THE EFFECT OF THE STORAGE SOLUTION, 3% GLUTARALDEHYDE, ON DENTIN FLEXURAL STRENGTH

By
CPT J. Clay Hastings, DMD

A THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Oral Biology in the Uniformed Services University of Health Sciences
FORT BRAGG, NC
2017

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

"THE EFFECT OF THE STORAGE SOLUTION, 3% GLUTARALDEHYDE, ON DENTIN FLEXURAL STRENGTH"

Is appropriately acknowledged and, beyond brief excerpts, is with the permission of the copyright owner.

J. Clay Hastings
Program
Uniformed Services University
Date: **05/15/2017**

Distribution Statement

Distribution A: Public Release.

The views presented here are those of the author and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences, the Department of Defense or the U.S. Government.

Submitted by Clay Hastings in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.
Accepted on behalf of the Faculty of the Graduate School by the thesis committee:

18 MAY 17
Date

Manuel Pelaez, DMD
Research Mentor

18 MAY 17
Date

Jason Bullock, DMD
AEGD Assistant Director

18 May 17
Date

Stacy Larsen, DDS
AEGD Program Director

IRBNet Number:
PI: CPT J. Clay Hastings, DC
Protocol Title: THE EFFECT OF THE STORAGE SOLUTION, 3% GLUTARALDEHYDE, ON DENTIN FLEXURAL STRENGTH
Initial Date Submitted: 16 DEC 2015
Revision Date:

Request for Determination for Research Meeting the Criteria for Research Not Involving Human Subjects (RNIHS)

1.0 PROTOCOL TITLE:

THE EFFECT OF THE STORAGE SOLUTION, 3% GLUTARALDEHYDE, ON DENTIN FLEXURAL STRENGTH

2.0 PRINCIPAL INVESTIGATOR:

Name: CPT J. Clay Hastings, DC
Title: Dentist
Department: DENTAC
Name/Address of Institution: Gruber Rd, Bldg H-3817, Fort Bragg, NC 28307AEGD Clinic
Phone: 910-396-5012
Outlook Email: john.c.hastings4.mil@mail.mil

2.1 OTHER INVESTIGATORS:

Name: CPT Vejay K. Ravindran, DC
Title: Dentist
Department: DENTAC
Name/Address of Institution: Gruber Rd, Bldg H-3817, Fort Bragg, NC 28307AEGD Clinic
Phone: 910-396-6925
Outlook Email: vejay.k.ravindran.mil@mail.mi

IRBNet Number:
PI: CPT J. Clay Hastings, DC
Protocol Title: THE EFFECT OF THE STORAGE SOLUTION, 3% GLUTARALDEHYDE, ON DENTIN FLEXURAL STRENGTH
Initial Date Submitted: 16 DEC 2015
Revision Date:

3.0 RESEARCH NOT INVOLVING HUMAN SUBJECTS DETERMINATION Please double click on the box that applies to your research and mark the "check" box.

3.1. Is the activity a **systematic** investigation **designed** to develop or contribute to **generalizable** knowledge? 32 CFR 219.102(d) *Generalizable knowledge consists of theories, principles, or relationships (or the accumulation of data on which they may be based) that can be corroborated by accepted scientific observation and inference that is applicable to other related situations, populations, or devices outside of the tested situation.*

☐ No. Activity is not research and does not require Clinical Investigation Service oversight.

☒ Yes. Proceed to 3.2. Because you are making a request for Research Not Involving Human Subjects determination, this should be checked "yes."

3.2 Does the research involve obtaining information about living individuals? 32 CFR 219.102(f)

☒ No. Activity is Research Not Involving Human Subjects. *Cadaver studies or animal studies would fall into this category.*

☐ Yes. Proceed to 3.3. This would almost always be checked "yes."

3.3. Does the research involve **intervention** or **interaction** with the individuals? 32 CFR 219.102(f). Intervention or interaction is some form of contact with the study subject.

☐ Yes. Activity is Human Subjects Research. Please contact usarmy.bragg.medcom-wamc.list.wamc-irb-admin@mail.mil for further instructions. You will have to submit your proposal as an "exempt" protocol or standard protocol application.

☒ No. Proceed to 3.4.

