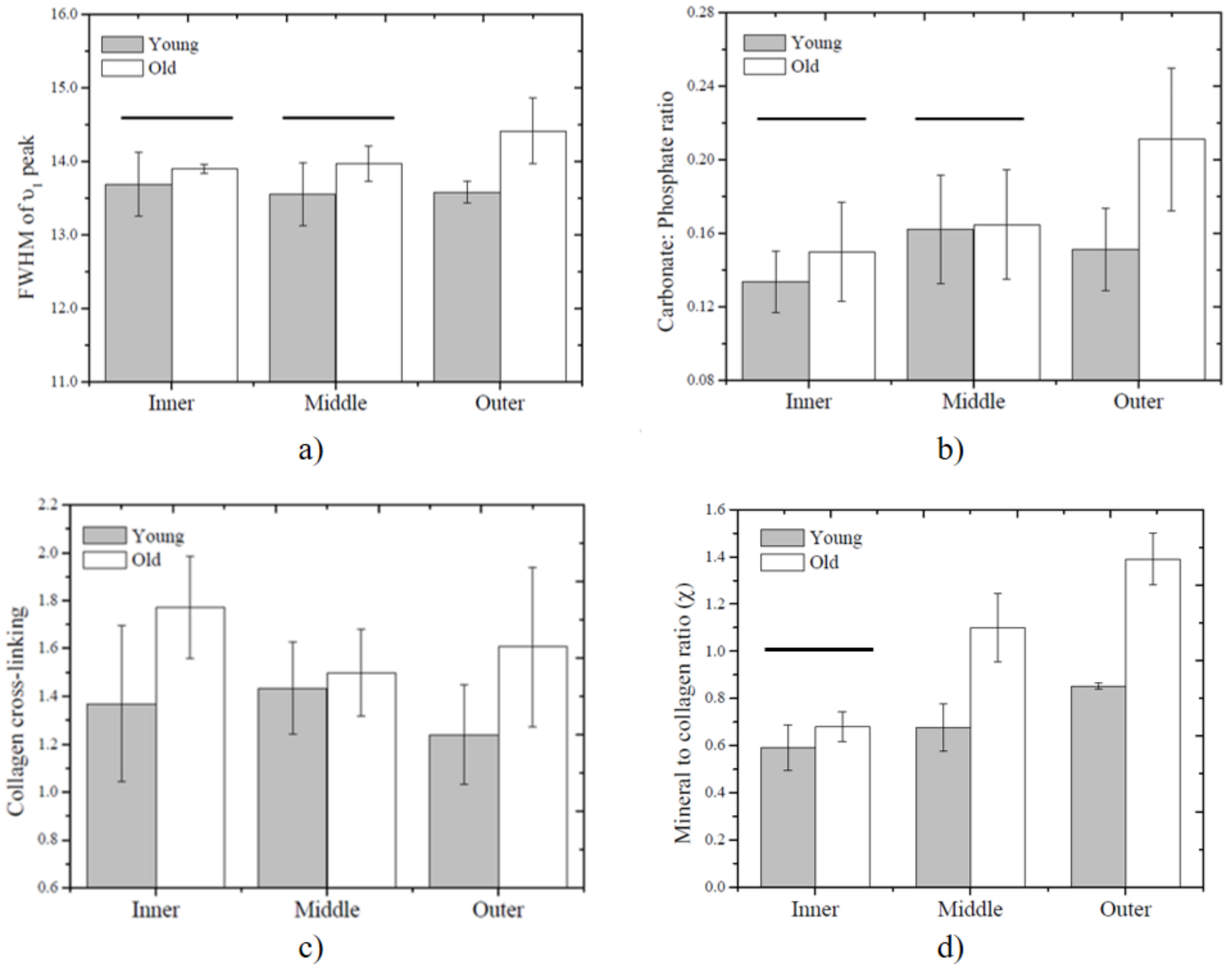


Figure 6. 4. Comparison of some dentin characteristics obtained using Raman Spectroscopy as a function of depth in the coronal dentin. a) Full width at half maximum (FWHM) of the ν_1 peak; b) Carbonate-to-phosphate ratio; c) Collagen cross-linking; d) Mineral-to-collagen ratio. Columns without significant differences are grouped with a line.

