Effect of Tannic Acid on Resistance to Vertical Root Fracture in Endodontically Treated Bovine Teeth

BY

Jorge Carlos Griswold
B.B.A., Augusta State University, 2009
D.M.D., Georgia Regents University, College of Dental Medicine, 2013

THESIS

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Defense Committee:

Dr. Mohamed Fayad, Chair
Dr. Ana K. Bedran-Russo, Advisor
Dr. Bradford Johnson, Advisor
DEDICATION

To my parents, who have always loved me unconditionally.

To Rachael, who has been a source of endless support and love during the challenges of school and life.
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<table>
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<th>Abbreviation</th>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>Ca(OH)$_2$</td>
<td>Calcium hydroxide</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>HOCl</td>
<td>Hypochlorous acid</td>
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<tr>
<td>HSD</td>
<td>Honest significant difference</td>
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<td>N</td>
<td>Newtons</td>
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<td>NaOCl</td>
<td>Sodium hypochlorite</td>
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<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>OCl$^-$</td>
<td>Hypochlorite ion</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<td>TA</td>
<td>Tannic acid</td>
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<td>VRF</td>
<td>Vertical root fracture</td>
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<td>W/V</td>
<td>Weight per volume</td>
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SUMMARY

Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are the most common irrigating solutions used during endodontic treatment. However, both solutions have the potential to cause negative effects on the mechanical properties of dentin. Tannic acid (TA) has demonstrated the ability to induce collagen cross-linking in cardiac muscles tissues. In addition, tannic acid has shown positive effects on the mechanical properties of dentin in previous studies.

The null hypothesis tested that the addition of tannic acid to the irrigation protocol and/or endodontic sealer will not have a significant effect on the resistance to vertical root fracture (VRF) in endodontically treated bovine teeth.

Thirty bovine primary anterior teeth were decoronated at the cemento-enamel junction to create 15mm long roots and were then randomly assigned into three groups according to the irrigation and sealer type: Group 1: 5.25% NaOCl, 17% EDTA, 5.25% NaOCl; then gutta-percha and Roth sealer; Group 2: 5.25% NaOCl, 17% EDTA, 20% TA; then gutta-percha and Roth sealer; Group 3: 5.25% NaOCl, 17% EDTA, 20% TA then gutta-percha and Roth sealer with 10% TA. The specimens received endodontic treatment and were tested for vertical loading force (Newtons) until fracture using the MTS testing system. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Tukey’s honest significant difference (HSD) as a post-hoc test, (α<0.05). A statistical significant difference was detected between group 1 (control) and group 2 (20% TA irrigation) as well as group 1 (control) and group 3 (20% TA irrigation and sealer with 10% TA).
SUMMARY (CONTINUED)

In conclusion, the addition of tannic acid to the irrigation protocol, as well as to endodontic sealer, had a significant effect on the resistance to VRF, thus the null hypothesis was rejected.
I. INTRODUCTION

A. Background:

The goal of root canal treatment is the prevention or treatment of apical periodontitis. The success of root canal treatment is dependent on proper cleansing and shaping of the root canal system followed by three-dimensional obturation to seal and prevent infection and/or reinfection of the root canal system (Schilder, 1967).

Irrigating solutions play an essential role in the disinfection of the root canal system during the cleansing and shaping phase of endodontic treatment. The removal of bacteria from areas untouched by instrumentation, estimated to be one-third of the root canal walls, is dependent upon the efficacy of irrigating solutions (Peters et al., 2001). According to Haapasalo et al. (2010) one of the most important properties of the ideal irrigating solution is the ability to dissolve organic and inorganic tissues facilitating the killing of bacteria and yeasts while having no caustic and cytotoxic effects. Another important feature is that the washing action of the irrigating solution should prevent the packing and extrusion of bacteria and dentin chips into the periapical tissues. Irrigating solutions also must be able to act as lubricants and reduce friction of the canal walls during instrumentation, to facilitate dentin removal and decrease procedural mishaps. Regardless of all desired functions, the ideal irrigating solution should not weaken tooth structure (Haapasalo et al., 2010).

Current irrigating solution protocols include NaOCl and EDTA at different concentrations to dissolve organic and inorganic tissues, reduce bacteria levels, and remove the smear layer. Although NaOCl is one of the most popular irrigating solutions, it was reported to have negative effects on the structure of dentin. In addition, the
prolonged use of NaOCl after EDTA may cause dentin to weaken and be more susceptible to fracture (Sim et al., 2001; Haapasalo et al., 2010). VRF prevalence of 30.8% was reported in extracted endodontically treated teeth after eight to ten years following treatment (Sjogren et al., 1990). Furthermore, VRF was the third most common reason at 10.9% for extraction of endodontically treated teeth in a public dental clinic study (Fuss et al., 1999).

