Residual Micro-strain in Root Dentin after Canal Instrumentation Measured with Digital Moiré Interferometry

by

Helena Lim

A thesis submitted in conformity with the requirements
for the degree of Master of Science

Graduate Department of Dentistry
University of Toronto

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Helena Lim, BSc, MA, DDS

Master of Science 2016

Graduate Department of Dentistry, Discipline of Endodontics
University of Toronto

Abstract

This study evaluated the residual micro-strain and the presence of micro-defects in root dentin following nickel-titanium instrumentation of canals in teeth maintained in hydrated and non-hydrated environments. Eighteen premolars were divided into two experimental groups (ProTaper Universal, Wave-One) and one control group (hand files). Nine specimens were maintained in deionized water and nine specimens were kept in ambient conditions for 72 hours. Digital moiré interferometry (DMI) was used to qualitatively determine the residual micro-strain distribution pattern in root dentin produced by instrumentation. Subsequently, micro-computed tomography (micro-CT) and polarized light microscopy (PLM) were used to detect the micro-defects. DMI showed a dentinal strain relaxation behavior in hydrated specimens whereas, root dentin from non-hydrated specimens showed localized regions of micro-strain concentrations. Micro-CT and PLM did not reveal obvious dentinal micro-defects in all groups. These findings suggested that the biomechanical response of root dentin to instrumentation was influenced by hydration.

ACKNOWLEDGMENTS

First and foremost, I would like to express my deepest gratitude to my principal supervisor Dr. Anil Kishen for providing me with the opportunity to work with him on this research project. Dr. Kishen was always supportive, motivating and his exceptional work ethics and enthusiasm was inspirational. Without his support and supervision, I would not have been able to complete this project. He has been the best teacher and mentor I have ever had and I feel truly privileged to have him as my principal supervisor. Second, I would like to thank Dr. Shimon Friedman for his guidance, support and encouragement during this project and throughout the graduate program. His meticulous eye for detail, invaluable feedbacks and countless hours of edits were greatly appreciated. I would also like to thank my scientific advisory committee member, Dr. Anuradha Prakki, for the constant support and feedbacks throughout my research endeavors. I am also very grateful to Dr. Alice (Fang Chi) Li for the countless hours of help during the execution of this project and manuscript correction. Her friendship and contribution was crucial in the completion of this research project. I would like to thank research technologist, Lisa Yu, in the Mouse Imaging Centre in the Hospital for SickKids for scanning samples using Micro-Computed Tomography and Dr. Wong from China for providing and sharing his expertise on customized digital moiré interferometry. Lastly, and most importantly, I would like to express my forever gratitude to my family for their prayers, love and support and special thanks to my husband, Peter, for sticking by me throughout this rollercoaster ride.

This research was supported by the Canadian Academy of Endodontics Endowment Fund, the American Association of Endodontist Foundation, and Natural Sciences and Engineering Research Council of Canada.

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I. GENERAL INTRODUCTION

Endodontic treatment is commonly performed to cure or prevent apical periodontitis so as to retain a functional tooth in the patient's mouth. Despite important improvements in the provision of endodontic treatment, a loss of 4 to 7% of endodontically treated teeth has been reported due to fracture within 5 years after treatment (1). Root fractures, specifically, were reported in 6% of the endodontically treated teeth in a recent 10-year survival study (2). Clinical studies and empirical observations have suggested that teeth are more likely to fracture as greater amounts of tooth structure are lost from disease or iatrogenic procedures, which includes access cavity preparation (6), root canal instrumentation (4, 7) and root canal filling (3-5).

Nickel-titanium (Ni-Ti) instruments are currently used in rotary or reciprocating motions to shape root canal systems with less procedural errors than hand files (4). Recently introduced single-file Ni-Ti system such as WaveOne (Dentsply Maillefer) is designed to shape the root canals with only one file, therefore, necessitating less time than full-sequence rotary instrumentation systems such as ProTaper Universal (PTU) (Dentsply Maillefer) (7). Concerns have been raised recently on the possible relationship between micro-defect formation in root dentin and canal instrumentation systems (7-11), but research data lack consensus (Table 2). Factors such as design and alloy of the instruments, cutting efficiency, number of instruments used and stress/strain concentration in dentin have been suggested as risk factors for dentinal micro-defect formation (4, 8, 10-12).

Previous studies that examined post-instrumentation micro-defects did not provide any information on the biomechanical response (localized stress/strain distribution) of root dentin post-instrumentation (4, 8, 10, 11, 13). This knowledge is crucial to understand the effect of instrumentation induced dentin structural changes and their influence on the mechanical integrity of teeth.

1. Dentin as a Biomaterial

1.1 Dentin Composition

Dentin is a mineralized and hydrated hard tissue that forms the major bulk of the human tooth. Dentin is composed of approximately 50 wt. % of inorganic materials, 40 wt. % of organic materials and 10 wt. % of water (14). The inorganic component is mostly formed of carbonated nanocrystalline apatite minerals, while the organic component is mostly composed of Type I collagen (90%) and small amounts of non-collagenous proteins (15). The collagen fibrils in dentin are 50-100 nm in diameter and they are oriented perpendicular to the direction of dentin formation (15). The non-collagenous proteins are made up of about 10% of the organic matrix and fall into several categories such as proteoglycans, glycosaminoglycans, sialoproteins and growth factors (16).

Dentin has fluid-filled dentinal tubules that transverse the entire bulk from the pulp chamber and canals to the periphery. The dimensions of dentinal tubules are smallest at the dentino-enamel junction and become wider toward the pulp (17). Each dentinal tubule is about 3 μm in diameter; however, after the deposition of

intratubular dentin, the superficial dentin narrows down to 0.6-0.8 μm (17). The amount of intratubular dentin decreases as tubules approach the pulp chamber and no intratubular dentin is present near the level of the pulp chamber (17). These variations in the structural components and architecture, the degree of mineralization (18) and direction of mineralized collagen fibrils (19) are believed to effect the mechanical properties of dentin. Inorganic and organic fraction contributes to the structural integrity of dentin, which is essential to retain the function of restored teeth (104).

1.2 Water in Dentin

Dentin contains 10% of water by weight and 20% by volume. Majority of the water (75%) is present in the dentinal tubules whereas the rest (25%) is in the mineralized matrix (20). In the matrix, water is in free or bound state. Free water, representing approximately 30% of dentin's total water, occupies the dentinal tubules, porosities and to some degree the matrix. Free water can be removed by exposing dentin to ambient condition and 85% of it is lost in the first 30 minutes of dehydration (21).

Bound water, representing approximately 70% of dentin's total water, is chemically bound to the hydroxyapatite and collagen fraction of the dentin. It requires much higher temperature to be removed (600°C) (104). The bound water forms a single layer of water molecules on the surface of hydroxyapatite by hydrogen bonds and weak van der Waals forces (22). Bound water is essential in stabilizing the triple

helix architecture of collagen molecules and each collagen tripeptide is known to have two water molecules (105). With an increase in the number of water molecules per collagen molecule, it expands laterally (23, 24).

The water content of dentin affects the elastic properties of dentin. Kinney et al. (1999) examined the elastic properties of human dentin and found that relaxation was potentially a function of hydration of dentin (25). Other studies have shown that water movement and properties of collagen fibril network affect the viscoelasticity of dentin (15, 23, 24, 26). Well-hydrated dentin typically displays a linear viscoelastic behavior (27). The water-filled dentinal tubules help dissipating the occlusal forces (28), which facilitates uniform distribution of strain throughout the entire bulk dentin (29). Moreover, it has been shown that the degree of hydration is related to the fracture toughness of dentin (30).

1.3 Mechanical Properties of Dentin

The mechanical properties of dentin are contingent on the fine balance between toughness and stiffness (27). The collagen component of dentin provides toughness and ultimate tensile strength, whereas the mineral component provides elastic modulus and compressive strength to dentin (31, 32). Moreover, water molecules provide a plasticization effect that increases the transient and viscous creep strains, while reducing the load sustainability in dentin (33). The intricate interactions of dentinal components and the microstructural arrangements result in the

mechanical properties of dentin such as young's modulus, fracture toughness, tensile strength and compressive strength (27).

Dentin has viscoelastic properties. Viscoelasticity is the property of a material that exhibits both viscous and elastic characteristics when undergoing deformation. In a viscoelastic system, the relaxation modulus (the ratio of stress to strain) changes with time and characterizes the response of a material to an applied stress. Strain placed on a viscoelastic material induces corresponding stress in that material, which decreases over time (stress relaxation) (33). When stresses are applied on dentin, its viscoelastic properties enable the dissipation of strain energy (34). However, when dentin is subjected to repeated stress or air-drying, the rate of stress relaxation becomes slower in dehydrated dentin when compared to hydrated dentin (33).

2. Fracture in Endodontically Treated Teeth

2.1 Prevalence of Fracture in Endodontically Treated Teeth

Endodontically treated teeth are weakened due to decreased or altered tooth structure (101). Dentin fracture initiates from a defect and it is due to a localization of high stress concentration (35). The most common type of fracture seen in endodontically treated teeth is vertical root fracture (VRF), defined as a complete or incomplete fracture initiated at any root level, that is usually buccolingual in

direction (13, 14, 36-43). Teeth with VRF have a poor prognosis, mostly ending up in extraction (36).