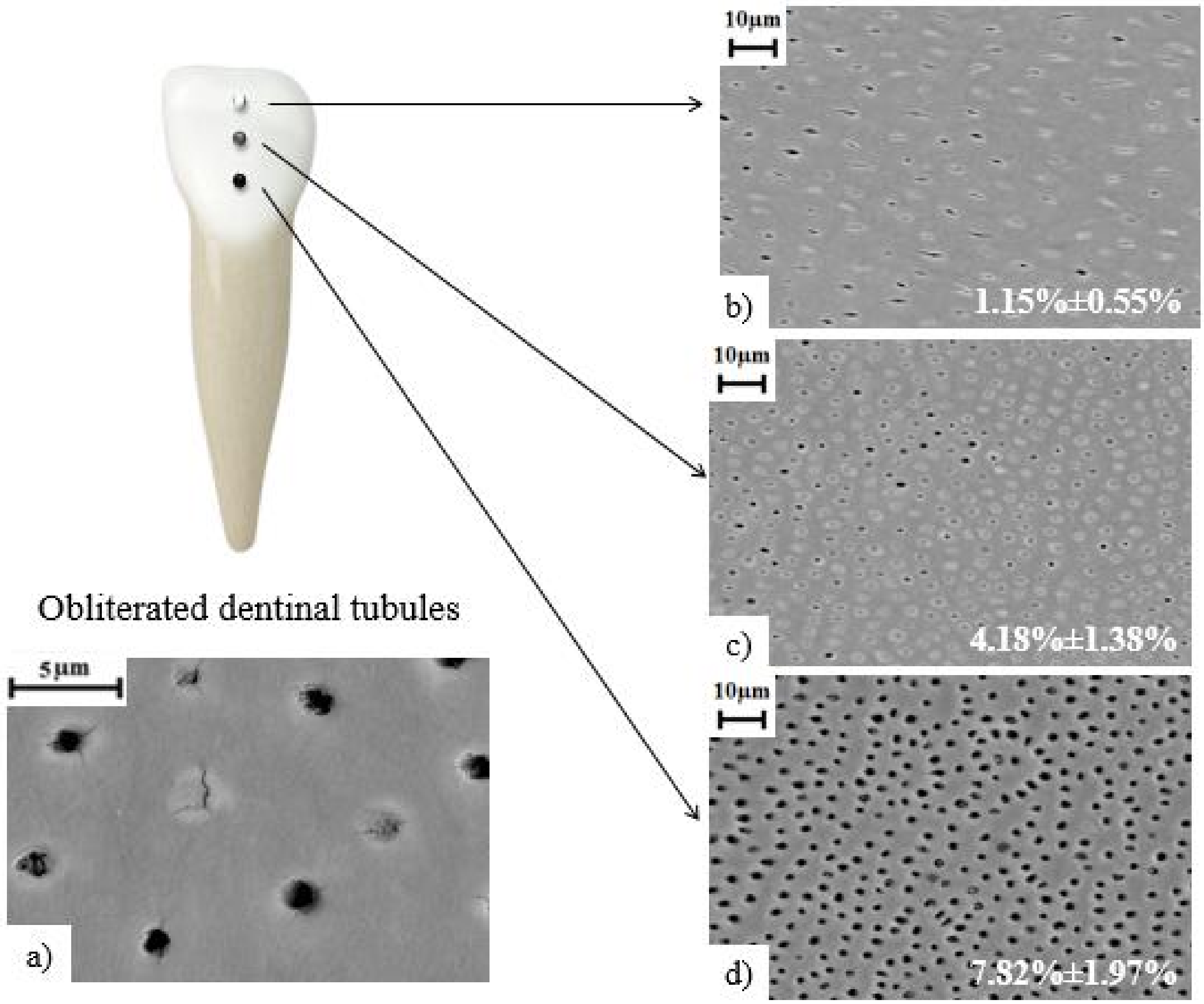


Figure 6. 5. Micrographs of the dentin microstructure. a) Obliterated dentinal tubules; b) Outer dentin; c) Middle dentin; Inner dentin. Note the obliterated dentinal tubules for the middle and outer regions. The percentages correspond to the fraction lumen area obtained for each region of dentin.

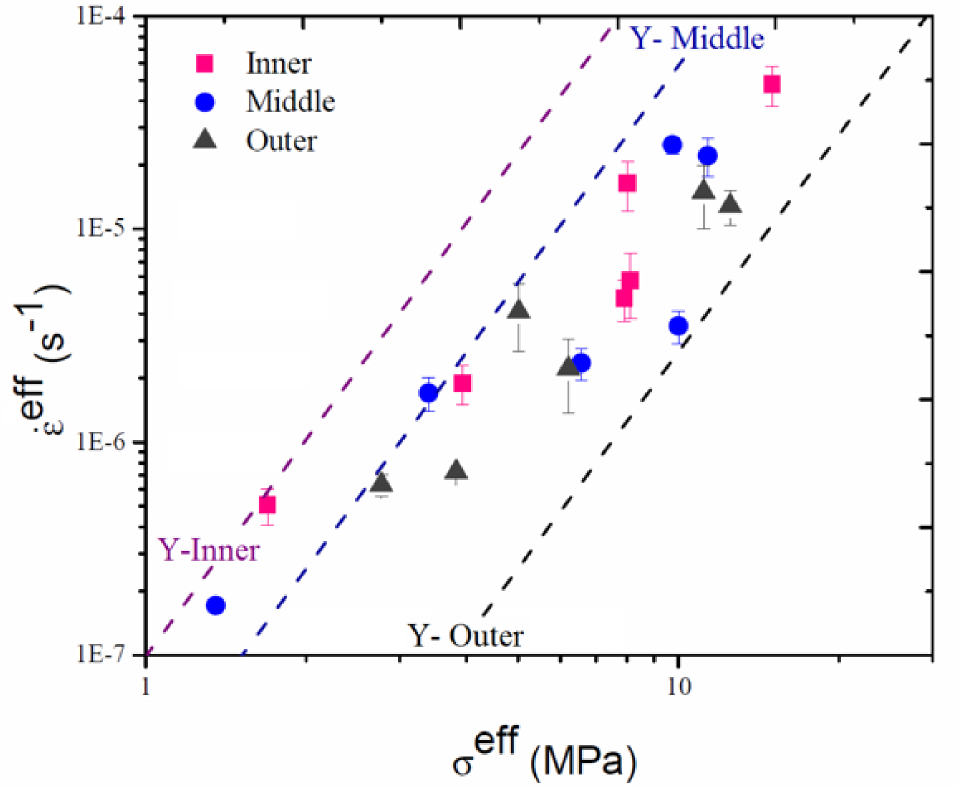


Figure 6. 6. Comparison of the effective stress and effective strain rate of young (lines) and old (markers) dentin.

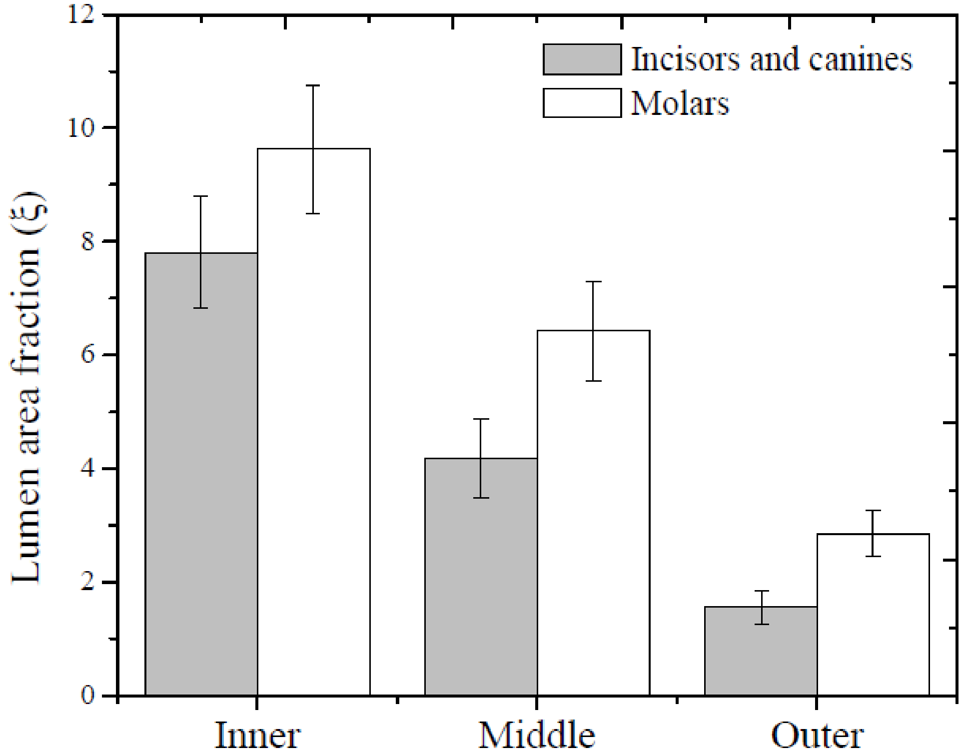


Figure 6. 7. Comparison of fraction lumen area (ξ) of old incisors and canines and molars.

Chapter 7

Ethnic Background influence on the aging process of dentin: Preliminary Results

7. 1 Introduction

Although the spatial variations in microstructure of dentin were well-recognized and extensively studied in chapter 3 for dentin from Colombian donors. Some authors have found significant differences between ages and sexes, when comparing results obtained for some dental features (Kumagai *et al.*, 2012). For example, some authors have suggested that a relation between ethnicity and race and some dental characteristics, such as tooth size (Merz *et al.,* 1991; Altherr *et al.*, 2007; Bishara *et al.*, 1989), enamel thickness (Hall *et al*., 2007) and tooth formation rate (Olze *et al.*, 2007) might exist. For instance, Merz *et al.* (1991) found that dental arches in black patients are significantly wider and deeper than those from white patients; similar results were found by Altherr *et al.* (2007), who reported larger teeth for black male patients in comparison with white donor samples. On the other hand, possible differences between tooth dimensions of permanent teeth in three ethnic groups: Egyptians, Mexicans, and the North Americans were analyzed by Bishara *et al.* (1989), finding not only significant differences between the three populations in tooth dimensions but between sexes, with males having larger canines and first molars than women. Regarding the dimensions of dental tissues; Hall *et al.* (2007) found thicker enamel on the distal aspect of a black donor tooth when comparing with white donors, this thicker enamel explained in some cases wider teeth, but not in all cases.