The search for new and better irrigating solutions continues. Tannic acid, a naturally occurring polyphenol, has demonstrated the ability to induce inter-and intra-molecular collagen cross-links in biological tissue (Jastrzebska et al., 2006). In addition, tannic acid demonstrated a positive effect in cross-linking of dentin collagen enhancing the mechanical properties of dentin (Bedran-Russo et al., 2009).
B. **Significance of the study**

Irrigating solutions are essential for successful root canal treatment. Protocols regarding irrigating solutions and the order in which they are used continue to be studied. Tannic acid, a naturally occurring polyphenol, may have the ability to reverse the negative effects of other irrigating solutions such as NaOCl by increasing collagen cross-linking.
C. **Specific Aims**

The purpose of this study was to investigate the effect of tannic acid on the resistance to vertical root fracture in endodontically treated bovine teeth. More specifically, to investigate the effect of tannic acid when used as an irrigating solution after following a conventional irrigation protocol as well as when added to endodontic sealer.
D. **Hypothesis**

The following null hypothesis was tested:

Addition of tannic acid to the irrigation protocol and/or endodontic sealer will not have a significant effect on the resistance to vertical root fracture in endodontically treated bovine teeth.
II. REVIEW OF THE LITERATURE

A. Sodium hypochlorite and ethylenediaminetetraacetic acid and their effect on dentin

Irrigating solutions are necessary during root canal treatment to remove debris from the root canal system (Baker et al., 1975). Irrigating solutions in the canal during instrumentation reduce mishaps such as file binding and separation and canal blockage with debris (VandeVisse and Brilliant, 1975).

Due to the tissue dissolution properties of NaOCl, it is the most commonly used irrigating solution in Endodontics. NaOCl possesses tissue dissolution properties. According to Estrela et al. (2002) hypochlorous acid (HOCl) releases chlorine ions to form chloramines. Hypochlorite ions (OCl⁻) also assist in the degradation and hydrolysis of amino acids. The antibacterial action of NaOCl arises by interfering with bacterial cell metabolism and essential bacterial enzymes. The high pH of NaOCl provides it with similar properties to calcium hydroxide (Ca(OH)₂) by the action of hydroxyl ions. The ions interfere with cell membrane integrity and bacterial enzyme activity (Estrela et al., 2002).

Sodium hypochlorite at different concentrations are currently used. The study by Stojicic et al. (2010) comparing tissue dissolution property demonstrated higher concentrations such as 5.25% to be most effective. NaOCl heated to 45 degrees Celsius led to an increase in tissue dissolution when compared to NaOCl at body temperature. Agitation of the irrigating solutions by ultrasonic methods also led to an increase in tissue dissolution when compared to needle delivery (Stojicic et al., 2010). Elimination of biofilm from the root canal system can be accomplished by the use of high concentration,
6% NaOCl. Lower concentration NaOCl is not effective at removing the biofilm; it only disrupts the biofilm leaving bacteria viable (Clegg et al., 2006). 5.25% NaOCl is safe to use and does not cause additional inter-appointment pain to the patient, a measure of clinical toxicity (Harrison et al., 1978). Nonetheless, several reports of NaOCl accidents exist in the literature.

Sodium hypochlorite has the potential to change the structure of dentin. Prolonged exposure of dentin to NaOCl negatively affects its flexural strength and elastic modulus (Sim et al., 2001). A study by Zhang et al. (2010) demonstrated collagen degradation and flexural strength were found to be negatively affected by high concentration NaOCl when exposed for longer than one hour. Low concentration 1.3% NaOCl did not cause negative changes to dentin when exposed for up to four hours (Zhang et al., 2010). The disintegration of the organic dentin matrix by NaOCl was demonstrated to be concentration-dependent. Intertubular and peripheral dentin alteration occurred after prolonged immersion in NaOCl. In addition, spontaneous cracking on the surface of dentin bars was noted when immersed in 5.25% NaOCl for 2 hours (Marending et al., 2007). Although the previous studies are benchtop studies, the findings infer a possible higher risk for fracture in endodontically treated teeth exposed to high concentration NaOCl.