A wide prevalence of VRF, ranging from 3-30%, has been reported in endodontically treated teeth (2, 44-52) (Table 1). This wide range may be explained by the variability in study designs and techniques used for the evaluation of VRF. For examples, studies that used radiograph to detect VRF reported a lower prevalence of VRF (3-5%) (44, 45, 48), whereas studies that evaluated VRF from extracted teeth reported a higher prevalence of VRF (11-30%) (46, 47, 49, 52). Among the teeth examined, both restored or non-restored (36) maxillary second premolars (27%) and mesial roots of mandibular molars (24%) most frequently displayed VRF (36). VRF was more commonly observed in females (52%) compared to males (47%). Furthermore, individuals between the ages of 41 to 50 years showed greater propensity for VRF (53).

2.2 Risk Factors of Fracture in Endodontically Treated Teeth

The high prevalence of VRF in endodontically treated teeth was attributed to dehydration effect, resulting in less elastic and more brittle behaviour in dentin after endodontic treatment (103). Studies by Bier et al. (2009) and Shemesh et al. (2009) that examined the relationship between micro-crack formation resulting from root canal instrumentation and the root filling procedure have sparked an interest in the association between endodontic procedures and crack formation (4, 8). Subsequently, additional studies have demonstrated the formation of dentinal

defects, such as micro-crack or craze lines, following endodontic procedures and argued that VRF is likely caused by the propagation of these initial dentinal defects when tooth is exposed to continual stresses from various iatrogenic procedures (42, 54, 56-59).

Studies have also considered other causes of VRF, such as spreader design (42, 55), root morphology (54, 56), post placement and corrosion (57, 58) and excessive force created during lateral condensation of root fillings (59). Wilcox et al. (1997) stated that the more tooth structure removed, the more likely a root is to fracture (54). Overall, clinical studies and empirical observations have suggested that, in general, teeth are more likely to fracture when more tooth structure is lost due to access cavity preparation (6) and root canal instrumentation during endodontic treatment (7, 8). However, to date, a precise understanding on the mechanism of VRF is lacking (60).

2.2.1 Effect of Ni-Ti Instrumentation on Mechanical Integrity of Dentin

Over the last 20 years, rotary Ni-Ti instruments have become the mainstream tools to mechanically prepare the root canal space. They have made mechanical instrumentation more efficient and predictable by improving cleaning and shaping and reducing the incidence of apical transportation, canal straightening and perforation in comparison to conventional stainless steel files (8). In general, Ni-Ti instruments are used in a low-speed torque-control setting and files rotate 360 degrees (61). ProFile (Dentsply Maillefer) and ProTaper Universal (Dentsply

Maillefer) are examples of commercially available instrument systems used in continuous 360 degrees rotation.

Recently, a new technique using reciprocating motion was proposed for root canal preparation. This approach relieves the stress on the files by counterclockwise (cutting action) and clockwise (release of the instrument) motions and, therefore, increasing its resistance to cyclic fatigue compared to continuous rotational motion (62, 63). WaveOne (Dentsply Maillefer) and Reciproc (Dentsply, VDW, Munich, Germany) are examples of single-file reciprocating systems. Furthermore, different types of Ni-Ti files have been introduced in recent years by treating the alloy with heat during the manufacturing process. The rationale for the heat-treatment process was to create instruments that are more flexible and having improved resistance to cyclic fatigue.

Despite the benefits, such as safety and shaping effectiveness of the rotary and reciprocating instrumentation, recent reports have suggested possible adverse impacts of these instruments (7-11). Related reports that evaluated the micro-defect formation in root dentin after canal instrumentation with Ni-Ti engine-driven instruments were mostly based on tooth sectioning methods. Shemesh et al. (2009) reported that canal preparation would result in significant number of dentinal defects such as fractures, craze lines and complete or incomplete cracks (8). Additionally, Bier et al. (2009) argued that the rotary kinematics of engine-driven Ni-Ti instrumentation was the main cause of micro-defect formation(4). Therefore, these studies concluded that strain accumulation in dentin during Ni-Ti

instrumentation may create dentinal defects in which VRF can initiate (54, 56, 64, 65).

Previously, studies have examined the incidence of crack formation following different types of instrumentation. Results of such studies have been inconsistent (Table 2). This inconsistency may be attributed to the wide variability in the different designs, tapers, preparation protocols, number of files, and kinematics Ni-Ti instrumentation systems. A finite element analysis simulation of shaping procedures with different Ni-Ti instrumentation systems have shown that the file design may affect apical stress and strain concentrations during instrumentation (64).

Studies have also evaluated the effect of different file motions on crack formation (7, 9, 61, 66-72), with inconsistent results. While reciprocating instruments produced more incomplete dentinal cracks at the apical level of canals than rotary instruments in one *in vitro* study (7), continuously rotated instruments produced more dentinal cracks compared to the reciprocating instruments in another *in vitro* study (9). Studies that reported that reciprocating instruments are more likely to induce micro-crack formation argued that reciprocating systems are usually a single-file system; therefore, a single large-tapered reciprocating file can result in substantial amount of dentin removal in a short period of time. Therefore, single-file reciprocating systems may increase the risk of micro-crack formation compared to the multiple-file conventional rotary systems, which involve more progressive and slower mechanical enlargement (7). However, majority of studies concluded

that rotary systems result in increased risk of crack formation compared to reciprocating systems (9, 61, 66-72).

Despite of many studies highlighting the association between instrumentation and micro-cracks, some studies could not find significant association between the kinematics of different instrumentation systems and crack formation (73-76). De-Deus et al. (2014) evaluated the root dentin before and after instrumentation with rotary and reciprocating systems using micro-computed tomography (micro-CT). Their result showed no clear causal relationship between micro-crack formation and root canal preparation, contrary to the previous micro-crack and instrumentation studies that had used root sections and light microscopy to establish a causal relationship between the two (62). Furthermore, no association was established between micro-cracks and different root canal instrumentation techniques in a cadaver model (73). In brief, the different results found in current literature may be due to different methodologies, such as study design and methods of evaluation.

2.2.2 Effect of Endodontic Treatment on the Moisture Content of Dentin

During endodontic treatment, the infected pulp tissue is eliminated and root canal system is chemically disinfected and dried prior to obturation (77). G. V. Black (1895) was the first to hypothesize that the changes in biomechanical properties in endodontically treated teeth was due to water loss. This compositional change will lead to brittleness of dentin, resulting in increased fracture prevalence of root-filled teeth (99). Helfer (1972) also demonstrated that root-filled teeth had about 9%

less moisture content in the dentin in comparison to vital teeth (100). Reeh and Messer (1989) concluded that endodontic treatment reduced tooth stiffness by 5% and this loss mainly resulted from access cavity preparation (78, 79).

Huang et al. (1992) examined whether endodontically treated teeth were more brittle in comparison to vital teeth and studied the effects of moisture on the biomechanical properties of dentin (79). Their result showed that regardless of the vitality of the samples, dehydration increased the stiffness and decreased the flexibility of dentin. Furthermore, dehydration had a clear effect on the biomechanical properties of dentin, such as fracture pattern, elastic modulus and proportional limit in compression. However, they argued that dehydration resulting from endodontic treatment could be compensated since endodontically treated teeth usually exist in an aqueous environment and since enamel or dentin are highly permeable.

Sedgely and Messer (1992) also examined whether endodontically treated teeth were more brittle compared to contralateral vital teeth from the same patient (80). They found no significant difference in the biomechanical properties (punch shear strength, toughness, hardness and load-to-fracture) between the endodontically treated and vital teeth. However, since both groups were stored in saline prior to experimentation, any difference resulting from dehydration of endodontically treated teeth was likely lost because storage in saline led to rehydration of dentin, thus allowing dentin to regain its mechanical properties. Papa and Messer (1994) evaluated the difference in moisture content of endodontically treated teeth and

vital teeth and found that endodontically treated teeth had 0.25% less moisture compared to vital teeth, which was statistically non-significant (81).

2.2.3 Effect of Hydration on Mechanical Integrity of Dentin

Toughness is the energy required to induce fracture and it is significantly reduced by dehydration (21, 82). Studies have shown that even partial loss of free water, which occurs when a tooth is exposed to ambient condition, produced significant alteration in the stress-strain behavior and resistance to fracture in dentin (21, 33, 83). One of the key studies that attempted to establish a direct link between the water content and mechanical behaviour of dentin was Jameson et al. (1993) (21). They reported that once dentin becomes exposed to atmospheric conditions, it started to lose water immediately (28, 82). Dehydration of dentin at 20 °C (50% relative humidity) led to about 30% moisture loss, which produced significant differences between the biomechanical properties of hydrated and dehydrated dentin (21). In dehydrated dentin, a decreased strain at fracture and reduced plastic energy during deformation resulted in a brittle behaviour. These changes were reversed after rehydration for 3 days. Kruzic et al. (2003) also found that dehydrated dentin had a significantly lower crack-initiation toughness compared to hydrated dentin (31).

Kishen and Asundi (2005) used a novel optical technique, the digital moiré interferometry (DMI), to evaluate the role of free water on the mechanical deformation of structural dentin by testing fully hydrated and dehydrated (20 °C for

72 hours) dentin specimens (29). They found that free water from dentin was lost in a biphasic manner when exposed to ambient room conditions. Rapid and highest degree (80-85%) of free water loss from dentinal tubules and porosities occurred in 30 to 120 min (depending on tooth section) (*phase-1*), followed by gradual loss of free water from the dentin matrix (*phase-2*). Fully hydrated dentin resulted in a strain response characteristic of a tough material, while dehydrated dentin resulted in a strain response characteristic of a brittle material (29).