3.4. Though you are not interacting or intervening with a living individual, you will be using their information. Is this information individually identifiable by the research team at any time?

☒ No. **Activity is Research Not Involving Human Subjects** and therefore does not require IRB approval. *This is the box that you will most likely check.*

☐ Yes. Proceed to 3.5.

3.5. Is the information private? 32 CFR 219.102(f)

☐ Yes. Activity is Human Subjects Research. Please contact usarmy.bragg.medcom-wamc.list.wamc-irb-admin@mail.mil for further instructions. You will have to submit your proposal on an "exempt" protocol or standard protocol application.

☒ No. **Activity is Research Not Involving Human Subjects** and therefore does not require IRB approval. *This would be information that is available in the public domain.*

4.0 EXPECTED COMPLETION DATE FOR STUDY (INCLUDING DATA ANALYSIS): DEC, 2016

5.0 SUMMARY: This study will test the flexural strength of dentin bars stored in various storage solutions. Dentin bars from extracted teeth will be immersed in storage solutions including bleach, thymol, glutaraldhyede and physiologic saline. These teeth will be sourced from dental providers at Ft. Bragg that are removing wisdom teeth as part of standard of care. Dentin bars will be cut and stored in the above storage solutions for two hours. The flexural strength will be tested using the Instron Machine located at the AEGD Clinic.

5.1 DATA COLLECTION METHODOLOGY AND STATE THE STUDY HYPOTHESIS OR RESEARCH QUESTION:

Hypothesis:

1. Dentin bars stored in 3% glutaraldehyde will have a reduced dentin flexural strength compared to dentin bars stored in our control solution, HBSS. The dentin bars stored in 0.05% Thymol and 5.25% NaOCl will have a reduced dentin flexural strength compared to dentin bars stored in our control solution, HBSS.

5.2 DESCRIBE THE TYPE OF DATA OR SPECIMENS TO BE STUDIED:

Study approval from the Fort Bragg WAMC Institutional Review Board, thirty extracted teeth were collected within a thirty day period, disinfected per the CDC guidelines and stored in HBSS until the teeth were prepared and sectioned into dentin bars. All of the dentin bar specimens required for testing were prepared within an eight hour time period and placed in our control solution, HBSS, until all dentin bars were prepared (Plotin, 2007). The dentin bars from each tooth were divided between the control solution (HBSS), 5.25% NaOCl, 0.05% thymol and 3% glutaraldehyde. Once the samples were prepared and after the dentin bars soaked in their respective storage solutions for three hours, a 3-point bend test was conducted to evaluate the effect of the

storage medium on the dentinal flexural strength.

Study Design

Thirty extracted teeth were collected, all teeth were human third molars, free of carious lesions, cracks, and restorations. The teeth were collected within a thirty day period, disinfected following the CDC guidelines and stored in HBSS. Eighty dentin bar specimens were prepared within an eight hour time period and equally distributed to one of four storage solutions; HBSS (n=20), 5.25% NaOCl (n=20), 0.05% thymol (n=20) and 3% glutaraldehyde (n=20). The specimens were stored, in solution, in an environment with 100% humidity at 37° C for three hours before the flexural strength was tested (Grigoratos, 2001).

Specimen preparation

To test the flexural strength of dentin, rectangular bars of dentin were created from freshly extracted human teeth, teeth collected within a 30 day time period. Maxillary and mandibular third molars, n=30, without caries or restorations extracted from male and female patients, 18 – 36 years of age were sampled for this study. Immediately after extraction, gross debris was removed from the samples w/ CaviWipes (Metrex Research, Romulus, MI USA), before being placed in HBSS.

For this experiment, the Exacta (*EC 330 Mini Saw New Exacta Jacksonville, FL, USA*) was used for removal of the occlusal enamel. The teeth were then affixed to acrylic bars (1 cm x 1 cm x 4 cm) with adhesive glue (Loctite Super Bonder, Henkel Corporation, North America) for sectioning with a double faced diamond disc (IsoMet 1000 Precision Diamond Saw, Buehler, an ITW Company, Lake Bluff, IL USA) mounted on a precision cutter (IsoMet 1000, Buehler, an ITW Company, Lake Bluff, IL USA), used at a low speed and under cooling, to serially cut the

specimens, with a distance of 1 mm between sections. The acrylic bars were rotated 90 degrees, and the specimens were serially cut again, with a distance of 1 mm between sections. The specimens were removed from the acrylic bars. Eighty rectangular dentin specimens (1 mm x 1 mm x >8 mm) were obtained, and the specimens from each tooth were randomly and equally distributed among the test solutions.