Ethylenediaminetetraacetic acid as an irrigating solution was introduced to Endodontics as an aid to the instrumentation of curved and calcified root canals (Nygaard-Otsby, 1957). EDTA acts a metal ion chelator, binding to calcium ions from mineralized dentin. EDTA has a near neutral pH value; however, during dentin demineralization, its pH turns acidic and decreases the efficiency of EDTA (Calt and
According to Hulsmann et al. (2003) EDTA decreases dentin hardness in as little as a five-minute exposure. Additionally, extending the exposure time to longer than 24 hours does not significantly increase the effect. The inorganic portion of the smear layer, composed of dentin debris, predentin, pulpal debris, and bacteria, is successfully removed by EDTA irrigation. Furthermore, EDTA increases dentin permeability, which may improve the activity of medicaments such as Ca(OH)₂. The ideal exposure time of EDTA to dentin is unknown at this time, but negative effects on the structure of dentin are seen after prolonged exposure (Hulsmann et al., 2003). A one-minute exposure to 10 mL 17% EDTA was sufficient to remove the smear layer. Excessive peritubular and intratubular dentin erosion was obvious after ten minutes (Calt and Serper, 2002).

Although reported as such, EDTA has no antibacterial activity and organic tissue dissolution power (Haapasalo et al., 2010). Accordingly, EDTA is used along with other irrigating solutions including NaOCl. Unfortunately, EDTA decreases the number of chlorine available in NaOCl rendering NaOCl’s antimicrobial and tissue dissolving effect ineffective (Zhender, 2006). Popular irrigation regimens include cleansing and shaping with NaOCl, then a final flush with 17% EDTA followed by NaOCl to obtain cleaner root canal systems (Yamada et al., 1983). The surfaces of exposed dentin after irrigation regimens of NaOCl with EDTA demonstrated an eroded appearance (Baumgartner and Mader, 1987). Dentin erosion was clearly demonstrated when NaOCl was used after EDTA. The effect of dentin erosion on dentin strength is not clear, but extensive erosion has been suggested to make endodontically treated teeth more susceptible to root fracture (Qian et al., 2011).
B. **Sealers**

The purpose of obturation is to prevent infection or reinfection of the root canal system. The standard materials for obturation are sealer cement and a central core material. According to Orstavik (2005) the sealer comes into contact with the tissues of the root canal system. The sealer has three roles including, “sealing off of the root canal system, entombment of remaining bacteria, and filling of voids and irregularities in the prepared canal space” (Orstavik, 2005).

Zinc-oxide-eugenol-based sealers have a long successful record of use in Endodontics. Rickert introduced the first zinc-oxide-eugenol sealer containing silver particles which stained teeth if left in the pulp chamber. Grossman created a variant, Roth sealer, without silver particles to prevent staining (Hargreaves and Cohen, 2011).

Leakage is of most importance for sealers to prevent the contamination of the root canal system. Zinc-oxide-eugenol-based sealers have displayed less apical leakage than glass-ionomer-based sealers (Brown, 1994). New clinically relevant methods to test leakage include the fluid filtration model and bacterial penetration setup (Orstavik, 2005). Unfortunately, more studies utilizing these methods are needed. Zinc-oxide-eugenol-based sealers and resin-based sealers such as AH Plus have shown a stable dimensional change with little shrinkage over a four-year period (Orstavik, 2005).

Zinc-oxide-eugenol-based sealers show toxicity in cell culture models as well as neurotoxicity to nerve cells. In addition, zinc-oxide-eugenol-based sealers cause inflammation upon contact with the periapical tissues similar to resin-based sealers (Dahl, 2005). Extrusion of sealer may activate trigeminal nociceptors and cause neurogenic inflammation with pain as the end result (Ruparel et al., 2014).
Strengthening of the root canal system via adhesive filling and resin core has been introduced with limited success. Resilon, a synthetic resin-based polycaprolactone polymer, used with a resin sealer claimed to form a bond at the interfaces of the core, sealer, and dentin. The creation of these interfaces was heavily questioned and studies showed that they did not create a monoblock root filling (Raina et al., 2007).

Bioceramic sealers composed of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, and other fillers are the latest available sealers claiming several properties of the ideal sealer such as no shrinkage, hydrophilicity, biocompatibility, and antibacterial (Hargreaves and Cohen, 2011; Zhang et al., 2010). Long term studies are needed to demonstrate their benefits.

C. **Structure of dentin**

Dentin is a mineralized connective tissue formed by differentiated cells called odontoblasts. Dentin is composed of a mineral phase, organic matrix, and water. The mineral phase constitutes approximately 70% by weight and 45% by volume, while the organic matrix makes up 20% and 33% respectively; and the remainder is water (Tjaderhane et al., 2012). The composition and structure of dentin differs by the location, function, and origin in the tooth (Tjaderhane et al., 2012).