Dehydration of collagen increases stiffness as a result of the formation of additional interpeptide hydrogen bonds that were previously inhibited by the hydrogen bonding with water (22). Furthermore, loss of free water from the dentin matrix is associated with shrinkage (82) and residual micro-strain (32). It could be recognized that a well-hydrated dentin typically displayed a linear viscoelastic behavior (84) and the fluid-filled tubules might function to hydraulically transfer and release stresses applied to the tooth (83).

In conclusion, deleterious effects of water loss on the collagen and proteoglycan matrix of dentin during endodontic treatment may act as a trigger for dentin deformation, inducing an increase in stress concentration and ultimately contributing to significant decrease in fracture resistance.

3. Critical Appraisal of Experimental Methods

There is a lack of consensus among studies that examined the relationship between micro-crack formations after canal preparation with different instrumentation systems. Overall, this inconsistency may be due to variations in tested system and preparation protocols, the type of irrigants and irrigation protocols, the classification of the defect, the sample storage condition, the sample selection and the observational methods used to examine the dentinal defects (85) (Table 3). Majority of studies have included Ni-Ti rotary instrumentation (ProTaper) systems as an experimental group, used mandibular teeth as their sample and sodium hypochlorite (NaOCl) with varying amounts and concentration as the irrigant. For analysis, direct observational method was used commonly with optical/light microscopy following sectioning of the root. The sectioning method is destructive in nature and precludes assessment of cracks along the longitudinal axis of the root (70).

3.1 Classification of the Defects

The lack of consensus regarding classification of defects formed in dentin may lead to misunderstanding, incorrect diagnosis and inappropriate treatment. The terms crack, defect, fracture, other defects, incomplete crack, craze line are often used interchangeably. Several studies lack clear definition of different types of defects in dentin. It is important to note that some studies included all incomplete cracks observed in root cross-sections extending either from the root surface or from the

canal lumen as defects created by instrumentation (8, 9, 11, 70, 86). However, incomplete cracks initiating from the outer root surface should not be classified as defects caused by instrumentation as these defects are most likely caused by the dehydration of the teeth or created during the sectioning procedures (85). Therefore, without clear definition and standardization of classification, these studies may have over- or under-estimated the occurrence of defects caused by instrumentation.

3.2 Sample Selection

There is a wide variation in human teeth related to root morphology and canal anatomy, dentinal structure, chemical composition, age related changes and biomechanical properties such as hardness and modulus of elasticity. This wide variability makes standardization of specimens and samples difficult. All these factors can act as potential confounding factors that can influence the appearance of dentinal defects and dentin's response to instrumentation (85).

3.3 Sample Storage Conditions

Sample storage condition is another factor to consider while studying crack generation because the degree of tooth hydration has been shown to play an important role in the resistance to fracture (21, 25, 33, 83). Experimental procedures can result in samples being exposed to ambient (non-hydrated) condition for long periods which was not well specified in the previous experiment

(85). Prolonged exposure to dry condition will lead to significant free water loss in dentin, resulting in brittleness and decreased toughness of dentin (21, 33, 83). Therefore, it is important to maintain samples in hydrated condition throughout the experiment. Moreover, several studies stored their samples in storage media, such as formalin (13, 56) and NaOCl (87), which might also cause severe changes to the micro-hardness, elastic modulus and flexural strength of dentin (88).

Given that many of the samples from previous investigations showed incomplete cracks extending from the outer surface of the root (8, 9, 11, 70, 86) it may be inferred that these cracks are likely caused by dehydration of dentin. Therefore, unless samples are kept in well-hydrated environment throughout the experiment and storage conditions are strictly standardized to prevent dehydration of the samples, it is erroneous to conclude that the post-instrumentation defects are due to instrumentation alone.

3.4 Methods for crack detection

A wide range of observational methods has been used to detect dentinal defects following endodontic procedures. This includes sectioning, scanning electron microscopy, micro-CT with and without contrast agents, stereomicroscopy, transillumination, endoscopy, infrared thermography, optical coherence tomography and transmission electron microscopy (85). Although many of these methods have shown great potential for endodontic research, some of them, such as sectioning and viewing it under the stereomicroscope, may provide questionable data.

3.4.1 Sectioning Methods

The most commonly used observational technique is a sectioning method where the samples are sectioned at different levels and viewed under high magnification microscopy (3, 9-11, 73). This method allows direct inspection of cracks on the root surface and provides information regarding the extension, propagation pattern and direction of cracks. A thin diamond blade is used to progressively slice the tooth into thin cross-sections, under running water to prevent heating and to minimize undesirable damage to the tooth structure. Most commonly, three or four sections are made at 1, 3, 7 and 9 mm from the root apex. Overall, the precision of the sectioning tool and method will determine the output quality of the sample (85).

The main limitation of this method is that it is destructive. Therefore, the possibility of crack generation during sectioning is challenging to negate. In addition, during sectioning, part of the sample is inevitably lost resulting in loss of information while the pre-instrumentation condition of dentin is often unknown. Furthermore, this technique does not support assessment of cracks propagating along the longitudinal axis of the root (75).

3.4.2 Stereomicroscope

A microscope is an optical instrument that uses a lens or multiple lenses to produce magnified images of objects too small to be seen by the unaided eye. The stereomicroscope is a type of optical microscopy, which typically utilizes light reflected from the surface of an object. This allows examination of objects that are

too thick and would appear opaque when viewed under conventional microscopy. It is designed for low magnification observation of a sample, usually conducted using magnification ranging from x8 to x100 under high-level illumination. Previous studies used stereomicroscopes to examine the cross-sectional root surface or evaluate non-sectioned apical root surface (12, 67).

The main limitation of this method is that the stereomicroscope does not allow full-range inspection of the 3-dimensional structure or an assessment of diffused damages or subsurface defects, which are early signs of dentin tissue damage (62). Moreover, if there are irregularities on the resected root surface, they may cause light to be reflected in a way that may mask the presence of micro-cracks (86). These limitations make this technique difficult to accurately identify early/initial dentinal defects, resulting in greater examiner variability.

3.4.3 Micro-computed Tomography (Micro-CT)

Micro-CT uses x-rays to create cross-sections of a 3-dimensional object that can be used to recreate a virtual 3-dimensional model without destroying the original object. Micro-CT was first applied to endodontic research about 13 years after it was developed (89). The main benefit of micro-CT is that it is a non-destructive reproducible method that allows for quantitative or qualitative evaluation of root canal systems (90). Unlike sectioning or stereomicroscopy, micro-CT allows assessment of the presence of cracks along the longitudinal axis of the root. Moreover, samples can be scanned before the experimental procedure, affording

detection of pre-existing defects. Considering that this a non-destructive method, the same sample can be scanned following the experimental procedure as well, allowing direct comparison with the pre-experimental scan, thus, serving as its own control.

The main limitation of micro-CT is that detailed 3-dimensional model of root canal systems is dependent on the resolution of computed tomography. Technically, the highest currently achievable micro-CT resolution *in vitro* ranges from 5 to 10 μm . With lower resolution, the exact reconstructions of the sample could not be achieved, therefore, resulting in underestimation of dentinal defects (85). Moreover, scanning and reconstruction procedures can take considerable time and this technique is not suitable for clinical use (80).

Overall, micro-CT provides a clear visualization of micro-cracks with high accuracy, allowing for evaluation of the presence, morphology and spatial location of dentinal cracks (85).

3.5 Digital Moiré Interferometry (DMI)

DMI is a highly sensitive, optical method for measuring in-plane deformations on the surface of a specimen. Commonly used optical systems in DMI have the following components: (i) illumination system, which consists of a coherent light source, (ii) beam expander and collimator, which can divide the input beam into two or more separate beams and directs them into the specimen grating, and (iii) camera system, which allows to capture and view the moiré fringe pattern (30) (Figure 1).

Figure 1. Schematic diagram of a two-beam moiré interferometer

3.5.1 Principle of DMI

DMI relies on the principle of optical diffraction and interference and provides the whole-field patterns with high spatial resolution and excellent clarity. It is based upon an optical phenomenon observed when two closely spaced gratings are superimposed. DMI results from the interaction of a deformed grating with an undeformed grating and the resultant light and dark bands are called moiré fringes. In the experimental setup, a high frequency (frequency of 1200 lines/mm) cross grating is replicated on the surface of the specimen and a virtual grating, usually of the same pitch, is placed adjacent to the specimen and aligned parallel to the

specimen grating. Following the experimental procedures, such as applying a load or instrumenting a canal space, if the surface undergoes any deformation it will be transferred to the grating with high accuracy. The virtual grating stays consistent, and, as a result, the deformed grating of the specimen and the virtual reference grating interact and give rise to an interference pattern of moiré fringes. The moiré fringe pattern is then captured and viewed through a CCD camera (30).

3.5.2 Applications of DMI

DMI is an established technique for high sensitivity determination of in-plane displacements on flat surfaces. This technique is used to study various materials including composite materials, polycrystalline materials and piezoelectric materials. It is also used to examine fracture mechanics, biomechanics, structural elements and structural joints, in the measurement of residual stress and strain gauge calibration (91). In dentistry, Wang and Weiner (1998) used DMI to study relationship between strain and structure in enamel and dentin (92). They found that the strain exhibited in enamel was significantly lower in comparison to strain in dentin. Kishen and Asundi (2001) used DMI to investigate the functional adaptation of dentin to thermal variations. They observed significant gradients in thermal strain and coefficient of thermal expansion within dentin (32). Furthermore, Kishen and Asundi (2005) used DMI to investigate the role of free water on the in-plane, mechanical strain response in dentin structure (29). They found that the water had an effect on the stress-strain response in both axial and lateral directions. In dehydrated dentin, a decreased strain at fracture and reduced plastic energy during

deformation resulted in brittle response and strain hardening in dentin. Furthermore, this DMI study showed the importance of free water in uniform distribution of strains within dentin structure (29).