The prepared specimens were stored, in their test solution, in an environment with 100% humidity at 37 degrees Celsius for three hours before each specimen was rinsed with HBSS per Grigoratos protocol in 2001.

The three-point bend test was conducted on a universal testing machine (Instron 5943 Single Column Tabletop Testing System, Instron Corporation, Norwood, MA USA), which was calibrated before testing per manufacturer guidelines. Due to the inability to procure a 3-Point Flexure Fixture that was compatible with the Instron 5943, the tensile fixture was utilized with the lower support points fixed a distance of 8 mm. The load cell was applied perpendicular to the long axis of the prepared dentin bars with a 1.0 mm / min crosshead speed until specimen fracture (Plotino, 2007). The primary outcomes were the Megapascals (MPa) reading on the Instron gauge at which each sample failed as well as the load at failure in newton's (N).

A one-way between subjects analysis of variance (ANOVA) was conducted to compare the effect of the different storage solutions on the maximum flexure stress (MFS) and the load at MFS of the dentin bar samples. P-values <0.05 were considered statistically significant. Data were analyzed using SPSS version 22.0 (SPSS, Chicago, IL USA).

5.3 NUMBER OF PARTICIPANTS:

A convience sample of 30 teeth will be utilized by this study.

Test	Medium	No. of Specimens
Group 1 control	Hank's Balanced Salt Solution	20
Group 2	5.25% NaOCL	20
Group 3	3% Glutaraldehyde	20
Group 3	0.5% Thymol	20

5.4 DESCRIBE ANY CODING OF DATA OR SPECIMENS, INCLUDING INFORMATION ON WHO HOLDS THE KEY TO THE CODE:

There will be no coding of Data.

5.5 MILITARY RELEVANCE: This test will bring awareness to the methods of storing extracted teeth. Dental residency's run by the military now mandate research projects. Many research projects have used extracted teeth and studied various properties. The present study will inform future students about potential reduced flexural strength resulting from a chosen storage medium.

5.6 MEDICAL APPLICATION: Same as 5.5.

6.0 PUBLICATION REQUIRMENTS: Proper WAMC publication clearance is required prior to all presentations, abstracts, and publications. The following require WAMC approval: reports involving WAMC subjects and/or patients, reports that cite WAMC in the title or byline, reports of WAMC approved clinical investigation or research, reports of research performed at WAMC, and reports of research conducted by WAMC assigned personnel.

The investigators will obtain proper OTSG publication clearance prior to all presentations, abstracts, and publications that involve traumatic brain injury, post-traumatic stress, poly-pharmacy,

pain, or suicide.

The investigators must provide to the Department of Clinical Investigation a listing of presentations, abstracts, and publications arising from the study.

IRBNet Number:
PI: CPT J. Clay Hastings, DC
Protocol Title: THE EFFECT OF THE STORAGE SOLUTION, 3% GLUTARALDEHYDE, ON DENTIN FLEXURAL STRENGTH
Initial Date Submitted: 16 DEC 2015
Revision Date:

7.0 SIGNATURES:
I verify that the contents of this proposal are accurate and that I have read and agree to comply with the statements above which outline my responsibilities as a Principal Investigator.

Principal Investigator Signature
Name and Date: J. CLAY HASTINGS, 11 JAN 2016

7.1 OTHER SIGNATURES FOR APPROVAL:
I concur with the submission of this proposal to the Department of Clinical Investigation for review and approval.