Dentinal tubules affect mechanical properties, the ability to resist occlusal forces, and dentin bonding. Dentinal tubules are surrounded by peritubular dentin, which may regulate communication between intertubular dentin and odontoblasts (Gotliv and Veis, 2007). Peritubular dentin is approximately 40% more mineralized than intertubular dentin; little collagen if any is present in peritubular dentin (Nanci, 2008). Intertubular
Dentin consists of a collagen fibril mesh oriented in a perpendicular direction to the dentinal tubules (Arola et al., 2012). The different composition leads us to believe a difference exists in elastic and fracture properties between the two types of dentin.

Dentinal tubules are present in larger quantities in the coronal section of the tooth than in the root. In addition, tubule width is larger near the pulp than near the dentino-enamel junction (Tjaderhane et al., 2012). Studies have suggested dentin in the apical portion of the root to be different. Dentinal tubule density decreases with increasing depth from the coronal third towards the apical third (Carrigan et al., 1984; Mjor et al., 2001). The dentinal tubules in the apical region can also be irregular in direction. In addition, lateral canals and resorptive defects are present as well as a decrease in dentin permeability has been seen (Tjaderhane et al., 2012).

### D. Mechanical properties of dentin

Dentin is an anisotropic natural material. The strength of dentin is dependent on the dentinal tubules orientation. Ultimate tensile strength is measured as the maximum stress that dentin can withstand. Dentinal tubules oriented parallel to the direction of the stress exhibited a significantly lower tensile strength (Lertchirakarn et al., 2001). Dentinal tubule density is also important for dentinal strength; a higher dentinal tubule density reveals a lower strength. These differences are due to the relative mineral and organic contents of dentin (Arola et al., 2012). Patient age has also been reported to decrease the ultimate tensile strength of dentin due to microstructural changes (Arola et al., 2012).

Fatigue crack growth is relevant to the survivability of teeth. The number of cycles needed to propagate an existing crack into one of critical length that can lead to
bulk fracture is called fatigue crack growth (Arola et al., 2012). Endodontic procedures have been reported to create dentinal defects and potential microcracks in dentin (Nalla et al., 2003). Due to the distribution of dentinal tubule densities in radicular dentin, the cervical third is at higher risk for fatigue crack growth (Ivancik et al., 2011; Arola et al., 2012). Nonetheless, the collagen fibrils in dentin provide toughness and exhibit some crack stopping behavior, while the mineral content provides strength to dentin (Kishen, 2006).

E. Collagen in dentin

The organic extracellular matrix of dentin is approximately 90% collagen, in which most of it is type I. Type I collagen is composed of a heteropolymer with two alpha1 chains and one alpha2 chain (Butler, 1984). Type I collagen has also been shown to be composed of a homopolymer consisting of three alpha1 chains; which may be important during dentin formation. Collagen, type III and V have been reported to be presented in dentin according to human and animal studies. Nonetheless the amount of type III and V are very small and inconsistent (Tjaderhane et al., 2012).

Intracanal agents such as NaOCl and Ca(OH)$_2$ can produce detrimental effects on dentin. Zhang et al. (2010) reported collagen degradation by NaOCl to be concentration-dependent and time-dependent. Collagen degradation appears to be a slow, continuous exposure to high concentration NaOCl which negatively impacts the flexural strength of dentin (Zhang et al., 2010).

Collagen fibrils are strengthened by the presence of cross-links, giving collagen tensile properties. Intra- and intermolecular crosslinks are induced to further stabilize
collagen in different tissues including dentin leading to an increase in ultimate tensile strength (Bedran-Russo et al., 2006). Chemical cross-linkers such as glutaraldehyde and plant derived proanthocyanidins mediate exogenous cross-links that have demonstrated improved dentin-resin bonding in restorative dentistry applications (Macedo et al., 2009).

**F. Bovine dentin**

Animal models to study dentin have been used for many years. A majority of the models involve rat and bovine dentin. Rat models are used to study dentinogenesis and effects in enamel and dentin development. Bovine models are used to study the mechanical properties of enamel and dentin. The composition of bovine dentin is similar to human dentin as reported in a literature review by Yassen et al. (2011). Dentinal tubules are present in similar numbers and share similar diameters. Bovine dentin is also similar in hardness, however, there is a higher amount of peritubular dentin present in older samples (Tjaderhane et al., 2012). Studies also found no significant difference between human and bovine dentin in ultimate tensile strength tests or modulus of elasticity (Sano et al., 1994). In addition, several studies have demonstrated that the bovine model is useful for microleakage studies (Yassen et al., 2011).

**G. Tannic acid**

Tannic acid, the commercial form of natural tannin, consists of a complex mixture of polygalloylglucoseesters. TA, a weak acid, has the ability to interact with collagen and lead to the formation of hydrogen bonds. The interaction modifies the surface topography
of collagen as well as the thickness and arrangement of collagen fibrils (Jastrzebska et al., 2006).