Most previous research on dentin has been made mostly from American donors. However, previous results reported on the chemical composition and microstructure of Colombian dentin has shown different results not only for the chemical composition but for some microstructural characteristics. For example, the results showed in Chapter 3 for the mineral-to-collagen ratio of Colombian dentin showed an increase in the mineral material as one approaches the DEJ, however the differences reported in this study along the crown were not statistically significant. Nevertheless, Ryou *et al.* (2011) found for samples of American (US) donors a lower amount of mineral material near DEJ when comparing with the regions near the pulp, and its differences were statically significant. Also, some microstructural differences have been found regarding tubule density. For instance, Ivancik *et al*. (2014) found significant differences in the tubule lumen diameters between the dentin of two groups from US and Colombia; while the US samples showed a significant change in the diameter of the lumens from the pulp to the DEJ, the average lumen diameter of dentin from the Colombian donors did not change significantly.

Despite of this, only a few studies on dentin mechanical behavior and how it might be affected by ethnicity have been carried out. For instance, Ivancik *et al*. (2014) found that the fatigue resistance properties of dentin from American donors are dependent on the location (varies from pulp to the DEJ), while Colombian dentin showed a homogeneous behavior without significant spatial variations. On the other hand, a comparison of the rate of crack growth between American and Chinese donors found that the coronal dentin of molars obtained from Chinese donors was larger than that of US patients. However, the microstructural analyses showed no significant differences in the average lumen diameter for the two groups (Bajaj *et al*., 2008). These differences have been explained not only by the ethnic backgrounds of the patient but by individual aspects as diet, water quality (fluoride content) and hygiene habits (Nikiforuk, 1970; Robinson *et al.,* 2004; Vieira *et al.*, 2005).

It is well known that the aging process of coronal dentin begins in the outer regions of the crown - near the DEJ - and its microstructural changes lead to a reduction in the fatigue strength and fracture toughness of dentin. Further, as the information comparing both microstructure and mechanical properties of dentin donors with different ethnic backgrounds is scarce, this chapter aims to compare the microstructure, chemical composition and mechanical properties (i.e. time dependent behavior) of outer dentin from residents of South America (Colombia), North America (USA) and Asia (China) in order to explore the potential importance of ethnic background and their dental hard tissues specific characteristics.

7. 2 Experimental investigation

Human dental pieces were obtained from selected patients after written consent and following all the protocols required and approved by the IRB of the Dentistry Clinic at Universidad Cooperativa de Colombia (UCC) located in Medellin (Colombia), University of Washington School of Dentistry located in Seattle (United States) and in the Tianjin Stomatology Hospital of Nankai University located in Tianjin, (China). Exclusion criteria included presence of caries and previous restorations.

The teeth were divided into three groups, named “Colombia” (N=20), “US” (N=21) and “China” (N=19) depending on the place of origin of the donor. Samples were in an age range between 18 and 85 years and there were almost an equal number of male and female samples in each group. The samples were also classified according to their age and named as: young (<30 years), middle age (30- 50 years) and old samples (> 50 years).

Immediately after extraction, all the specimens were prepared and tested according to the procedure explained in section 5.3 for indentation tests, microstructural and chemical composition analysis.

7. 3 Experimental results

Microstructure

The microstructure of dentin from selected samples of each age group is shown in Figure 7.1. The microstructures shown for each group correspond to outer dentin from China (Fig 7.1a, 7.1b and 7.1c), Colombia (Fig 7.1d, 7.1e, and 7.1f) and United States (US) (Fig 7.1g, 7.1h and 7.1i). As evident from the figures, a peritubular dentin ring can be seen surrounding each dentinal tubule regardless of donor age. When comparing the samples according to age, dentinal tubules in the young samples seem to be open, while as for the old samples a considerable number of obliterated dentinal tubules can be seen.

A quantitative comparison of the microstructural characteristics of dentin and how it changes according to age is shown in Figures 7.2. The tubule density in the three origin groups and ages can be seen in Figure 7.2a, where a reduction in tubule density can be observed with increasing donor age in all three origin regions. For the young donor's groups, the average tubule density was 22,000 tubules/mm^2 without significant differences between the three origins analyzed ($p>0.05$). While for the old group, an average of 14,000 tubules/mm^2 was found, with a significant difference in the US group; finding an average of 9,500 tubules/mm^2.

A comparison of the average tubule diameter for the three age groups is shown in Figure 7.2b. In the young donor group, the average lumen diameter was 1.34 ± 0.02 µm, with very few differences between the three groups of origin. While for the old group an average of 1.23 ± 0.02 µm was found. For the middle-age group an average diameter of 1.22 ± 0.09 µm was found but with a higher variation, which shows that during this age there is a transition in the tissue with some partially opened and closed tubules. Overall, there is a reduction in tubule density and diameter of dentinal tubules for the three regions of origin analyzed with increasing donor age ($p \leq 0.05$). A comparison of the results according to their origin shows that the differences for tubule density and diameter of dentinal tubules for the young samples were not statistically significant ($p>0.05$), while for the old samples a lower diameter of dentinal tubules was found for the US group ($p \leq 0.05$).

Spherical Indentation Tests

Figure 7.3 shows the evolution of indentation depth (h) in dentin as a function of time for selected applied loads of an old sample from China. The results of all the groups analyzed showed an initial "instantaneous" elastic deformation followed by a decreasing indentation rate (transient creep regime). Thereafter, the response exhibited an approximately constant indentation rate corresponding to the steady-state creep regime. The indentation rate ($\dot{h}$) was extracted from the steady-state region of each test performed. From the experiments were obtained the effective stress (s^{eff}) and the effective strain rate ($\dot{\varepsilon}^{eff}$) by using Eqs. (5.4) and (5.6), respectively to get the results shown in figure 7.4. The results obtained for all the groups followed a power law behavior. The individual power-law exponents obtained for each group are listed in Table 7.1. The results correspond only to the old and young samples given the difficulty to find enough samples to build the middle-age group response.

From the results can be seen that the young dentin showed a similar power-law exponent regardless of the donor origin, finding an average value of *3.11*. On the other hand, a reduction in the power-law exponent was found for the old group when comparing with the young donors for all origins analyzed. When comparing the reduction between old and young donors according to their origin, a reduction of almost of 50% was found for the China and US group, while for the Colombia group a reduction of only 27% was found.