Service Chief Signature
Name and Date: LTC MANUEL PELAEZ, 11 JAN 2016

Department Chief Signature
Name and Date: COL STACY LARSEN, 11 JAN 2016

FOR USE BY DEPARTMENT OF CLINICAL INVESTIGATION STAFF ONLY

Regulatory Affairs Review

Name and Date

Statistical Review

Name and Date

Submitted by Clay Hastings in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.
Accepted on behalf of the Faculty of the Graduate School by the thesis committee:

Date	Manuel Pelaez, DMD Research Mentor
Date	Jason Bullock, DMD AEGD Assistant Director
Date	Stacy Larsen, DDS AEGD Program Director

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my AEGD Program Director, COL Larsen; Assistant Director, AEGD Assistant Program Director, MAJ Bullock; Research Mentor, LTC Pelaez; and Statistician, Mr. Thomas Beltran for their guidance, support, and developmental assistance throughout the duration of my research project. Thanks to my classmates and staff, dental assistants, and laboratory assistants. Last but certainly not least, my amazing wife Jennifer, who blessed me with two beautiful girls, Savannah and Scarlett, for allowing me to sacrifice so much valuable family time over the past two years while providing me with ending love and support without which I would have not been able to successfully complete this program.

COPYRIGHT

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

THE EFFECT OF THE STORAGE SOLUTION, 3% GLUTARALDEHYDE, ON DENTIN FLEXURAL STRENGTH

is appropriately acknowledged and, beyond brief excerpts, is with the permission of the copyright owner.

J. Clay Hastings
2-yr AEGD Program, Fort Bragg
Uniformed Services University
Date: 06/01/2017

ABSTRACT

Statement of the problem:

Extracted teeth are widely used in dental research. The current protocol is to store extracted teeth in storage solutions which serve as a disinfectant and a storage medium. Researchers have not determined if storage solutions could alter the flexural strength of dentin.

Methods and Materials:

Freshly extracted third molars were disinfected with Cavicide wipes. These teeth were stored in Hank's Balanced Salt Solution (HBSS) to remain hydrated until they were utilized for sample preparation. Dentin bars measuring 1mm by 1mm by 10mm were cut using a precision saw, IsoMet Low Speed Saw. Twenty Dentin bars were immersed in one of four solutions; HBSS, Sodium hypochlorite, thymol, and glutaraldehyde. The bars were stored for three hours. A 3-point bend test was then conducted on the Instron 5943 Single Column Table Top System to test the flexural strength of each dentin bar sample.

Results:

Dentin bars immersed in glutaraldehyde demonstrated flexural strength consistent with the control, HBSS. The results confirmed that sodium hypochlorite would decrease the flexural strength on dentin. Sodium hypochlorite was the only solution that had a significant affect on the flexural strength of dentin.

Conclusions:

Within the limits of this study dentin bars from extracted teeth stored in 3% glutaraldehyde for three hours showed no significant change in the flexural strength when compared to HBSS. Army research may want to consider the effects of their storage medium on the teeth that are to be utilized for in vitro research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA – Analysis of Variance

CDC – Centers for Disease Control and Prevention

cm – centimeters

HBSS – Hank's Balance Salt Solution

HBV – Hepatitis B virus

HIV – Human Immunodeficiency Virus

HSD – Honest Significant Difference

MFS – Maximum flexure stress

MPa – Megapascal

mm – millimeters

N – Newton

NaOCl – Sodium Hypochlorite

WHO – World Health Organization

INTRODUCTION

Before becoming commercially available, novel dental materials are first tested in *in vitro* utilizing extracted human teeth, which must first be disinfected and stored until used for testing purposes (Kishen, 2005). The easiest storage is to immerse the teeth in an aqueous solution, however, the effect of the storage solution on the tooth structure could result *in vitro* testing conditions that cannot be replicated *in vivo*. It is critical to understand and evaluate the effects of the storage solution on the extracted teeth, and the internal tooth structure, such as dentin.

Dentin specimens are commonly used for *in vitro* dental research, evaluating dentin permeability, hydraulic conductance, and bond strengths (Goodis, 1993). The flexural strength of dentin should be evaluated because it is the flexural strength that determines the fracture resistance of a sample (Plotino, 2007).

Storage solutions, conditions, and duration are not standardized for extracted teeth that are to be used for research (Goodhis, 1993). Strawn concluded that there were changes in surface chemistry of dentin based on storage solution and duration, both of which must be considered when studying dentin (Strawn, 1996). To determine if the storage solution of extracted teeth affects dentin flexural strength, the extracted teeth will be collected within a 30 day period, disinfected according to Centers for Disease Control and Prevention

(CDC) guidelines and placed in Hank's balanced salt solution (HBSS) until the specimens are prepared.