As reported by Jastrzebska et al. (2006), TA has the ability to function as a collagen crosslinking agent. The formation of hydrogen bonds occurs between amine as well as amide –NH groups from collagen and hydroxyl groups from tannic acid as indicated by previous studies (Jastrzebska et al., 2006).

A search of the Endodontic literature revealed a study by Bitter (1989) on the use of 25% TA solution as a root canal irrigating solution. TA more effectively removed the smear layer than NaOCl and hydrogen peroxide (Bitter, 1989). Studies for restorative dentistry have demonstrated dentinal tensile strength and elastic modulus increased after exposure to TA (Koide and Daito, 1997). Also, TA was tested as a replacement to phosphoric acid etchant. Dentin exposed to tannic acid for two hours revealed intact collagen unlike collagen that appeared denatured after phosphoric acid exposure (Okamoto et al., 1991).

Tannic acid at a neutral pH has also demonstrated the potential to significantly affect dentin matrix properties and resin-dentin bond strengths (Bedran-Russo et al., 2009). Modulus of elasticity was significantly affected by exposure to TA, where the effect was concentration- and time-dependent. The formation of hydrogen bonds by tannic acid was speculated as the possible mechanism for the positive effects after exposure to TA (Bedran-Russo et al., 2009). At last positive trends although not significant were demonstrated for flexural strength, collagen biodegradation rates, and denaturation temperatures for dentin exposed to 20% tannic acid solution (Iampaglia, 2012, unpublished data).
H. **Vertical root fracture**

Vertical root fracture is one of the modes of failure of endodontically treated teeth. Prevalence of VRF differs vastly by different studies. VRF was the third most common cause for extraction with a reported prevalence of 10.9% by Fuss et al. (1999). The study included 564 teeth that were extracted in a dental clinic in which 147 were endodontically treated (Fuss et al., 1999). A long-term study by Sjogren et al. (1990) eight to ten years after root canal treatment also looked at VRF and its prevalence in extracted endodontically treated teeth. Out of 356 endodontically treated teeth, 68 teeth were extracted, in which 21 teeth or 30.8% were extracted due to root fracture (Sjogren et al., 1990). A recent study in Tokyo, Japan reported 233 teeth or 31.7% out of 736 teeth, were extracted due to VRF. They also reported that 93.6% of these teeth were endodontically treated (Yoshino et al., 2014).

Risk of VRF in endodontically treated teeth may be related due to cleansing and shaping, and obturation procedures. Risk factors include excessive tooth structural loss due to mechanical preparation, effects of irrigating solutions and medicaments such as NaOCl and Ca(OH)$_2$, and restorative procedures such as post and core placement. The negative effects of NaOCl to dentin’s mechanical properties are well recognized. In addition, NaOCl’s combined use with EDTA can make dentin more prone to fracture (Kishen, 2006). Long-term use of Ca(OH)$_2$ can also negatively affect the fracture resistance of dentin (Rosenberg et al., 2007). Reports of excessive wedging forces created during lateral condensation are also available and they are known to be a risk factor for VRF (Holcomb et al., 1987).
I. **Studies utilizing a vertical loading force to test fracture**

Several studies evaluating different dentin properties have been completed, with the variable of interest being the load to fracture. A study comparing biomechanical properties of vital and endodontically treated teeth used the Shimadzu testing machine at a constant crosshead speed of 0.1 mm per minute in order to vertically load roots until fracture occurred (Sedgley and Messer, 1992). A study comparing root fracture resistance using two different root canal sealers used a similar technique as the previous study. An Instron testing machine at a rate of 1.0 mm per minute was used to apply a vertical loading force to fracture roots (Apicella et al., 1999). At last, different canal filling systems including gutta-percha and Resilon were compared using similar methodology as previously described using an Instron testing machine at a rate of 1.0 mm per minute to vertically load roots until fracture occurred (Kazandag et al., 2009).
III. MATERIALS & METHODS

A. Study design

The aim of the study was to investigate the potential for TA to reinforce the remaining dentin and therefore improve resistance to VRF in endodontically treated bovine teeth. To carry out the objective, a benchtop study was designed.

Slaughtered, frozen bovine heads were obtained from a slaughterhouse in Chicago, IL. Bovine primary anterior teeth were extracted and stored in distilled water with 0.5% thymol solution. Extracted bovine primary teeth were decoronated at the cement-enamel junction or below to create 15mm long roots.