Chemical Composition

Figure 7.5 shows the results obtained for the mineral-to-collagen ratio from young and old donor teeth for the three origin regions analyzed. An increase in the mineral-to-collagen ratio was found when comparing the young and old donors' groups and independent of the region of origin. A higher mineral-to-collagen ratio is related with a more mineralized tissue (i.e. higher mineral content). This increase in the mineral content with aging is related with the obliteration of dentinal tubules due to the deposition of secondary dentin. An increase of around 55% was found for the Colombia and China groups in comparison with young tissues, while for the US group the mineral-to-collagen ratio is twice higher in the old donors than in the young samples.

When comparing the results for young samples according to their region of origin, the US and Colombia samples showed to be statistically equal ($p>0.05$), while the China samples have a statistically higher mineral-to-collagen ratio when comparing with the other two regions (~40% higher mineral-to- Collagen ratio). For the old donor's samples, the China and US samples are statistically equal ($p>0.05$); meanwhile the Colombian samples are statistically lower.

7. 4 Discussion

Only a few studies have been developed aiming at understand the effect of ethnic differences on the microstructure and mechanical properties of dentin. In this study, the microstructure, chemical composition and viscoelastic properties of outer dentin of three different regions (China, Colombia and United States) were studied.

The microstructure for the young samples (<30 years) were within the ranges previously reported for tubule density and diameter of dentinal tubules regardless of the origin of the donor (Pashley, 1989, 2015; Ivancik *et al.,* 2014). Average values of 22,000 tubules/mm^2 and 1.33 μm were found for the tubule density and diameter of dentinal tubules, respectively. Surprisingly, although the microstructure characteristics were consistent between the three origin groups in the young samples, for the old samples (>50 years) a decrease in tubule density was found, but this decrease is more evident for the US group. For the Colombian and Chinese groups a decrease of approximately 30% in tubule density compared to the group of young donors was found, while for the US donors a decrease of almost 50% was found. In the case of the diameter of dentinal tubules, a similar decrease (~7.0%) was found for the three origin regions when comparing with the young samples.

A decrease in the microstructural parameters was also found for the middle-age group. However, as can be seen in Figure 7.2b, the data corresponding to the diameters of the dentinal tubules show a high standard deviation. This variation may be related to the fact that a transition occurs in the microstructure in that age range (30-50 years) in which there is a variety of partially open tubules and with different diameters. This decrease in tubule density and in the diameter of dentinal tubules for the old and middle-age groups is related with the progressive deposition of secondary dentin since the tooth enters into occlusion and continues throughout life; this dentin is deposited in dentinal tubules decreasing its diameter progressively and in the long term obliterating it completely (Gómez de Ferraris and Campos Munoz, 2009).

In order to identify how significant is the change in tubule density taking place with aging, with respect to the amount of initial dentinal tubules (young donors), the proportion of obliterated dentinal tubules was calculated for each sample according to:

$$Obliterated\ dentinal\ tubules\ (\%) = \frac{Nr.Obliterated\ tubules}{Nr.tubules} * 100, \qquad (7.1)$$

Measured estimates for the proportion of obliterated dentinal tubules for each sample were evaluated and shown in Figure 7.6. As expected, the proportion of obliterated dentinal tubules in the young donor's groups is approximately zero in all three origin regions until about 30 years of age, where an increase in the number of obliterated dentinal tubules can be seen. In the Chinese group, a maximum proportion of obliterated dentinal tubules of 47% was found in a 53-year-old donor. While for the US, the maximum value obtained was 41% in a 47-year-old (although the oldest sample is of 83 years), and in the Colombian samples the maximum obliteration found was 9% in a 47-year-old sample. The analysis of this results and how fast obliteration of dentinal tubules occurs, draw attention to two aspects: The increase on the proportion of obliterated dentinal tubules with age is higher in the China and US groups when comparing with the Colombian samples. On the other hand, the obliteration of dentinal tubules does not seem to increase proportionately with age, as some samples of the middle-age group have higher obliteration than samples of almost 90 years; this result may imply that the obliteration of dentinal tubules (aging) could not only depend on the donor age but on some characteristics and customs of the donor.

The viscoelastic behavior of outer -young and old- dentin was studied using spherical indentation, the results obtained in terms of indentation depth and time were used to calculate the effective stresses (s^{eff}) (Eq. 5.4) and strain rates ($\dot{\varepsilon}^{eff}$) (Eq. 5.6); using the solution previously proposed for the spherical indentation of a creeping solid material (Mulhearn and Tabor, 1960) The relationship between effective stress and effective strain rate presented in figure 7.4 showed that all the groups of dentin analyzed exhibited a non-linear creep behavior, which is indicative of a non-linear viscous solid. The results obtained in table 7.1 showed that the young samples for all the origins analyzed have a similar stress exponent, with an average value of *3.11.* This stress exponent is similar to the stress exponents reported on earlier studies on other hard tissues like bone (Rimnac *et al.*, 1993), where a stress exponent of 5.2 was found for compact bovine bone using uniaxial tensile test. However, Bowman *et al.* (1994) analyzed the creep behavior of cortical bone using compressive tests, from the results the creep curve seems to be independent of the apparent density of the tissue and the steady-state creep rate vs. applied normalized stress were fit to a power-law with an exponent of 17.65, an exponent considerably higher than the one found for the dentin in this study. These differences in the stress exponent between studies could be a result of the magnitude of the loads used during the tests. Fondrk *et al.* (1988) analyzed the creep behavior of cortical bone using tensile test; from his results two different regimes of creep behavior were found, a regime associated with lower stresses and a regime associated with higher stresses, where higher stress exponents were found. The limit between these two regimes was found to be near 73 MPa for human bone and 117 MPa for bovine bone, which correspond to 360 and 580 N, for their test configurations respectively (Fondrk *et al.*, 1988). During mastication, the forces to which the tooth is subjected can vary depending on the type of tooth and food, but can be near 100N for molars, 40 for premolars and 50 N for incisors (Bates *et al.,* 1976); enamel absorbs most of these loads due to its higher stiffness and only a low proportion of these reach dentin; whereby the dentin during normal function never will be above the higher stress creep regime. However, the studies reported on the creep behavior of hard tissues seem to agree that organic content (i.e. collagen) and its characteristics are responsible for the time-dependent deformation behavior of the tissue (Bowman *et al.*, 1999; Deymier-Black *et al.,* 2012; Shen *et al.,* 2011).

A comparison of the stress exponent obtained for old donors shows a decrease for all the regions of origin. Since the creep behavior of dentin depends on the collagen characteristics, this decrease with aging could be attributed to a lower formability of collagen due possibly to an increase in collagen crosslinking (Bentley, 1979; Walters and Eyre, 1983), and dehydration (Toto *et al*., 1971; Wang *et al.,* 2012). Additionally, the obliteration of dentinal tubules found in the microstructural analysis may have an effect on the mobility of dentinal fluid within the dentin and therefore limiting the movement of the organic phase.