The CDC guidelines for infection control in dental health-care settings states the surface of the extracted teeth must be cleaned of visible blood and debris, and disinfected with an EPA-registered hospital disinfectant with intermediate-level activity (i.e., tuberculocidal claim). Additionally the teeth should be heat-sterilized by using an autoclave cycle for 40 minutes. The CDC does acknowledge it is unknown whether the autoclave affects dentin structure to the point where the dental materials and dentin relationship would be affected for research. (CDC, 2003)

During the author's literature review, the author noted that a thymol solution was a disinfectant and/or storage solution utilized prior to the samples being tested, in several recent studies. Marcelino (2014) and Hope (2012) utilized 0.01% thymol solution. These studies did not provide an explanation as to why a thymol storage medium was selected for their study.

In an effort to identify acceptable storage mediums commonly utilized, this author contacted a representative of the Gordon J. Christensen Clinicians Report, a publication of the CR Foundation, on 27 December 2016, a non-profit, educational research institute. Following inquiry regarding their chosen storage solution for extracted teeth prior to testing, a member of the Science Team

responded via a Public Relations Coordinator. The CR Foundation Science Department, as of DEC 2016, stores freshly extracted teeth in water prior to use in bonding studies, and those teeth are used within the first six months following extraction. Studies have shown that storage in water can interfere with certain properties – for example, ion exchange with oral structures. The CR Foundation recognizes that water storage seems to be the most widely used and simplest method, so they continue to utilize it. Teeth stored for longer than six months are used for other research, including bur cutting, endodontic research, and radiography research. In addition, the CR Foundation noted that they have chosen to store freshly extracted teeth in a dilute (0.5%) solution of Chloramine T for periods of three to seven days for disinfection purposes. They acknowledged that teeth undergo rapid and significant post-mortem changes.

To the author's knowledge, no previous studies, other than the study conducted along side the researcher by Dr. Ravindran, have compared the flexural strength of dentin bars placed in the four solutions, 5.25% sodium hypochlorite (NaOCl), 0.05% thymol, 3% glutaraldehyde, and the control (HBSS). The researcher has been unable to determine if a standardized storage solution is utilized for extracted teeth in preparation for research. The aim of the present study was to evaluate the dentin flexural strength of dentin bars stored in three widely used storage solutions with a focus on 3% glutaraldehyde.

DENTIN

The outermost layer of teeth is enamel, the inner layer is dentin. Dentin the inner layer of human teeth, is frequently utilized *in vitro* research. Dentin is formed by odontoblasts that are contained within the pulp (Shababange, 2013). The volume percentage of dentin is approximately 50 – 70 vol% inorganic (hydroxyapatite), 20 – 25 vol% organic material (type I collagen major protein), and 10 – 25 vol% fluid (water). The collagen protein constitutes 90% of the organic matrix, and is often referred to as the collagen matrix (Tate, 1991). Changing the content of the organic matrix, affects the ability of dentin to interact with resin restorations. The challenge of dentin bonding in restorative dentistry, is considered more difficult and less predictable (Sturdevant's Art and Science of Operative Dentistry, 2013).

In dentistry, research is constantly evaluating and trying to improve the resin-dentin adhesion. The stability and durability, of resin-dentin adhesion or bond strength is secondary to the resin monomers and their ability to penetrate collagen fibers and the resulting hybrid layer that is formed (Toledano, 2006). The dentin/resin interface of the hybrid layer can be affected by the degradation of the denuded collagen matrix (Perdigao, 2013). NaOCl acts as an oxidizing agent and may degrade the dentin organic matrix (Marending, 2007). Any change in the properties of dentin can influence the dentin/resin interface (Marshall, 1997). Several studies have shown NaOCl as an endodontic irrigant modifies the structure of intraradicular dentin.