Kishen and Asundi (2005) also used DMI to examine the relationship between macroscopic mechanical stress and strain gradients within the root dentin structure (93). The strain analysis showed a reduction in strain from the cervical to the apical third of the root dentin and root dentin displayed uniform distribution of normal strains.

3.5.3 Advantages of using DMI to Study Deformation

The following are advantages of using DMI: (i) It is a real-time technique whereby displacement fields can be viewed as loads are applied (ii) It is a whole-field non-contact method with high sensitivity to in-plane displacement in U and V field (iii) It has a high spatial resolution; therefore, measurements can be made in minute regions of interest within high signal to noise ratio. DMI has a large dynamic range in their measurements; hence, it is suggested to be compatible with small and large displacements, with no correlation requirements (30).

Although DMI is a useful experimental technique to provide sensitive and accurate strain information in microstructures, to date there are no reports on its applications to determine residual micro-strain in root dentin subsequent to Ni-Ti instrumentation of the root canals.

3.5.4. Summary of Current Literature

A wide range of the prevalence of VRF, ranging from 2-20%, in endodontically treated teeth has been reported (44-46). VRF represents one of the most difficult clinical problems in dentistry to diagnose and treat. Being considered one of the most common cause for tooth extraction in endodontically treated teeth (44-46), a thorough understanding of the etiology and risk factors related to VRF is crucial in clinical practice to ascertain the prognosis of endodontically treated teeth. Many *in vitro* studies have highlighted the propensity for crack formation after routine endodontic procedures such as root canal preparation, root filling, retreatment and post preparation (4, 8, 58, 59). Clinical studies and empirical observations have suggested that, in general, teeth are more likely to fracture when more tooth structure is lost to disease or iatrogenic procedure (3, 4).

Several studies have investigated the mechanism behind increased propensity for cracks and fractures in endodontically treated teeth. It has been suggested that the high prevalence of VRF in endodontically treated teeth can be attributed to dehydration, resulting in less elastic and more brittle dentin after endodontic treatment (103). The water molecules in dentin matrix offer the critical fracture toughening mechanisms and viscoelastic properties to dentin (21, 82). Therefore, even partial loss of free water, that occurs when a tooth is exposed to ambient condition, would produce significant alteration in the stress-strain behavior and resistance to fracture in dentin (21, 33, 83). Thus, it can be concluded that degree of hydration is an important factor that can influence resistance to fracture of teeth.

A close critical appraisal of the methods used to study dentin cracks showed that the large discrepancy in the incidence of micro-defect formation may be attributed to large variations in experimental methods (85). Therefore, it is apparent from the literatures that, currently, there are lack of consensus among studies that examined the relationship between micro-crack formations after canal preparation and an exact cause of VRF is still unclear and debatable.

To promote the understanding of the biomechanical response of root dentin to instrumentation, this study applied a novel device, DMI. While the biomechanical response of root dentin to canal instrumentation and its effect on the dentin's mechanical integrity has been addressed (4, 8, 10, 11, 13), determining residual strain is a challenge. With the use of DMI, which provides sensitive and accurate strain information in microstructures, this study determined residual micro-strain in root dentin subsequent to Ni-Ti instrumentation of root canals.

II. OBJECTIVES AND HYPOTHESIS

1. Objectives

The objectives of this study were to (1) determine residual micro-strain in root dentin subsequent to Ni-Ti instrumentation of canals using DMI and (2) to explore its correlation with formation of dentinal micro-defects, in teeth maintained in hydrated and non-hydrated environments.

Specifically the study aimed to:

- Determine the residual micro-strain distribution pattern in root dentin produced by rotary and reciprocating Ni-Ti instrumentation using customized DMI.
- Detect the presence of subsurface damage and micro-defects in root dentin using Micro-CT and Polarized Light Microscope (PLM).

2. Hypothesis

We hypothesized that:

- Residual micro-strain will form subsequent to rotary and reciprocating Ni-Ti instrumentations in teeth maintained in hydrated and non-hydrated environments.
- Formation of residual micro-strain will correlate with the formation of micro-defects in root dentin.

III. ARTICLE

(Submitted for publication)

Residual Micro-strain in Root Dentin after Canal Instrumentation Measured with Digital Moiré Interferometry

Helena Lim MA, DDS, Fang-Chi Li DDS Cert Endo, Shimon Friedman DMD,

Anil Kishen BDS, MDS, PhD

Discipline of Endodontics / Dental Research Institute, Faculty of Dentistry, University of
Toronto, Toronto, Canada

****Corresponding author**

Dr. Anil Kishen

Professor, Discipline of Endodontics & Dental Research Institute, Faculty of Dentistry,
University of Toronto, Toronto, M5G 1G6, Canada

E-mail: anil.kishen@utoronto.ca

Abstract

Background: Residual micro-strain influences the resistance to crack propagation in a biomaterial. **Objective:** This study evaluated the residual micro-strain and micro-defects formed in dentin after canal instrumentation in teeth maintained in a hydrated and non-hydrated environments. **Methods:** Canals of 18 extracted human premolars with single root canals were instrumented in accordance with 3 groups: PTU –ProTaper Universal (S1, S2, F1, F2) used in rotation; WO – Wave-One (Primary) used in reciprocal motion; Control – hand files. Half the specimens (3/group) were maintained in deionized water (hydrated) and half in ambient conditions (22°C, 55%RH) for 72 hours (non-hydrated). Customized high-sensitivity digital moiré interferometry (DMI) was used to qualitatively evaluate pre- and post-instrumentation dentinal micro-strain. Subsequently, specimens were examined for dentinal micro-defects with micro-computed tomography (micro-CT) and polarized light microscopy (PLM). **Results:** DMI showed only minor changes in post-instrumentation micro-strain in hydrated dentin in all groups, suggestive of a strain relaxation behavior. Non-hydrated dentin in all groups showed localized concentration of post-instrumentation micro-strain, which appeared higher in WO than in the other groups. No dentinal micro-defects were detected by micro-CT and PLM in hydrated and non-hydrated specimens in all groups. **Conclusions:** This study suggested that the biomechanical response of root dentin to instrumentation was influenced by hydration. Reciprocating, rotary and hand instrumentation of well-hydrated roots did not cause an increase in residual micro-strain or formation of micro-defects in root dentin.

Keywords: Residual micro-strain, micro-defects, canal instrumentation, dentin dehydration, moiré interferometry

Introduction

Endodontic treatment is commonly performed to treat or prevent apical periodontitis in order to retain a functional tooth. Despite important improvements in the provision of endodontic treatment, up to 4 to 7% of endodontically treated restored teeth are lost within 5 years after treatment because of fractures (1). Root fractures, specifically, were reported in 6% of endodontically treated teeth in a recent 10-year survival study (2). Studies have suggested that, in general, teeth are more likely to fracture when more tooth structure is lost to disease or iatrogenic procedures (3-5), including access cavity preparation (6) and root canal instrumentation during endodontic treatment (7, 8).

Currently, different nickel-titanium (Ni-Ti) instrument systems are commonly used in rotary or reciprocating motion to shape root canals with minimal procedural errors (4). The WaveOne system (Dentsply Maillefer, Ballaigues, Switzerland) is designed for use of a single reciprocating instrument to expedite canal shaping compared to full-sequence rotary instrumentation systems such as ProTaper Universal (PTU) (Dentsply Maillefer) (7). Recent reports of micro-defect formation in root dentin after canal instrumentation with Ni-Ti engine-driven instruments have suggested possible adverse impacts of these instruments (7-11), but research data have been inconsistent. While reciprocating instruments produced more incomplete dentinal cracks at the apical level of canals than full-sequence rotary instruments in one *in vitro* study (7), continuously rotated instruments produced more dentinal cracks compared to the reciprocating instruments in another *in vitro* study (9). In contrast, no association could be established between micro-cracks and different root canal instrumentation techniques in a cadaver model (73).

Factors such as instrument design, alloy, cutting efficiency, size, taper and number of instruments used, as well as stress concentration, have been suggested as contributing factors in formation of dentinal micro-defects (4, 8, 10-12).

Dentin is a porous biologic composite with 50 wt. % inorganic phase, 40 wt. % organic phase and 10 wt. % water (14), comprised of free- and bound water. Free water, representing approximately 30% of dentin's total water, occupies the dentinal tubules, porosities and some of the matrix. Bound water, representing approximately 70% of dentin's total water, is tightly bound to the hydroxyapatite and collagen fraction of the dentin (5). Water molecules offer the critical fracture toughening mechanisms and viscoelastic properties to dentin matrix (21, 82), so that even partial loss of free water produces significant alteration in the stress-strain behavior and resistance to fracture in dentin (21, 33, 83). The loss of free water, occurring when a tooth is exposed to ambient room conditions, induces residual strain in dentin (5). Residual strain is the permanent strain experienced in a material when stressed beyond the elastic limit; it can alter the material's response to forces and crack propagation, and thus influence the material's mechanical integrity (94).