Tannic acid was obtained from Acros Organics (Fair Lawn, NJ, USA). A 20% (w/v) solution in saline adjusted to pH 7.4 using sodium hydroxide (NaOH) was made on the day it was used. 5.25% NaOCl (James Austin, Mars, PA, USA) and 17% EDTA (Roydent, Johnson City, TN, USA) were used as irrigating solutions in this study. Roth sealer (Roth International Limited, Chicago, IL) was mixed with TA and a 10% (w/v) mixture prepared to use as sealer.

It was determine the sample size for each group to be ten teeth based on previous studies. A similar study by Apicella et al. (1999) used the same sample size.

B. Tooth specimen preparation

Thirty bovine primary anterior teeth were extracted and stored in distilled water with 0.5% thymol solution. The selected extracted bovine primary teeth were decoronated at the cement-enamel junction or below to create 15mm long roots. The selected teeth had similar root canal cross-sections, lacked curvature, and had closed
apices. The teeth were inspected under an operating microscope (Global, St. Louis, MO, USA) for microcracks. The teeth were randomly assigned to three groups.

Six (6) mm of the apical root end of the teeth were embedded in 25mm self-cure orthodontic resin blocks exposing 9mm of the coronal end of the teeth. The self-cure resin was allowed to polymerize for several hours. The long axis of the teeth was checked with a protractor to verify proper angulation. The teeth were stored in a sealed container containing distilled water not in direct contact with the roots. The container was placed in an incubator at 37 degrees Celsius for two days.
C. Test groups

The teeth were randomly assigned to three groups.
The irrigating solution protocol groups were: group 1 (control): 5ml 5.25% NaOCl for 3 minutes, 2.5ml 17% EDTA for 3 minutes, followed by a final rinse of 2.5ml 5.25% NaOCl for 3 minutes; group 2 and 3: 5ml 5.25% NaOCl for 3 minutes, 2.5ml 17% EDTA for 3 minutes, followed by a final rinse of 2.5ml 20% TA for 3 minutes. The obturation/sealer protocol groups were: group 1 (control): gutta-percha and Roth sealer; group 2: gutta-percha and Roth sealer; group 3: gutta-percha and Roth sealer with 10% TA.

**Table I**

TEST GROUPS

<table>
<thead>
<tr>
<th>Groups</th>
<th>Irrigation</th>
<th>Obturation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 (Control)</strong></td>
<td>5ml 5.25% NaOCl for 3 minutes, 2.5ml 17% EDTA for 3 minutes, 2.5ml 5.25% NaOCl for 3 minutes</td>
<td>Gutta-percha and Roth sealer</td>
</tr>
<tr>
<td><strong>Group 2 (20% TA irrigation)</strong></td>
<td>5ml 5.25% NaOCl for 3 minutes, 2.5ml 17% EDTA for 3 minutes, 2.5ml 20% TA for 3 minutes</td>
<td>Gutta-percha and Roth sealer</td>
</tr>
<tr>
<td><strong>Group 3 (20% TA irrigation + Roth with 10% TA)</strong></td>
<td>5ml 5.25% NaOCl for 3 minutes, 2.5ml 17% EDTA for 3 minutes, 2.5ml 20% TA for 3 minutes</td>
<td>Gutta-percha and Roth sealer with 10% TA</td>
</tr>
</tbody>
</table>

**D. Root canal treatment**

The root canals of the teeth were cleansed and shaped using Endo-Sequence (Brasseler, Savannah, GA, USA) 0.04 tapered files. The protocol used was: The canals were enlarged with Gates Glidden burs, sizes 2 and 3 (Roydent, Johnson City, TN, USA). The crown-down technique was used to enlarge the canals until a size 60/0.04 was
obtained as the master apical file size. The root canals were irrigated with 2ml 5.25% NaOCl after each file. Remaining dentin thickness of approximately 1mm on the coronal aspect of the root was visible following cleansing and shaping, then the irrigation protocol was completed.

The canals were dried with paper points (Roydent, Johnson City, TN, USA). The canals were then filled with Endo-Sequence gutta-percha (Brasseler, Savannah, GA, USA) via continuous wave compaction and thermoplastic injection (B & L Biotech, Fairfax, VA, USA) according to the obturation/sealer protocol. The teeth were stored for ten days to allow for setting of the sealer as described above.