The reduction in the power- law exponent with aging was found to be different in each origin group. The most significant decreases were in the Chinese and US groups, where the stress exponent of the old groups correspond to almost half the value found for young samples. These results are in agreement with those found for the microstructural analysis and the proportion of obliterated dentinal tubules, where the samples of these two regions (China and US) have the highest number of obliterated tubules. As mentioned earlier, a lower proportion of open tubules can limit the mobility of dentinal fluid and therefore its ability to plasticize the collagen (Nalla *et al*., 2005). The results obtained for the mineral-to-collagen ratio seem to support the decrease in the stress exponent in the US old samples, since the mineral-to-collagen ratio found in the old samples group doubles the value of the young donors samples. For the Chinese samples, although the increase in the mineral-to-collagen ratio is less significant (55%), the young samples of this group were found to be more mineralized in comparison with samples of the other two regions. Furthermore, a reduction of only 27% was found in the stress exponent of the Colombian samples with aging, which is reflected in the low proportion of obliterated dentinal tubules found in the old samples.

After the analysis of these results a question remain: ¿Does the origin of the donor (or location where aging occurs) have an effect on the mineralization degree during the development of dentin or its aging process?

From the results obtained in the chemical composition analysis of the China young samples, a significant higher mineral-to-collagen ratio was found when comparing with the other two regions. These results agree with those reported by Bajaj *et al.* (2008) regarding the fatigue crack growth response of US and China dentin. In this study the rate of crack growth of the China samples were 100 times greater than that in the dentin of US donors. Likewise, the apparent stress intensity threshold of the China samples was lower than the US.

The results showed that some of the characteristics of dentin and how the aging process take place (and its effect on the mechanical properties) could be related to the ethnic characteristics of the donor. The ethnic characteristics of a person, should not to be confused with its race. Ethnicity involves ways of being, norms, manners, and rituals; while the race is a biological feature that typically is described in relation to physical descriptions such as skin color or facial features (Williams and Wilson, 2001). The effect of ethnicity on the chemical composition, mechanical properties and aging of hard tissues has been previously reported. The most studied has been the bone tissue, where differences in bone mineral density according not only to age but ethnicity have been reported. For example, Looker *et al.* (1998) reported that Black-Americans had a higher bone mineral density in the femoral neck and hip compared to White-American men; while Marshall *et al.* (2008) analyzed osteoporotic fractures of elder donors, finding that Black-American and East Asian-American men reported higher bone strength compared to White-American men due to greater volumetric bone mineral density. Other results regarding the possible effect of ethnicity on the properties and aging of bone can be found in the literature (Nelson *et al.*, 2011; Travison *et al.*, 2008).

According to these results, it is possible to think that as dentin and bone are two hard tissues with similar characteristics, ethnicity will also be an important factor in determining some of the characteristics of the dental tissue. Although it is well known that the aging process of dentin (increase in mineral content) occurs differently to bone (decrease in mineral content), it has been reported that the aging process depends largely on individual characteristics determinated by gender, genetic, medication status, dietary intakes, oral health and water quality (Rosen *et al.* 2009).

The identification of differences in the microstructure of the aged dentin (i.e. proportion of obliterated dentinal tubules) between the three regions analyzed raises questions pertaining to the principal cause that could be related with differences in the water quality between the three regions, nutritional status of the donor and oral cleaning habits. However, one of the main limitations of this study is that in the samples used were not taken into account individual characteristics of the donor besides age and gender.

7. 5 Conclusions

According to the results obtained, the following conclusions were drawn:

1. A reduction in the tubule density was observed with increasing donor age for the three regions of origin analyzed: China, Colombia and United States. For the young samples the average tubule density was 22,000 tubules/mm^2 ($p>0.05$). While for the old group an average of 14,000 tubules/mm^2 was found, with significant difference in the US group (9,500 tubules/mm^2).
2. The average lumen diameter of the young sample was 1.34±0.02 µm, with very few differences between the three groups of origin ($p> 0.05$). While for the old group an

average of 1.23±0.02 μm was found. The lower diameter of dentinal tubules was found for the US group ($p \leq 0.05$).

3. The time dependent deformation behavior of dentin was studied using instrumented spherical indentation. The experimental results showed that the deformation behavior for both young and old samples followed a power law behavior. From the results can be seen that the young dentin showed a power-law exponent of *3.11* regardless of their origin. A reduction in the power- law exponent was found for the old group. A reduction of almost of 50% was found for the China and US group, while for the Colombian group a reduction of only 27% was found.
4. The largest proportion of obliterated dentinal tubules found was of 47% in the China group in a 53-year-old sample. While for the US and Colombian samples, the maximum values obtained were 41% and 9%, respectively, in both cases in a sample of 47-year-old sample.
5. The mineral-to-collagen ratio of the young US and Colombia samples showed to be statistically equal ($p>0.05$), while the China samples have a statistically higher mineral-to-collagen ratio (~40% higher). An increase in the mineral-to-collagen ratio was found in the old samples for all the regions; the results obtained showed that the old China and US samples are statistically equal ($p>0.05$); meanwhile the Colombia samples are statistically lower.
6. The differences found on the obliteration of dentinal tubules, chemical composition and mechanical properties between the regions analyzed showed that the aging process of dentin could be related not only with ethnicity but also to individual characteristic of the donor.

7. 6 Tables

Table 7. 1. Power-law parameter obtained for each group of dentin analyzed.

Origin	Age Group	*n*
China	Young	3.23
	Old	1.63
Colombia	Young	3.00
	Old	2.18
US	Young	3.10
	Old	1.67