While the biomechanical response of root dentin to canal instrumentation and its effect on the dentin's mechanical integrity have been addressed in several investigations (4, 8, 10, 11, 13) determination of residual strain is a challenge. A method widely used as the precise metrology in several science and engineering fields is digital moiré interferometry (DMI), a whole-field, non-contact method based on optical interferometry. DMI has been used to study the strain-structure relationship in dental hard tissues subjected to compressive forces (29, 32, 95) and the influence of hydration on the thermal strain

response to dental hard tissues (96). DMI can also be applied to determine micro-strain in root dentin with high sensitivity and resolution (95). The objectives of this study were to determine residual micro-strain in root dentin subsequent to Ni-Ti instrumentation of canals using DMI and to explore its correlation with formation of dentinal micro-defects, in teeth maintained in hydrated and non-hydrated environments.

Materials and Methods

Experiments were conducted in two stages. First, the residual micro-strain in root dentin, before and after rotary or reciprocating instrumentation, was determined with DMI in teeth maintained in hydrated and non-hydrated environments. Second, the presence of dentinal subsurface micro-defects was examined with micro-computerized tomography (micro-CT) and with polarized light microscopy (PLM). The Research Ethics Board of the University approved the collection of teeth for this study (protocol #29929).

Specimens

Sample size calculation was not conducted due to the qualitative nature of this study. Eighteen extracted human premolars with complete root formation and single straight root canals were stored in deionized water. Only non-carious teeth with fully developed roots, without visible cracks, fractures or root resorption (internal or external) were selected. Selected teeth were exposed radiographically (Digora Soredex, Helsinki, Finland) in the mesio-distal and bucco-lingual planes and the canal dimensions used to standardize the sample. Additionally, teeth were imaged with micro-CT (SkyScan 1172, Toronto, Canada) at an isotropic resolution of 8 μm (pre-treatment scan) to detect any preexisting craze lines or micro-defects in root dentin.

Endodontic access cavities were prepared with diamond burs (F392-016; Axis Dental, Coppell, TX) at high speed under water-spray cooling, and extended till the roof of the pulp chamber was completely removed. Canals were negotiated with ISO size 10 K-type files (Flexofile; Dentsply Maillefer) to the major apical foramen and the working length (WL) established 0.5 mm shorter. A glide path was established with ISO size 15 K-type files while irrigating canals with 10 mL of deionized water.

Specimens were standardized by resecting the roots at 13 mm length from the buccal cusp tip using a water-cooled low-speed diamond saw (UKAM, Precision smart cut, Valencia, California, USA). Specimens were then maintained for 72 hours before diffraction grating replication and experimental procedures, 9 specimens in deionized water (hydrated) and 9 specimens in ambient conditions (22°C, 55% RH) (non-hydrated).

Residual micro-strain assessment

A high frequency cross-line diffraction grating, the deformation-sensing element in DMI, with a frequency of 1200 lines/mm, diffraction efficiency of 10% and intensity variation of <15%, was replicated on the apical resected surface of the root with a thin layer of epoxy (J-B MarineWeld, Sulphur Springs, TX) and kept in ambient conditions (22°C, 55% RH) for 12 hours to set. The grating lines were aligned parallel and perpendicular to the bucco-lingual axis of the root's cross-section. The distance between adjacent grating lines, referred to as the pitch of grating, was 0.833 μm . After completion of grating replication, all specimens were randomly divided into 2 experimental and 1 control group ($n = 3$), and their canals instrumented as follows: PTU group – PTU instruments S1, S2, F1 and F2 used in full rotation; WO group – WaveOne Primary instrument used in

reciprocating motion; C group (control) – ISO size 20 and 25 K-type files. Instrumentation followed the manufacturer's instructions with new instruments used for each specimen. Due to sensitivity of grating material to large amount of liquid, all instrumentations were done without any irrigation.

Customized DMI, which consist of a moiré interferometry system linked to a digital image processing system was used to visualize post-instrumentation residual micro-strain in root dentin (Fig. 1), using a virtual reference grating formed by the interference of two mutually coherent beams from a diode laser ($\lambda = 670$ nm) that were incident on the specimen plane at a fixed angle. Patterns of moiré fringes (resulting from interference between the deformed specimen grating and the virtual reference grating), representing residual micro-strain distribution, were acquired using a high resolution CCD camera. Samples were stored in deionized water following the experiment. The strain distribution patterns for each specimen at the buccal, lingual, mesial and distal regions of interest in proximity to the root canal lumen (Fig. 2), were determined as follows:

- From the U-field fringe pattern, the strain (ϵ_x) in X direction (mesial-distal; Fig. 2 C, D) is given as: $\Delta U / \Delta x = P / \Delta x$, where U = relative displacement in x direction between two points; P = pitch of the reference grating; Δx = fringe spacing in x direction).
- From the V-field fringe pattern, the strain (ϵ_y) in Y direction (buccal-lingual; Fig. 2 A, B) is given as: $\Delta V / \Delta y = P / \Delta y$, where ΔV = relative displacement in y direction; Δy = fringe spacing in y direction).

The high sensitive, moiré fringe analysis in this manner allow only for qualitative assessment of the residual micro-strain distribution in root dentin in response to canal instrumentation.

Detection of subsurface micro-defects in root dentin

After completion of the DMI phase of the study, all 18 specimens were imaged with micro-CT (post-treatment scan) to detect dentinal micro-defects, using isotropic resolution of 8 μm and 3-D software (Amira 5.2.2, Visage Imaging, Berlin, Germany) for visualization. Subsequently, specimens were cross-sectioned using water-cooled low-speed saw to render 1 mm thick root dentin slices including the surface examined for residual micro-strain. These root dentin slices were examined with PLM (Leitz Wetzlar microscope, Wetzlar, Germany) under x16 and x40 magnification and micrographic images captured (AxioCam, Carl Zeiss, Oberkochen, Germany). Any subsurface micro-defects initiating from the root canal lumen observed in the micro-CT and PLM images were related to the regions of micro-strain concentration, examined with DMI, in the root dentin to explore possible correlation.

Results

Residual micro-strain in root dentin

In PTU group (Fig. 3 A), the mesial region in hydrated specimens displayed minor increase and decrease fluctuation in residual micro-strain after each instrumentation step, from S1 through F2, without localized micro-strain concentration. In non-hydrated specimens, the mesial region displayed a pronounced increase in residual micro-strain after instrumentation with S1 and a further pronounced increase after instrumentation

with F1 (Fig. 3A), with localized regions of concentration (Fig. 2 A, B arrow). Buccal, lingual and distal regions of interest also showed the similar pattern of residual micro-strain following the instrumentation.

In WO group (Fig. 3 B), the distal and buccal regions of the hydrated specimens did not display residual micro-strain. The mesial and lingual regions displayed minor increase in residual micro-strain, in the range observed in hydrated PTU specimens, without localized micro-strain concentration. In non-hydrated specimens, all four regions surrounding the canal lumen displayed concentrated, prominent increase in residual micro-strain.

In C group (Fig. 3 C), no or very minor residual micro-strain was evident in hydrated specimens. In non-hydrated specimens, the mesial, distal and buccal regions displayed varying degrees of increase in residual micro-strain; this increase appeared smaller compared to the WO group and within the range observed in the PTU group. The lingual region displayed a minor decrease in residual micro-strain.

Subsurface micro-defects in root dentin

No obvious micro-defects in root dentin were detected by micro-CT or PLM in any of the examined specimens, both hydrated and non-hydrated.

Discussion

In vitro studies have reported a wide range of incidence of post-instrumentation micro-defects in root dentin (4, 7, 8, 67) using mostly destructive models to detect micro-defects, such as sectioning teeth for light microscopic examination (4, 7, 8, 10, 11). To promote the understanding of the biomechanical response of root dentin to

instrumentation, this study applied DMI to assess post-instrumentation residual micro-strain and explored its possible correlation with formation of micro-defects in root dentin. A customized DMI was used to enable detection of residual micro-strain in specific regions of interest in dentin. The protocols for specimen preparation and DMI were previously calibrated (95). Because spatial gradients in the microstructure and elastic modulus of dentin markedly influence micro-strain, assessment of the nature of strain distribution in dentin with DMI is generally qualitative (95).

Assessment of residual micro-strain patterns was coupled with examination for micro-defects to explore potential association between these two occurrences in root dentin in response to canal instrumentation. Dentin representing the apical third of examined roots was specifically targeted for assessment considering that crack-initiation leading to vertical root fracture frequently occurs at the radicular portion of the tooth (7). Pre- and post-instrumentation high-resolution micro-CT was selected as a non-destructive, 3-dimensional detection method of root dentin micro-defects, as previously described (62). It was corroborated by PLM examination. Because polarized light oscillates in only one plane, it produces birefringence upon interaction with the constituents of dentin (97) which, in turn, improves visualization of subsurface defects compared to ordinary transmission light microscopy.

ProTaper Universal and WaveOne instruments used in rotation and reciprocation, respectively, were assessed in this study because of the contrasting reports on their biomechanical impact on root dentin resulting in dentinal micro-defects (7-10, 73). Hand-files were used as control based on previous reports suggesting that their use does not result in dentinal micro-defects (10, 61). Sub-groups of specimens were maintained in

hydrated and non-hydrated environments to explore potential differences in the biomechanical response of root dentin to instrumentation. The impact of the loss of free water on the stress-strain behavior and resistance to fracture in dentin has previously been demonstrated (21, 33).