The composition of dentin is not fixed, the size of dentin tubules varies relative to the position on the tooth, the age of the tooth, the presence or absence of disease (Toto, 1971). As teeth age they contain less water than young teeth, 10 – 20 years of age (Toto, 1971). Dehydrated dentin has been described as brittle with decreased strength (Jameson, 1994). Extracted teeth not utilized immediately for research, must be cleaned free of blood and debris, disinfected per CDC guidelines (2003), and stored until the specimen is to be used for research. Outhwaite et al, in 1976, showed that an increase in temperature increases dentin permeability; therefore, it is reasonable to speculate that a decrease in temperature would result in decreased dentin permeability.

STORAGE SOLUTIONS

The storage conditions of extracted teeth can alter the dental tissue and mechanical properties of teeth (Muhleman, 1964), which can affect the results of *in vitro* testing during the experimental process. While preserving the hydration of the dental hard tissues, the aqueous storage solution can result in mineral leaching (Strawn,1996). The mechanical properties of enamel and dentin are dependent on their mineral content (Tesch, 2001). In contrast to previous research, Marcelino et al, found that a short application, 10 minutes, of chemical agents did not reduce or compromise the flexural strength of dentin (Marcelino, 2014).

Storage solutions utilized in previous research include HBSS (Aydin, 2015), glutaraldehyde (Aydin, 2015), NaOCl (Grigoratos, 2001), and thymol (Kivanc, 2009 and Aydin, 2015). Aydin et al, concluded that 0.2% glutaraldehyde, HBSS, 0.1% NaOCl or 0.1% thymol were acceptable for *in vitro* tests, however, long-term studies of the storage solutions were needed to evaluate the change in the mechanical properties (Aydin, 2015). The effect of these storage solutions on dentin and the flexural strength of dentin have received little investigation.

Hank's Balanced Salt Solution

HBSS Is a balanced salt solution is made to specific physiological pH and isotonic concentration. The osmolality of HBSS closely matches the cells of the tooth (calcium, magnesium, sodium, phosphate, and chloride ions), balanced to preserve dental tissues (Aydin, 2015). HBSS was selected as the control solution for this study for the properties listed above.

Sodium hypochlorite (NaOCl)

NaOCl is an inexpensive commonly used disinfectant or bleaching agent that is capable of dissolving vital and necrotic tissue by breaking down protein into amino acids (Johnson, 2009). The American Association of Endodontists published in the Winter 2011, Endodontic – Colleagues for Excellence, a concentration of 5.25% NaOCl in as little as 40 minutes was effective in removing *Enterococcus faecalis* (AAE, 2011). At 40 minutes the flexural strength and

modulus of elasticity of dentin may be reduced by exposure to 5% NaOCl (Sim, 1996). Grigoratos immersed dentin bars in 3% and 5% sodium hypochlorite and found decreased dentin flexural strength. He proposed the sodium hypochlorite interfered with the collagen matrix composition, resulting in brittle dentin (Grigoratos, 2001).

0.05% Thymol

Thymol is a naturally occurring biocidal agent with strong antimicrobial properties (Stappert, 2006). A 0.5% thymol solution was utilized as storage solution before testing flexural strength of extracted premolars (Shafiei, 2014). After testing was completed, the researcher discovered an article, which stated, a thymol solution is widely used to store teeth prior to mechanical testing (Aydin, 2015).

3% Glutaraldehyde

Glutaraldehyde has been used in medicine since the 1960s (Booth, 1998). Glutaraldehyde is recognized as a potent sterilizing agent. It has a broad-spectrum biocidal activity, affecting Gram-positive and Gram-negative bacteria, spores, and viruses such Hepatitis B virus (HBV) and Human Immunodeficiency Virus (HIV) (Urbani, 1990). The 19th List of Essential Medicines by the World Health Organization (WHO) has 2% glutaraldehyde as one of the four disinfectants listed (WHO, 2015). It has been reported that 2% glutaraldehyde results in a 15% decrease in hardness of bovine dentin after 20 minutes,

however, after 2 days of storage resulted in a 15% increase in dentin hardening (Wemes, 1984). Teeth stored in glutaraldehyde, HBSS, NaOCl, and thymol can be used for mechanical *in vitro* tests up to 2 months after being initially placed in the solution, however, at 12 months of storage, the microhardness of enamel and dentin was significantly decreased (Aydin, 2015).