7. 7 Figures

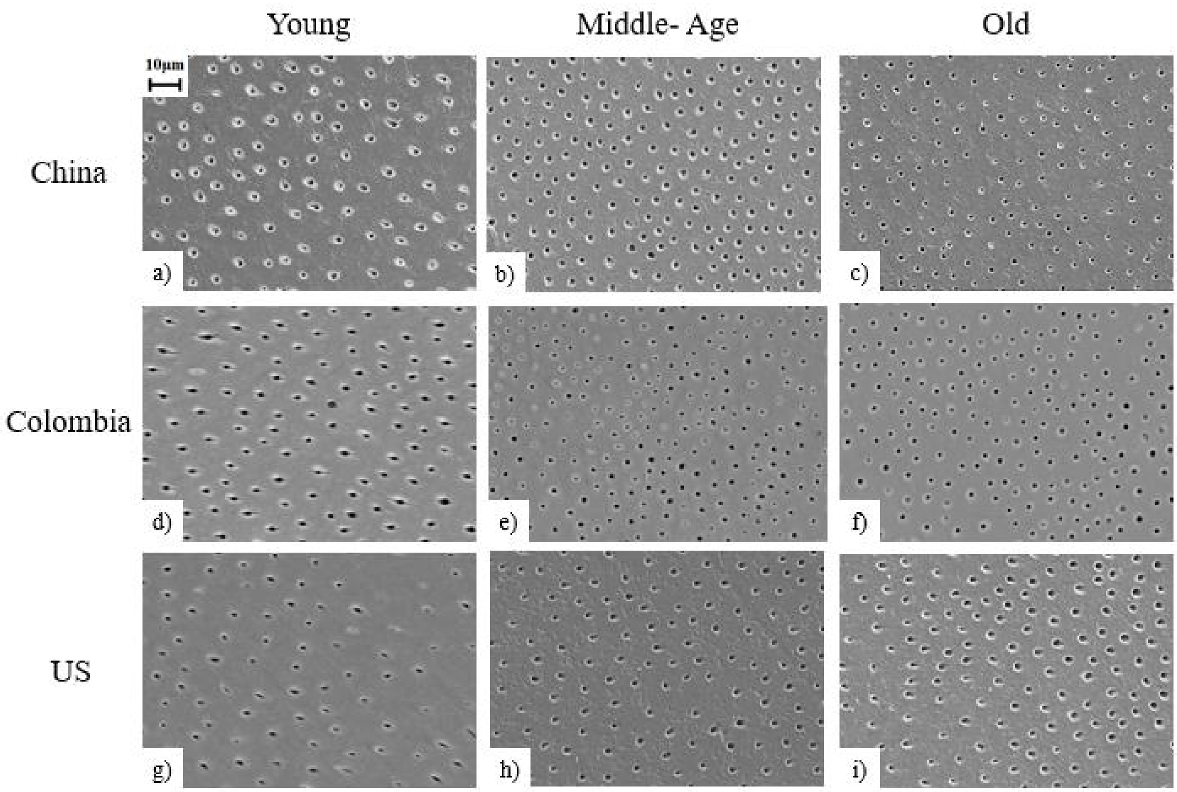


Figure 7. 1. Micrographs of the outer dentin microstructure. a, b, c) China donors; d, e, f) Colombia donors; g, h, i) United States donors.

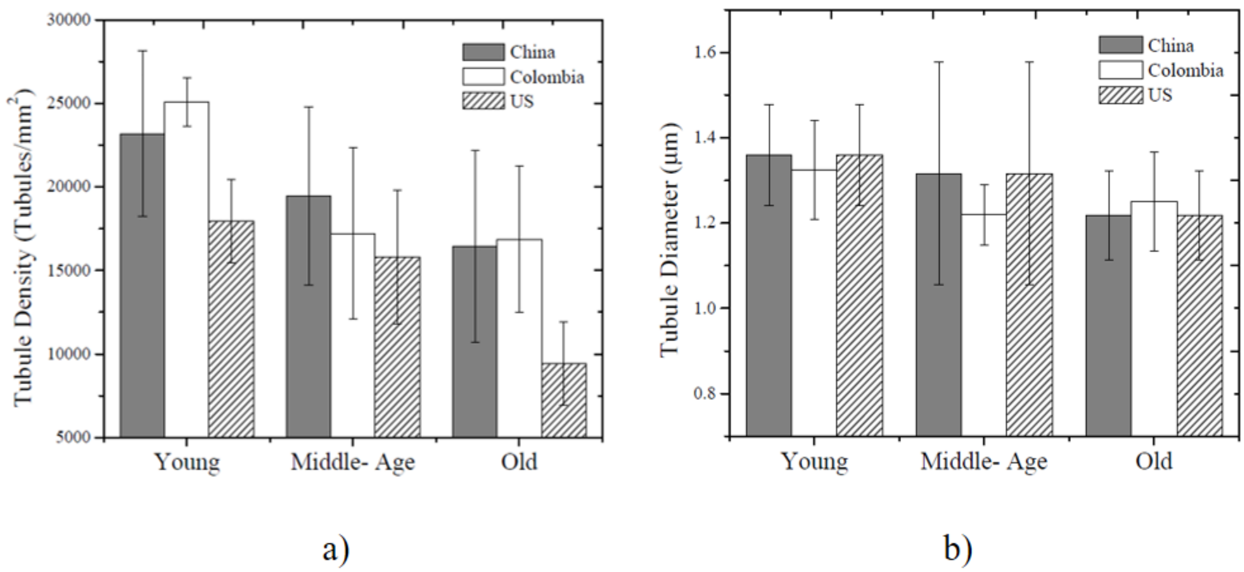


Figure 7. 2. A comparison of the microstructure as a function of age in the outer dentin from donor teeth of China, Colombian and US. a) lumen density; b) lumen diameter.

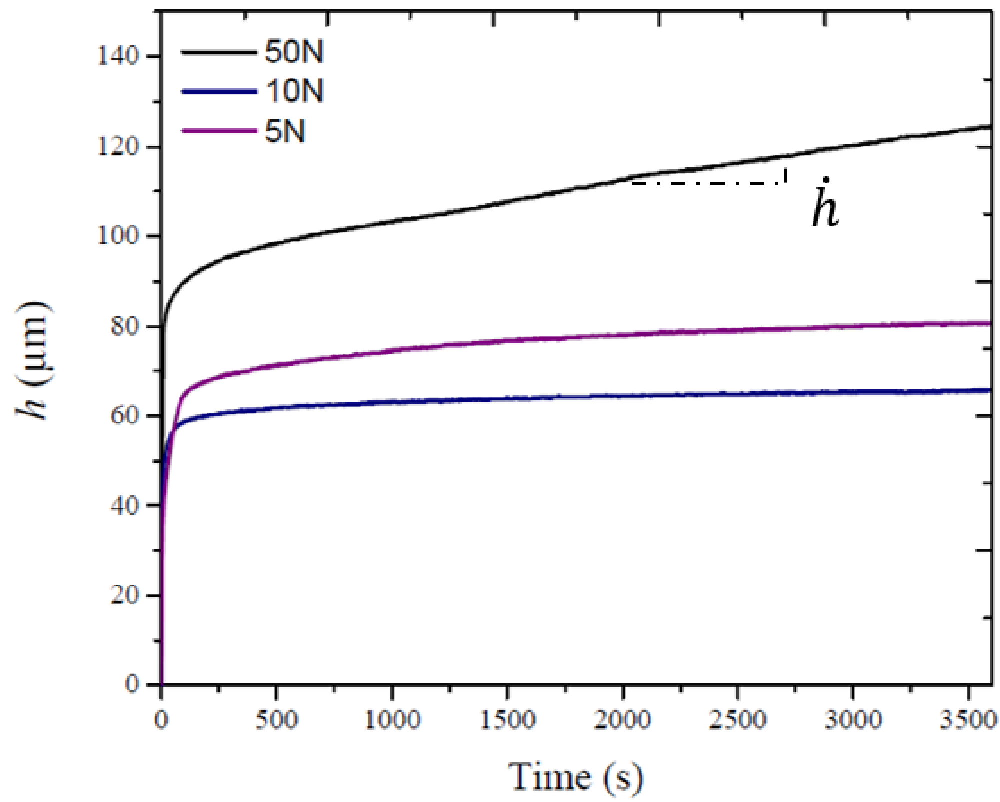


Figure 7. 3. Selected indentation depth versus time results for outer china dentin at applied loads of 5, 10 and 50 Newtons. The results correspond to a Chinese donor of 56 years of age.