In the present study, the response to instrumentation of hydrated root dentin was characterized by no change or minor increase or decrease in residual micro-strain, regardless of the instrumentation method. This finding suggested that the residual micro-strain observed in the hydrated roots was below the proportional limit of dentin, with instrumentation resulting in release of residual strain, time-dependent viscoelastic relaxation, and redistribution of internal stress. On the other hand, the response to instrumentation of non-hydrated root dentin was characterized by increase in residual micro-strain and localized regions of strain concentration. This finding suggested that the increased residual micro-strain teeth observed in the non-hydrated roots exceeded the yield strength of dentin, resulting in strain concentration and localized yielding (permanent deformation), such that might lead to micro-cracks in root dentin (21).

Well-hydrated dentin typically displays a linear viscoelastic behavior (84). Water molecules provide a plasticization effect that increases the transient and viscous creep strains and reduces the load sustainability in dentin (84). Strain placed on a viscoelastic material induces corresponding stress in that material, which decreases over time (stress relaxation) (33). When dentin absorbs stress, its viscoelastic properties enable dissipation of energy (34). But when dentin is subjected to repeated stress or air-drying, the rate of stress relaxation becomes slower than in hydrated dentin (33). Free water from dentin is lost in a biphasic manner when exposed to ambient room conditions (5). Rapid and

highest degree (80-85%) of free water loss from dentinal tubules and porosities occurs in 30 to 120 min (depending on tooth section) (*phase-1*), followed by gradual loss of free water from the dentin matrix (*phase-2*) (5). Loss of free water from the dentin matrix is associated with shrinkage (82) and residual micro-strain (5).

In the present study, the hydrated roots, which were maintained in ambient environment for 12 hours during grating procedure, would have lost most of the free water from dentinal tubules, whereas the non-hydrated roots, maintained in ambient environment for 72 hours, would have lost free water from dentinal tubules and the dentin matrix. Thus, the observation of higher residual micro-strain in the root dentin in the non-hydrated roots was likely attributed to loss of additional free water (5). Among the groups that were examined, WO group displayed the biggest difference in residual micro-strain when comparing the root dentin maintained in hydrated environment to those maintained in non-hydrated environment. This is consistent with the previous study that reported that the reciprocating instruments were more prone to promoting dentin micro-defects because the use of single large-tapered reciprocating file, which cuts large amounts of dentin at once have the tendency to aggravate more dentinal defects than gradual enlargement with multi-filed rotary system (7). Moreover, previous studies showed that the incidence of dentinal defects was significantly lower when canal is instrument with hand-files than any mechanical instrumentation (10, 61).

Despite increased residual micro-strain in the non-hydrated roots in response to instrumentation, no micro-defects in root dentin were detected by micro-CT or PLM, in contrast to post-instrumentation dentinal micro-defects reported previously (4, 7, 8, 67). This inconsistency might be attributed to differences in specimen preparation, sample

selection, methods used to detect dentinal defects and the classification of defects (85). Because several studies (9, 11, 86) regarded any lines observed in the section, extending from the outer root surface into the dentin or from the root canal lumen to the dentin, as defects, they might have overestimated the incidence of defects caused by instrumentation. Moreover, it has been reported that some of the earlier studies on dentin crack formation did not specify or standardize the storage and experimental conditions with respect to dentin hydration (85); teeth were stored in preservation medium such as formalin (13, 56) and sodium hypochlorite (87), which could compromise the mechanical characteristics of dentin (88). Therefore, unless the level of free water is standardized throughout the experiments, it cannot be concluded that any defects observed post-instrumentation are due to the instrumentation procedure alone.

In summary, the biomechanical response of root dentin to root canal instrumentation was influenced by dentin hydration. In hydrated roots instrumentation with hand, reciprocating or rotary Ni-Ti instruments did not result in residual micro-strain concentrations. Corresponding instrumentation of non-hydrated roots caused localized micro-strain concentration and reduced strain relaxation, but this biomechanical impact did not result in detectable dentinal micro-defects.

Acknowledgements

Supported in part by the American Association of Endodontists Foundation and the Canadian Academy of Endodontics Endowment Fund.

The authors deny any conflicts of interest related to this study.

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Figure legends

Figure 1. Schematic diagram of the digital moiré interferometer

Figure 2. Shows a typical digital moiré fringe patterns in root dentin in specimen maintained in non-hydrated environment

(A) V-field moiré fringe patterns prior to instrumentation; (B) After instrumentation

(C) U-field moiré fringe patterns prior to instrumentation; (D) After instrumentation

The red box indicates the region of interest in proximity to the root canal lumen and the red arrow indicates the localized regions of high strain concentration

Figure 3. Shows a typical pattern of residual micro-strain of a sample from each group:

(A) Post-instrumentation (PTU group) residual micro-strain of the hydrated (in blue) and non-hydrated (in red) specimens at the mesial region of interest after instrumentation with PTU rotary S1, S2, F1 and F2 files. Buccal, lingual and distal regions of interest also showed the similar pattern of residual micro-strain following the instrumentation

(B) Post-instrumentation (WO group) residual micro-strain of the hydrated (in blue) and non-hydrated (in red) specimens at the buccal, lingual, mesial and distal regions of interest after the instrumentation with Primary WaveOne reciprocating file

(C) Post-instrumentation (C group) residual micro-strain of the hydrated (in blue) and non-hydrated (in red) specimens at the buccal, lingual, mesial and distal regions of interest after instrumentation with size 20 and 25K-type files

Figure 1.

Figure 2.

Figure 3.

IV. DISCUSSION

Previous studies have reported a wide range of incidence of micro-defects in root dentin post-instrumentation (4, 7, 8, 67). These studies highlighted instrumentation-induced dentinal defects as trigger points of VRF, which consequently would impact tooth survival (54, 64, 65). The main limitations in majority of these studies were that they used destructive models to detect micro-defects. Examining the dentinal defects by sectioning the tooth and viewing it under transmitted light microscope were the most commonly used approach (4, 8, 56, 67). These experimental methods can reduce the overall impact of the outcome measure by precluding inspection of the 3-dimensional structure, because they only allow examination of the defects present on the cross-sectioned root surface. Though presence of micro-defects or diffuse damage in dentin might contribute to decreased resistance to fracture in endodontically treated teeth, little is known about their occurrence and accumulation of diffuse damage in instrumented root canals.

To examine the biomechanical response of root dentin to instrumentation, this study applied a novel method, DMI. This method was widely used for precision metrology in several science and engineering fields. It was applied in dental biomechanics to study the strain-structure relationship in dental hard tissues subjected to compressive forces (29, 32, 95) and the influence of hydration on the thermal strain response of dental hard tissues (96). Unlike other conventional mechanical tests, DMI is a very specialized whole-field nondestructive optical technique that allows detection of micro-strain from the root dentin with high

spatial resolution (95). In this study, customized DMI was used to examine post-instrumentation residual micro-strain in specific regions of interest in dentin with high sensitivity (95).

Moiré fringes, which represented contours of displacement components, were analyzed using an image processing software. The moiré patterns defining the U displacement field are formed by the interaction of x family of the specimen grating lines with the virtual grating and the V displacement field is formed by the interaction of the y family of specimen grating lines with the virtual grating (95). The grating was replicated on the root surface cross-section at the junction of apical and middle third. This region was chosen because crack-initiation factors leading to VRF are known to occur at the radicular portion of the tooth, progressing towards the crown (95). Assessment of the strain distribution in dentin with DMI is generally qualitative because: (1) spatial gradients in the microstructure and elastic modulus of dentin markedly influence micro-strain and these features could vary significantly among different teeth and (2) high sensitive nature of DMI (95).

Visualization of micro-defects in dentin and comparison with residual micro-strain patterns was intended to explore the potential association between residual micro-strain and micro-defects in root dentin. Pre- and post-instrumentation micro-CT images of the samples were captured at 8 μm resolution (62). Considering that micro-CT is a non-destructive 3-dimensional method, this method afforded comparison of the pre- and post-instrumentation images of the same sample.

PLM was used to examine the subsurface micro-defects and early structural changes in bulk dentin. Polarized light oscillates in only one plane and has a single wavelength. The inorganic crystals and collagen fiber of structural biomaterials is capable of splitting a polarized light into two, resulting in *birefringence*. This property is observed in structures like enamel and dentin because of their inherent crystalline nature (97). Improved visualization of the microstructural features of dentin ensues when examined under polarized light, which may not be perceived under transmitted light microscopy. Previous investigation has applied PLM combined with digital image processing to estimate micro-damages in dentin (97).

For experimental groups, ProTaper Universal system and WaveOne instruments used in rotation and reciprocation motion, respectively, were chosen because previous studies showed contrasting reports on their biomechanical impact on root dentin resulting in micro-defects (7-10, 73). As a control group, hand-files were used because previous studies reported that instrumentation with hand-files did not lead to dentinal micro-defects (10, 61). Furthermore, to explore potential affect of hydration in the biomechanical response of root dentin to instrumentation, specimens were subdivided into hydrated and non-hydrated subgroups.

A previous study showed that about 80% of free water is lost in 2 hours at 22°C and about 86% of bound water is lost in 2 hours at 105°C (32). Therefore, in this study, the hydrated samples would have lost most of the free water from dentinal tubules, whereas the non-hydrated samples would have lost free water from both dentinal tubules and the dentin matrix. However, both groups would have maintained bound

water in the dentin matrix because they were kept under room temperature. Thus, the observation of higher residual micro-strain in root dentin in the non-hydrated roots was most likely attributed to the loss of additional free water from the dentin matrix (5).