E. **Root Fracture Resistance testing**

The endodontically treated specimens were mounted in an MTS universal testing system (MTS model 1125, Eden Prairie, MN, USA) one at a time. The vertical loading force technique used is similar to the technique used by Apicella et al (1999). A cone shaped rod (2.mm in diameter) was mounted on the MTS testing system to apply a vertical loading force. A rod was placed on top of the canal opening of each tooth. A loading force at a rate of 1.0mm per minute was applied until root fracture occurred. The load was recorded in Newtons (N). The fracturing load force was verified by observing the drop in loading force in the computer, which occurred at the same time of the crack sound. Visual confirmation of the fracture was also completed.
**Figure 2.** Diagrammatic representation of the root ready for root fracture resistance testing

**Figure 3.** Cone shaped rod mounted on the MTS universal testing system

**Figure 4.** Root with visible fracture after root fracture resistance test
IV. STATISTICAL ANALYSIS

The data was analyzed using one-way ANOVA (SPSS for Windows, Version 19, SPSS Inc., Armonk, NY) for comparison of the three groups. Tukey’s HSD was used as a post-hoc test. A value of alpha = 0.05 was used for all statistical tests. Power calculation was also completed.
V. RESULTS

The means and standard deviations of the vertical loading force to fracture are presented on Table II. The vertical loading force in the three groups were compared using one-way ANOVA revealing a statistical significant difference among the three groups (p=0.002). The mean vertical loading force for group 1 (control) was 196.95 ± 31.26 N; group 2 was 252.95 ± 49.83 N, and group 3 was 278.66 ± 56.82 N.

Tukey’s HSD test was utilized as a post-hoc test to determine differences among groups. There was a significant difference between group 1 (control) and group 2 (20% TA irrigation) where p=0.034. There was also a significant difference between group 1 (control) and group 3 (20% TA irrigation + Roth sealer with 10% TA) where p=0.002. There was no statistical significant difference between groups 2 and 3 (p=0.453).

Power calculation revealed an observed power of 92.7%.

TABLE II
MEANS WITH STANDARD DEVIATION OF VERTICAL LOADING FORCE

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample size</th>
<th>Mean ± SD (in Newtons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>10</td>
<td>196.95 B ± 31.26</td>
</tr>
<tr>
<td>Group 2 (20%TA irrigation)</td>
<td>10</td>
<td>252.95 A ± 49.83</td>
</tr>
<tr>
<td>Group 3 (20% TA irrigation + Roth with 10% TA)</td>
<td>10</td>
<td>278.66 A ± 56.82</td>
</tr>
</tbody>
</table>

SD= standard deviation; TA= tannic acid; different letters indicate statistically significant differences between groups (p<0.05)
Figure 5. Means of vertical loading force for each group

<table>
<thead>
<tr>
<th>Group vs.</th>
<th>Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>Group 2 (20% TA irrigation)</td>
<td>0.034</td>
</tr>
<tr>
<td>Group 1 (control)</td>
<td>Group 3 (20% TA irrigation + Roth with 10% TA)</td>
<td>0.002</td>
</tr>
<tr>
<td>Group 2</td>
<td>Group 3</td>
<td>0.453</td>
</tr>
</tbody>
</table>

TABLE III

TUKEY’S HDS POST-HOC TEST TO DETERMINE STATISTICALLY SIGNIFICANT DIFFERENCE AMONG GROUPS
VI. DISCUSSION

The aim of this study was to investigate the potential for TA to improve resistance to VRF in endodontically treated bovine teeth. One control and two test groups were compared and analyzed. Bovine primary teeth were utilized in this study due to accessibility, availability, and similarity with human dentin’s mechanical properties.

The methodology used in this study has been used in the past to provide viable information on root fracture resistance. Sedgley et al. (1992) used it to compare fracture resistance of endodontically treated teeth vs. vital teeth. Apicella et al. (1999) used it to compare root fracture resistance using two different root canal sealers. Utilizing a vertical loading force (1.0mm/min) supplied by an MTS testing system, this study tested resistance to vertical root fracture and the stress to fracture (N) was used as measurements.

This study utilized TA as an irrigating solution due to TA’s ability to act as a collagen cross-linking agent. TA interacts with collagen and forms hydrogen bonds modifying collagen fibrils’ thickness and arrangement (Jastrzebska et al., 2006). Dentin exposed to TA demonstrated significant increases in tensile strength and elastic modulus in a restorative dentistry study (Koide and Daito, 1997). In addition, TA positively affected dentin matrix properties and increased modulus of elasticity of dentin (Bedran-Russo et al., 2009).

In this study, irrigation with 20% TA exhibited a statistically significant increase in resistance to VRF. This study went further and added 10% TA (w/v) to Roth sealer. Groups two and three had the same irrigation protocol with 20% TA; the only difference was the addition of 10% TA to Roth sealer. The addition of 10% TA to Roth sealer
demonstrated a statistically significant increase when compared to the control group. However, there was no statistically significant difference between groups two and three. In other words, the addition of 10% TA to Roth sealer, did not affect the resistance to VRF when 20% TA is used as an irrigating solution.