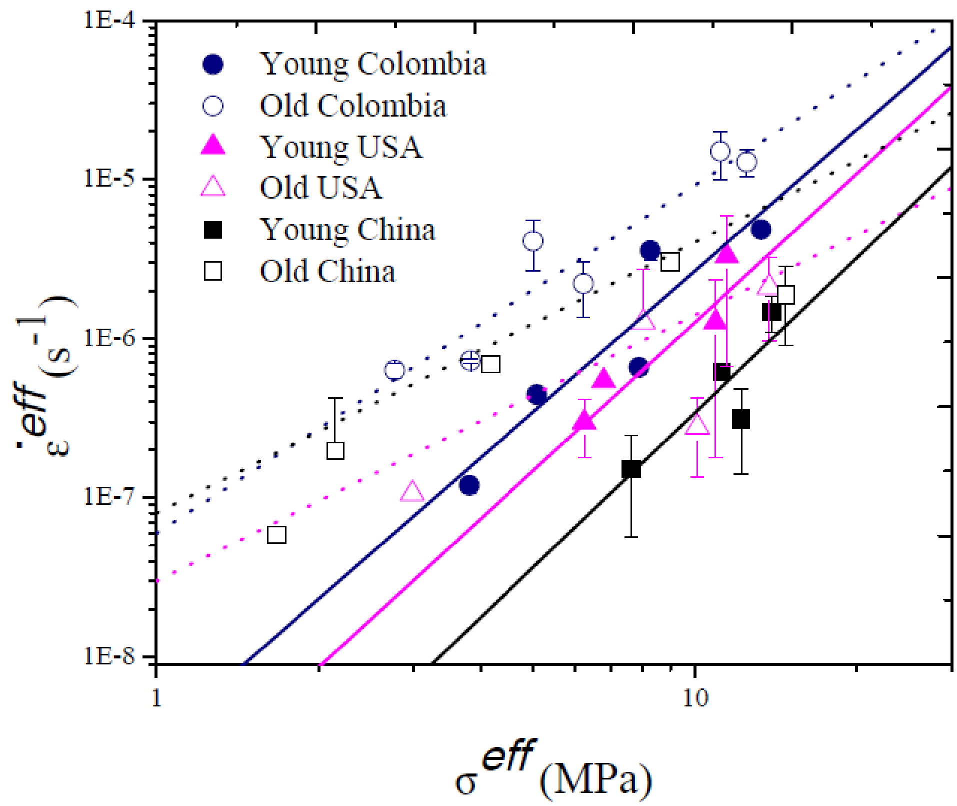


Figure 7. 4. Comparison of the experimental steady-state effective stress and effective strain for the outer dentin of the three groups analyzed (markers) with the power-law fit (lines).

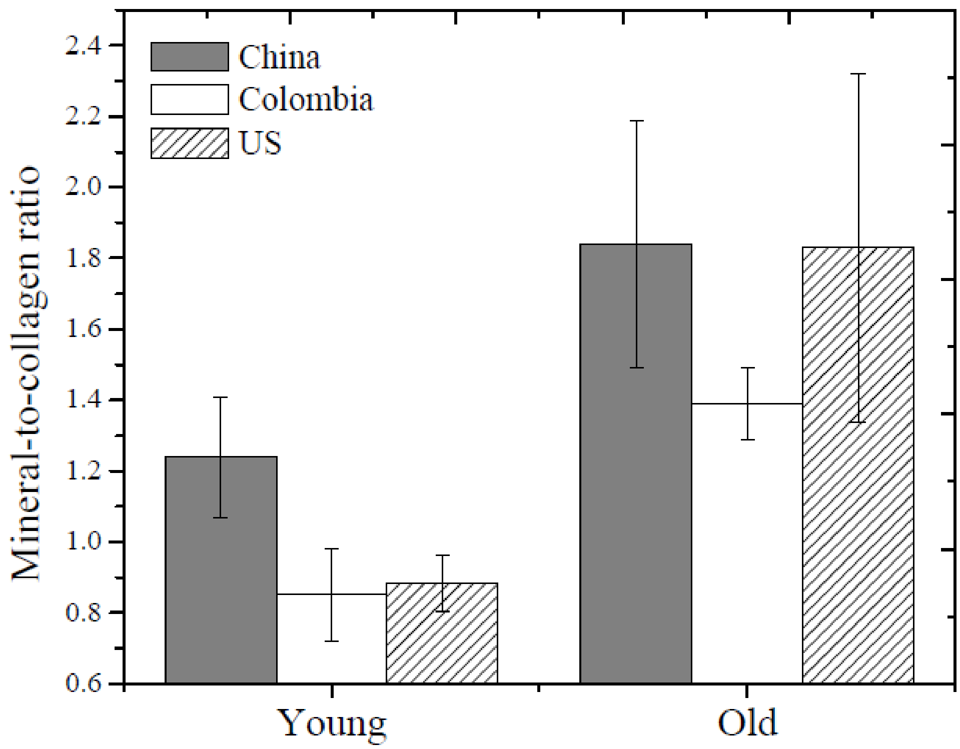


Figure 7. 5. Mineral-to-collagen ratio of outer dentin from young and old donor teeth according to the donor's origin region.

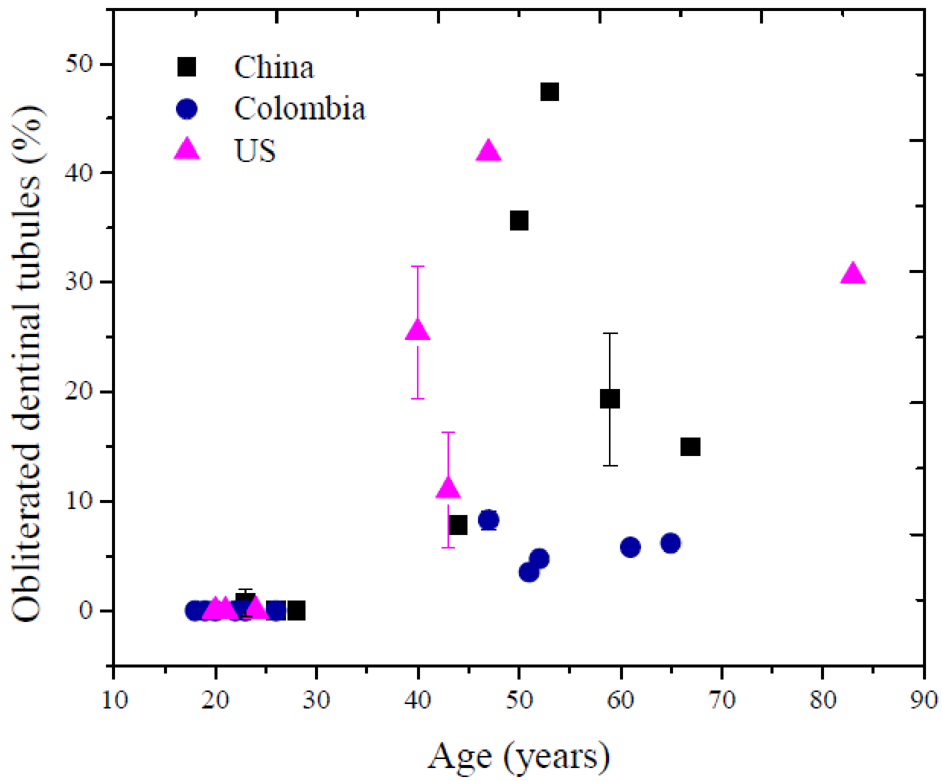


Figure 7. 6. Change in the proportion of obliterated dentinal tubules with aging.