In teeth maintained in hydrated environment, we observed generalized decrease or no changes in the residual micro-strain and no localized region of micro-strain concentration post-instrumentation. This result can be explained by the fact that, when initial residual strain is within or below the proportional limit, instrumentation produces conspicuous reversible deformation in dentin. Hence, in hydrated samples, root canal instrumentation resulted in the release of residual strain and redistribution of internal stress. This response of dentin can be attributed to the time-dependent viscoelastic relaxation in dentin.

On the other hand, in teeth maintained in non-hydrated environment, a generalized increase in residual micro-strain and localized regions of high strain concentration were observed post-instrumentation. When initial residual strain is above the yield strength, instrumentation may induce localized yielding. Therefore, in non-hydrated samples, increased residual micro-strain exceeded the yield strength of dentin, resulting in the strain concentration and localized yielding, which might lead to micro-cracking (30).

Despite the observation of increased residual micro-strain in the non-hydrated roots in response to instrumentation, we did not observe any micro-defects in root dentin via micro-CT or PLM. This finding was contradictory to the previous reports

of post-instrumentation dentinal micro-defects (4, 7, 8, 67). This inconsistency might be due to differences in experimental and observational methods and the inconsistent classification of defects among studies (85). Thus, without proper standardization of experimental methods and analysis between studies, it could lead comparisons of the results to erroneous interpretations.

V. CONCLUSION

In hydrated specimens, instrumentation with hand, ProTaper Universal and WaveOne Ni-Ti instruments did not result in residual micro-strain concentrations, whereas in non-hydrated specimens, instrumentation with ProTaper Universal and WaveOne resulted in localized micro-strain concentrations and reduced strain relaxation. However, Micro-CT and PLM did not reveal obvious dentinal micro-defects in specimens from all groups, in both hydrated and non-hydrated specimens. This study suggested that the biomechanical response of root dentin to canal instrumentation was influenced by hydration in dentin.

VI. Future Directions

Further research is required to quantitatively examine the degree of hydration after root canal treatment and their influence on dentin micro-crack as a function of time. Moreover, a quantitative study to compare post-instrumentation residual micro-strain among different instrumentation groups will promote evaluation of

differences in residual micro-strain among the groups. It is important to develop a non-invasive technique to measure residual micro-stain without sectioning the samples. Lastly, an *ex vivo* model to study the micro-strain experienced during the endodontic procedure will promote the understanding of the biomechanical impact of instrumentation in root dentin.

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VIII. Appendix

Table 1. Summary of current literature assessing the epidemiology of vertical root fracture of endodontically treated teeth

Authors, Year	Operator	Dx Method	Follow-up	% of VRF
Sjögren, 1990 ⁽⁴⁵⁾	Dental students	Extraction	8-10 years	31
Morfis, 1990 ⁽⁴⁸⁾	Endodontist	Radiographic & Clinical exam	At least 3 years	3.7
Vire, 1991 ⁽⁴⁹⁾	NA	Extraction	NA	13
Fuss, 1999 ⁽⁴⁶⁾	General dentists	Extraction	NA	11
Caplan, 1997 ⁽⁵⁰⁾	NA	Radiographic & chart entries	7 years	20
Heft, 2000 ⁽⁵¹⁾	NA	Clinical exam	2 years	10.3
Zadik, 2008 ⁽⁵²⁾	NA	Extraction	NA	11
Borén , 2015 ⁽²⁾	Endodontist	Dental record/speaking to referring dentist or patients	10 years	6

Table 2. Summary of current literature evaluating dentinal defects formed in root dentin after root canal preparation with various Ni-Ti instrumentation systems

Authors/year	Dentinal Defect Formation
Shemesh, 2009 ⁽⁸⁾	More defects: GG, GT
Bier, 2009 ⁽⁴⁾	No defect: unprepared, hand files, S-ApeX

	Defects: GT 4%, Profile 8%, ProTaper Univ. 16%
Burklein, 2013 ⁽⁷⁾	Complete cracks: Reciproc > Rotary Incomplete apical cracks: Reciproc/WO > Rotary
Liu, 2013 ⁽⁶⁷⁾	Cracks: SAF, Reciproc < ProTaper Univ., One Shape
Ustun, 2015 ⁽⁶¹⁾	Incomplete fractures: All groups (ProTaper Univ., ProTaper Next, Reciproc)

Table 3. Summary of the methodology and main findings of studies evaluating the incidence of dentinal defects after different endodontic treatment procedures

Authors, Year	Aim/Methods	Main Findings
EX VIVO		
Barreto. 2012 ⁽⁹⁸⁾	Aim: to evaluate the ex vivo effects of root canal preparation,	- MC by itself did not induce VRF. - The filled groups presented a

	<p>filling techniques, and mechanical cycling (MC) on the incidence of dentin defects and VRF.</p> <p>Methods: Ex vivo. 70 extracted teeth divided into 6 groups: 1) unprepared, 2) unprepared/MC; groups prepared by Gates Glidden, Protaper universal files – 3) absence of root canal filling, 4) passive technique, 5) lateral compaction, and 6) tagger's hybride technique. MC on all but unprepared group. Roots sectioned and viewed under stereomicroscope. Chi-square test conducted.</p>	<p>similar incidence of other defects, although VRFs were observed only when the MC was associated with pressure filling techniques - 13.3% (lateral compaction) and 33.3% (Tagger's hybrid) of the cases.</p>
Shemesh, 2009 ⁽⁸⁾	<p>Aim: To evaluate ex vivo the incidence of defects in root dentine before and after root canal preparation and filling.</p> <p>Methods: Ex vivo. 80 extracted mandibular premolars divided into 4 groups: 1) unprepared; all other groups prepared with Gates Glidden and Sys GT files, 2) filled with GP and AH26 master cone, 3) passive insertion of secondary GP pts, 4) lateral compaction. Roots sectioned and viewed under a microscope.</p>	<ul style="list-style-type: none"> - No defects in the roots with unprepared canals. - Overall difference between the groups was sig. - Canal preparation alone created sig. more defects than unprepared canals. - Total number of defects after lateral compaction was sig. larger than after noncompaction canal filling. - Root canal preparation and filling of extracted teeth created dentine defects such as fractures, craze lines and incomplete cracks
IN VITRO		
Bier, 2009 ⁽⁴⁾	<p>Aim: to compare the incidence of dentinal defects (fractures and craze lines) after canal preparation with different Ni-Ti rotary files.</p> <p>Methods: 260 mandibular premolars – 1) unprepared, 2) manual flexofiles, 3) protaper, 4) profile, 5) sysGT, 6) S-ApeX. Sectioned and viewed under a microscope.</p>	<ul style="list-style-type: none"> - No complete fracture (through and through) in any of the samples - Sig. difference in the appearance of defects between the groups. - No defects in unprepared, prepared with handfiles and S-ApeX. - Defects: GT 4%, Profile 8%, Protaper 16% - Some endo prep methods might damage the root and induce dentinal defects - Definition of Defect: at least one of three sections showed either a craze

		line, partial crack, or a fracture.
Sathorn, 2005 ⁽⁵⁶⁾	<p>Aim: to determine whether rotary Ni-Ti canal preparations strengthen the roots. Finite element analysis (FEA) of stresses in the canal walls of selected fractured roots was also undertaken to determine whether the observed fracture load and pattern could be predicted from the prepared canal shape.</p> <p>Methods: 50 teeth – 1) 25 with hand file (SS K-file), 2) 25 with rotary (Profile). After obturation, all teeth were subjected to loading, using a spreader tip mounted in an Instron testing machine, until fracture. Light microscope with 20X used to determine the fracture pattern.</p> <p>- FEA models were developed by digitizing a single cross-section of each root at the mid root level. Linear elastic isotropic analysis and eight-node hexahedral elements were chosen.</p>	<p>- No significant difference of fracture load between the two techniques was found (Rotary Ni-Ti canal prep did not reduce fracture susceptibility of the roots).</p> <p>- BL (36%) and MD (38%) fractures were observed in almost the same frequency and compound fracture was found in 26%. (BL fracture didn't predominate in this study, in contrast to previous root fracture studies)</p> <p>- MD fracture occurred more often in a rotary Ni-Ti group, and BL fracture occurred more often in hand instrumentation group.</p> <p>- Stress pattern in $\frac{3}{4}$ FEA models correlated well with the observed fracture patterns, whereas the fourth couldn't be predicted reliably because of the uniform stress distribution. The predictability of the pattern of fracture was reduced when the canal shape was round, because of the lack of a highly localized tensile stress area. Crack initiation can occur anywhere around a smooth, round canal surface unless the external root morph leads to highly localized stress.</p>
Kim, 2010 ⁽⁶⁴⁾	<p>Aim: to compare the stress distribution that may be generated in apical root dentin during rotary instrumentation in a curved canal with NiTi files featuring different shaft geometries (constant taper, progressive changing taper, and noncutting round shaft).</p> <p>Methods: Stresses were calculated using finite element (FE) analysis. 3D finite element analysis was</p>	<p>- ProTaper Universal induced the highest stress concentration in the root dentin and had the highest tensile and compressive principal strain components at the external root surface (ProTaper > Profile > LightSpeed)</p> <p>- The calculated stress values from ProTaper Universal, which had the biggest taper shaft, approached the strength properties of dentin. Light-Speed generated the lowest stresses.</p> <p>- Stiffer file designs generated</p>