The possible mechanism for increase in resistance to VRF was TA’s ability to act as a collagen cross-linking agent. Collagen, type I, present in the organic extracellular matrix of dentin possibly exhibited modification of its fibrils’ arrangement and thickness as seen in previous studies, such as Jastrzebska et al. (2006). The modification and extent of the collagen cross-linking density determines the mechanical behavior of dentin. Formation of hydrogen bonds between amine and amide –NH groups from collagen and hydroxyl groups from TA increased the collagen cross-linking density, thus increasing mechanical properties (Jastrzebska et al., 2006).

Adhesive filling and resin core materials have been introduced with limited success with claims of increased resistance to VRF. Resilon, a synthetic resin-based polycaprolactone polymer is used with Epiphany, a self-etching primer and methacrylate-based sealer. Initial studies reported an increase in resistance to VRF using Resilon/Epiphany (Teixeira et al., 2004; Hammad et al., 2007). However, Tay and Pashley (2007) demonstrated in independent studies that Resilon/Epiphany is no better than gutta-percha and conventional sealers in sealing the root canal system. Microshear bond testing and push-out tests also revealed weak bonding between Resilon/Epiphany and root dentin, making a mechanically homogeneous unit or secondary monoblock unable to form in the root canal system (Tay and Pashley, 2007). EndoRez, a resin-based material and ActiV GP, a glass-ionomer based material have attempted to improve
resistance to fracture of endodontically treated teeth by creating tertiary monoblock systems. Unfortunately, EndoRez has shown weak bonding and ActiV GP, a single-cone technique, has shown poor coronal seal with increased leakage and no increase in resistance to fracture (Tay and Pashley, 2007).

Tannic acid has the advantage that it may be easily introduced to the irrigation protocols for endodontic treatment, unlike new obturation systems. However, irrigation with 20% TA following 5.25% NaOCl produced a brown precipitate. The removal of the organic component of the smear layer also needs to be considered if NaOCl or other antibacterial solutions are not used after 17% EDTA. A proper sequence of irrigating solutions would need to be established. Due to the weak acidity of TA, the solution may need to be buffered to prevent negative effects on dentin. The addition of TA to endodontic sealer would require a closer look at the sealer properties.

Although no studies have investigated TA’s effect on resistance to VRF, the results of this study are consistent with other studies in which TA demonstrated a positive effect on dentin (Koide and Daito, 1997; Bedran-Russo et al., 2009). From the results of this study, it can be gathered that irrigation with 20% TA and addition of 10% TA to Roth sealer increases the resistance to VRF.
VII. CLINICAL RELEVANCE AND LIMITATIONS

In this study, an attempt to replicate clinical conditions was made. The use of complete roots instead of dentin beams as specimens and protocols regarding cleansing and shaping, and obturation were clinically relevant.

A limitation of this study includes the use of bovine teeth instead of human. However, similar mechanical properties between bovine and human dentin have been found (Tjaderhane et al., 2012). In addition, bovine teeth were used in this study to have similar specimens in regards to age, root canal cross-section, and curvature. Another limitation includes the fracturing load force, which was a static force in this study. A cyclic loading force would be more clinically relevant as this force is similar to how VRF occurs in vivo.
VIII. FUTURE RESEARCH

The positive results of TA in this study should lead to further research of TA as an irrigating solution and/or endodontic sealer in Endodontics. Research should aim to evaluate the addition of TA to sealer following a conventional irrigation protocol. Different concentrations of TA in sealer should also be evaluated. A significant area of study should include the evaluation of the effects of TA to the properties of sealer such as leakage and setting.
IX. CONCLUSIONS

In this study, 20% TA as an irrigating solution and 10% TA in Roth sealer positively affected the resistance to VRF following endodontic treatment in bovine teeth. Thus the null hypothesis is rejected.

The results of this study are promising and similar in that TA appears to have a positive effect on the mechanical properties of dentin as in previous studies.
X. CITED LITERATURE


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NAME: Jorge Carlos Griswold

EDUCATION: D.M.D., Georgia Regents University, College of Dental Medicine, Augusta, GA, 2013

B.B.A., Augusta State University, Augusta, GA, 2009

Teaching Experience:
Teaching Assistant, Pre-Doctoral Technique Course, Department of Endodontics, University of Illinois at Chicago College of Dentistry, Chicago, IL, 2013

Resident Clinical Instructor, Department of Endodontics, University of Illinois at Chicago College of Dentistry, Chicago, IL, 2014-2015

Professional Memberships:
American Association of Endodontists
American Dental Association
The Edgar D. Coolidge Endodontic Study Club

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