Chapter 8

Conclusions

This chapter summarizes the main conclusions of this dissertation:

Chapter 2: Review of previous research on the mechanical behavior and aging of human dentin

The aging process of dentin aging causes a change in dentin microstructure, product of the obliteration of dentinal tubules; these changes have been related with an increase in mineral content. However, the literature review shows no studies aimed at determine and quantify the changes in chemical composition of aged coronal dentin (spatial variations). These changes might be related with an increase in mineralization, crosslinking of collagen or changes in crystallinity.

Dentin hardness has been shown to have considerable local variations for young patients. For aged dentin, although it has been reported that hardness increases with aging, the changes obtained have been related to the amount of "free spaces" (i.e. dentinal tubules density), but not with chemical composition changes that occur in the tooth. For this correlation old dentin can include other additional changes that occur with aging such besides obliteration of dentinal tubules.

The viscoelastic properties of coronal dentin have been measured in the bulk dentin. However, the fact that dentin is an anisotropic material, and that mechanical properties change spatially within the tooth suggests that the viscoelastic properties of dentin will too. Also, only one study regarding the viscoelastic properties of aged dentin is available, considering intertubular and peritubular dentin, ignoring how these properties could change along different regions of dentin.

Chapter 3: Effect of aging on hardness, microstructure and chemical composition of dentin

The tubule density in the dentin of young and old donor teeth ranged from approximately 22,000 to 35,000 tubules/mm^2. The tubule diameter ranged from approximately 1.2 μm to 1.8 μm. There was a significant decrease in tubule density from the pulp to the DEJ in both young and old dentin ($p \leq 0.05$).

There was a significant difference ($p \leq 0.05$) in the measures of occlusion ratio between the outer (4.71%) and middle dentin (2.50%) of the old donor teeth. The largest proportion of obliterated dentinal tubules being near the DEJ and decrease towards the pulp.

A significant decrease in hardness was found with increasing distance from the DEJ for both young and old donor teeth ($p \leq 0.05$). A larger hardness was found in the old dentin when compared to young dentin. There were significant differences ($p \leq 0.05$) in the hardness in the outer and middle dentin between young and old patients.

The mineral-to-collagen ratio was found to increase with proximity to the DEJ for both young and old patients. This behavior is opposed to previous results reported in the literature for dentin from US donors, which in turn raises questions whether the chemical composition of dentin and its percentages of organic material and collagen might be affected by ethnicity.

The outer dentin exhibited the highest mineral-to-collagen ratio, while lower values were found in the areas near the pulp. A greater mineral-to-collagen ratio was found for the old dentin, with the highest values found in the outer dentin.

A comparison of the hardness and mineral-to-collagen ratio distributions showed that the regions with the highest mineral-to-collagen ratio exhibited the highest hardness values.

Chapter 4: Importance of Tubule Density to the Fracture Toughness of Dentin

A quantitative analysis of the changes in tubules occurring in coronal dentin with aging showed that the primary difference between young and old dentin was a reduction in the lumen area fraction. In the outer dentin there was nearly a 40% reduction in the lumen area with aging. This difference is attributed to obliteration of dentinal tubules with formation of sclerotic dentin.

An approach previously proposed to study the mechanical behavior of porous materials was employed to model the fracture toughness of dentin and the influence of spatial variations in dentinal tubules. The model showed a strong correlation to experimental results previously reported in the literature for the dentin of young donor teeth. However, results for the old dentin were not in agreement due to differences in the mechanisms of fracture.

Chapter 5: Time dependent deformation behavior of human dentin

The time dependent deformation behavior of coronal dentin was studied using instrumented spherical indentation. The experimental results showed that the deformation behavior followed a power law behavior, which was appropriately captured by an extension to previously proposed models for the time dependent deformation behavior of structural materials. The model accounts for the spatial variations of chemical composition (e.g. mineral to collagen ratio) and microstructure (e.g. tubule density) of coronal dentin. Regions with higher mineral to collagen ratio exhibited a stiffening effect, with reduced strain rate for indentation loads. Similarly, a reduction in tubule density also caused a decrease in the deformation behavior. The power law creep exponent for dentin was found to have a value of *n=3.38* which was independent of the spatial variations in composition and microstructure. Furthermore, this parameter was found to be dependent on the specific composition of the collagen present in the tissue and not on the amount.

Chapter 6: Contributions of aging on the time dependent deformation of dentin

The time dependent deformation and chemical composition of aged dentin was evaluated in outer, middle and inner coronal dentin. The experimental results showed to follow a power law deformation behavior with a decrease in the stress exponent when comparing with young dentin.

Significant changes in the mineral to collagen ratio, crystallinity and carbonate to phosphate ratio were found with increasing donor age. Most of these changes were found to be more significant in the middle and outer dentin.

A model previously proposed to predict the time dependent deformation response of dentin was employed to model the creep behavior of aged dentin. The model showed a strong correlation between the experimental and the predicted results. A decrease in the time-dependent behavior of middle and inner aged dentin was found when compared with young dentin.

Chapter 7: Ethnic Background influence on the aging process of dentin

A reduction in the tubule density was observed with increasing donor age for the three regions of origin analyzed: China, Colombian and United States. For the young samples the average tubule density was 22,000 tubules/mm^2 ($p>0.05$). While for the old group an average of 14,000 tubules/mm^2 was found, with a significant difference in the US group (9,500 tubules/mm^2).

The average lumen diameter of the young sample was 1.34 ± 0.02 μm, with very few differences between the three groups of origin ($p > 0.05$). While for the old group an average of 1.23 ± 0.02 μm was found. The lower diameter of dentinal tubules was found for the US group ($p \leq 0.05$).

The time dependent deformation behavior of dentin was studied using instrumented spherical indentation. The experimental results showed that the deformation behavior for both young and old samples followed a power law behavior; from the results, can be seen that the young dentin showed a power-law exponent of *3.1* regardless of their area of origin. A reduction in the power- law exponent was found for the old group, a reduction of almost of 50% was found for the China and US group, while for the Colombia group a reduction of only 27% was found.

The largest proportion of obliterated dentinal tubules found was 47% in the China group in a 53-year-old sample. While for the US and Colombian samples, the maximum values obtained were 41% and 9%, respectively, in both cases in a sample of 47-year-old sample.

The mineral-to-collagen ratio of the young US and Colombia samples showed to be statistically equal ($p>0.05$), while the China samples have a statistically higher mineral-to-collagen ratio (~40% higher). An increase in the mineral-to-collagen ratio was found in the old samples for all the regions; the results obtained showed that the old China and US samples are statistically equal ($p>0.05$); meanwhile the Colombia samples are statistically lower.

The differences found on the obliteration of dentinal tubules, chemical composition and mechanical properties between the regions analyzed, showed that the aging process of dentin could be related not only with ethnicity but individual characteristic of the donor.

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