	used to assess the stresses. 13 mm long curved root was modeled. The simulated canal was designed to have a sufficient lumen size for all file models to rotate inside. FE models of Profile, ProTaper Universal, and LightSpeed LSX were rotated within a curved root canal. The stress and strain conditions resulting from the stimulated shaping actions were evaluated in the apical root dentin.	higher stress concentrations in the apical root dentin during shaping of the curved canal, which raises the risk of dentinal defects that may lead to apical root cracking. Thus, stress levels during shaping and fracture susceptibility after shaping vary with instrument design.
Burklein, 2013 ⁽⁷⁾	<p>Aim: Evaluate the incidence of dentinal defects after root canal preparation with reciprocating instruments (Reciproc and WaveOne) and rotary instruments</p> <p>Methods: 100 human central mandibular incisors were assigned to 5 groups (n=20/group) – instrumented with reciprocating single-file system Reciproc, WaveOne and the full-sequence rotary Mtwo and ProTaper. One group was left unprepared as control. Roots were sectioned and evaluated under microscope.</p>	<ul style="list-style-type: none"> - No defects observed in controls - All canal preparation created dentinal defects. - Reciproc was associated with more complete cracks than the full-sequence files. - Both reciprocating files produced more incomplete cracks apically compared to rotary files (not sig. different)
Liu, 2013 ⁽⁶⁷⁾	<p>Aim: Compare the incidence of root cracks in the apical root surface and/or canal wall after instrumentation with the Reciproc, OneShape and SAF</p> <p>Methods: 100 single rooted Mand incisors with a single canal were assigned to 5 groups – instrumented with ProTaper, SAF, Reciproc, or OneShape. Control group had access opened but non-instrumented. Roots were sectioned and evaluated under microscope.</p>	<ul style="list-style-type: none"> - Cracks were seen in 50% of ProTaper treated teeth, 35% of the OneShape group and 5% of the Reciproc group. - No cracks were seen with the SAF. Results were sig with Reciproc and SAF. - AF and Reciproc created less cracks than ProTaper and OneShape files.
Lam,	Aim: to determine fracture loads	- The mean fracture load: 10.2

2005 ⁽¹³⁾	<p>in tooth roots after canal preparation using different techniques.</p> <p>Methods: MB roots of 39 extracted mandibular molars – 3 groups (13 root/group): prepared by SS hand-file (K-files), and two rotary NiTi techniques (Lightspeed and Greater Taper files).</p> <p>After obturation, a vertical load was applied by means of a spreader inserted into the canal until fracture occurred.</p>	<p>± 4.4kg for K-files; 15.7 ± 9.1kg for Lightspeed; 13.2 ± 6.1kg for Greater Taper files, but differences were not statistically sig.</p> <ul style="list-style-type: none"> - Most fracture lines were incomplete fractures on the B surface, followed by proximal and compound fractures. - Greater apical enlargement (Lightspeed) or increased canal taper (Greater taper files) did not increase fracture susceptibility of tooth roots.
Adorno, 2009 ⁽¹²⁾	<p>Aim: to compare the effects of root canal preparation techniques and instrumentation length on the development of apical root cracks.</p> <p>Methods: 40 extracted mandibular premolars with straight roots were randomly selected and mounted on resin blocks with simulated periodontal ligaments, and the apex was exposed. Four groups (10 teeth each): a) Step-back preparation (SB) with SS files (SF) using root canal length (RCL) to guide instrumentation length; b) SB using RCL – 1mm; c) crown-down preparation (CD) with Profile using RCL; and d) CD with PF using RCL – 1mm. Digital images of the instrumentation sequence were compared for each tooth.</p>	<ul style="list-style-type: none"> - Instrumentation length had significant effects on the first file that produced a crack and also on the file that produced dentinal detachment. - No significant effect of preparation technique on the development of apical cracks
IN-VIVO		
Chen, 1999 ⁽¹⁾	<p>Aim: compare endodontically vs. nonendodontically treated teeth with respect to clinical features, including patient age and gender and tooth types and VRF.</p> <p>Methods: 315 consecutive cases of VRF occurring in 274 Chinese</p>	<ul style="list-style-type: none"> - 87% had 1 fractured tooth; the others had 2 or 3 fractured teeth. - Of all VRF, 40% occurred in non-endodontically treated teeth. - Difference in VRF in endo or non-endodontically treated teeth: VRF in non-endodontically treated anterior teeth

	<p>patients during a 13 year period were reviewed.</p>	<p>seldom occurred; endodontically treated teeth showed higher tendency to fracture in D roots of mandibular molars, premolars, and ant teeth (may be related to weakening of the root structure by endo tx or post insertion).</p> <ul style="list-style-type: none"> - Endodontically treated and non-endodontically treated VRF have certain uncommon contributing factors such as: endo treatment and post insertion in endo treated teeth and moderate to severe attrition in non-endo treated teeth. Biologic or anatomical variations of the endo treated and the non-endo treated tooth are imp factors of VRF. - VRFs on endo treated teeth may have diff pattern of crack initiation and propagation.
<p>Cohen, 2006 ⁽⁵³⁾</p>	<p>Aim: The most common dental procedure that is thought to cause VRFs is overzealous endodontic treatment. However, increased amount of dentin removal doesn't necessarily correlate to increased fracture susceptibility. The purpose of this study is to explore the demographic profile of factors associated with VRFs, as gathered in an identical process from three different geographical sites.</p> <p>Methods: Different variables were investigated and statistically evaluated as to their correlation with the presence of VRFs. Specifically analyzed were gender, tooth location, age, radiographic and clinical findings, bruxism, and pulpal status. The data were collected from 3 diff endodontists, from 3 diff geographic locations, comprising a total of 227 teeth.</p>	<ul style="list-style-type: none"> - VRFs are statistically more prevalent in mandibular molars and max premolars. <p>VRF prevalence:</p> <ul style="list-style-type: none"> - Nonvital w/ prev. RCT: 49% - Nonvital w/ no RCT: 39% - Vital: 12% - Presence of previous RCT existed 47%, and was found to occur more often than a pulpal status of vital or nonvital. This may indicate that endodontictx may increase the tooth's predisposition of fracturing. - VRFs are associated with periradicular bone loss, pain to percussion, extensive restorations, and seem to occur more often in females and older pts. - VRFs are not necessarily related to periapical bone loss, a widening of the PL space, associated periodontal pockets, a sinus tract, particular pulpal status, or bruxism.

Testori, 1993 ⁽⁴⁴⁾	<p>Aim: Clinical study was done on 36 original cases of VRF along with the data gathered from 32 cases published previously in the literature.</p> <p>Methods: Data collected from this report were derived from two sources – 1) observation of 36 cases of VRF between Jan 1988-Jan 1991. 2) Cases involving fractures published in the lit starting from 1973</p>	<ul style="list-style-type: none"> - VRF most frequently occur in posterior teeth (premolar & molar) in patients between 45-60 yr of age. Smaller the M-D diameter of the root, the greater the incidence of fracture. Premolars, MD roots of upper molars, and M root of lower molars have these characteristics. Anatomical factors play a sig rol in the incidence of root fractures. - The average elapsed time between the endodontic treatment and the subsequent dx of VF was found to be approx. 10 years (no sig diff relative to type of tooth or depth of PD) - Evidence and symptoms most often found are mild pain in the area of the fractured tooth often accompanied by swelling and fistula, along with a deep pocket in just one area of the attachment surrounding the tooth. - The sign most often revealed by x-ray is a radiolucent periradicular band.
Meister, 1980 ⁽⁵⁹⁾	<p>Aim: To identify the causes of VRFs and the diagnostic signs normally present.</p> <p>Methods: 32 cases of VRFs were studied in an attempt to identify the causes and dx signs normally present.</p>	<ul style="list-style-type: none"> - In all of the patients except two, osseous defects were present and could be probed. - Majority (66%) had only mild pain or a dull discomfort. 75% showed diffuse widening of the PDL space. - Excessive force during lateral condensation of the GP caused 84% of the fractures. A secondary cause was the forcing or tapping of inlays or dowels into place. - Majority (78%) of the patients were over the age of 40. - In all but 9 of the cases, treatment consisted of the extraction of the involved teeth.
Tang, 2010 ⁽³⁾	<p>Aim: to identify and reduce the risks for potential tooth fractures.</p> <p>Methods: an overview of the risk factors for potential tooth</p>	<ul style="list-style-type: none"> - Post-endodontic tooth fractures might occur because of the loss of tooth structure and induced stresses caused by endodontic and

	<p>fractures in endodontically treated teeth on the basis of literature retrieved from PubMed and selected journal searches.</p>	<p>restorative procedures such as access cavity preparation, instrumentation and irrigation of the root canal, post-space preparation, post selection, and coronal restoration and from inappropriate selection of tooth abutments for prostheses.</p> <ul style="list-style-type: none"> - Tooth type, canal wall thickness and root canal diameter and cross-sectional shape, root canal preparation instruments and preparation methods, and the size of the master apical file might all be involved in the increased risk for the tooth fracture during and subsequent to endodontic therapy. - Over-instrumentation of root canals with excessive removal of dentin and the presence of noncircular canals and thin canal walls, particularly with certain tooth types, increase the risk for root fracture. The effect of various Ni-Ti rotary files is somewhat controversial, with some studies reporting an increased risk for craze lines and dentin cracks and reduced root fracture resistance compared with using hand files.
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