3-POINT LOADING TEST

Fracture resistance or flexural strength of dentin is a commonly tested parameter. It is the gold standard to observe alterations of mechanical properties in mineralized tissues. (Aydin, 2015). A three-point loading system utilizing central loading on a simply supported beam can determine a small deflection or fracture of rectangular dentin bars (American Society for Testing & Materials, 1989).

HYPOTHESIS

Dentin bars stored in 3% glutaraldehyde will have a reduced dentin flexural strength compared to dentin bars stored in our control solution, HBSS. The dentin bars stored in 0.05% Thymol and 5.25% NaOCl will have a reduced dentin flexural strength compared to dentin bars stored in our control solution, HBSS.

MATERIALS AND METHODS

Study approval from the Fort Bragg WAMC Institutional Review Board, thirty extracted teeth were collected within a thirty day period, disinfected per the CDC guidelines and stored in HBSS until the teeth were prepared and sectioned into dentin bars. All of the dentin bar specimens required for testing were prepared within an eight hour time period and placed in our control solution, HBSS, until all dentin bars were prepared (Plotin, 2007). The dentin bars from each tooth were divided between the control solution (HBSS), 5.25% NaOCl, 0.05% thymol and 3% glutaraldehyde. Once the samples were prepared and after the dentin bars soaked in their respective storage solutions for three hours, a 3-point bend test was conducted to evaluate the effect of the storage medium on the dentinal flexural strength.

Study Design

Thirty extracted teeth were collected, all teeth were human third molars, free of carious lesions, cracks, and restorations. The teeth were collected within a thirty day period, disinfected following the CDC guidelines and stored in HBSS. Eighty dentin bar specimens were prepared within an eight hour time period and equally distributed to one of four storage solutions; HBSS (n=20), 5.25% NaOCl (n=20), 0.05% thymol (n=20) and 3% glutaraldehyde (n=20). The specimens were stored, in solution, in an environment with 100% humidity at 37^{o} C for three hours before the flexural strength was tested (Grigoratos, 2001).

Specimen preparation

To test the flexural strength of dentin, rectangular bars of dentin were created from freshly extracted human teeth, teeth collected within a 30 day time period. Maxillary and mandibular third molars, n=30, without caries or restorations extracted from male and female patients, 18 – 36 years of age were sampled for this study. Immediately after extraction, gross debris was removed from the samples w/ CaviWipes (Metrex Research, Romulus, MI USA), before being placed in HBSS.

For this experiment, the Exacta (*EC 330 Mini Saw New Exacta Jacksonville, FL, USA*) was used for removal of the occlusal enamel. The teeth were then affixed to acrylic bars (1 cm x 1 cm x 4 cm) with adhesive glue (Loctite Super Bonder, Henkel Corporation, North America) for sectioning with a double faced diamond disc (IsoMet 1000 Precision Diamond Saw, Buehler, an ITW Company, Lake Bluff, IL USA) mounted on a precision cutter (IsoMet 1000, Buehler, an ITW Company, Lake Bluff, IL USA), used at a low speed and under cooling, to serially cut the specimens, with a distance of 1 mm between sections. The acrylic bars were rotated 90 degrees, and the specimens were serially cut again, with a distance of 1 mm between sections. The specimens were removed from the acrylic bars. Eighty rectangular dentin specimens (1 mm x 1 mm x >8 mm) were obtained, and the specimens from each tooth were randomly and equally distributed among the test solutions.

The prepared specimens were stored, in their test solution, in an environment with 100% humidity at 37 degrees Celsius for three hours before each specimen was rinsed with HBSS per Grigoratos protocol in 2001.

The three-point bend test was conducted on a universal testing machine (Instron 5943 Single Column Tabletop Testing System, Instron Corporation, Norwood, MA USA), which was calibrated before testing per manufacturer guidelines. Due to the inability to procure a 3-Point Flexure Fixture that was compatible with the Instron 5943, the tensile fixture was utilized with the lower support points fixed a distance of 8 mm. The load cell was applied perpendicular to the long axis of the prepared dentin bars with a 1.0 mm / min crosshead speed until specimen fracture (Plotino, 2007). The primary outcomes were the Megapascals (MPa) reading on the Instron gauge at which each sample failed as well as the load at failure in newton's (N).

A one-way between subjects analysis of variance (ANOVA) was conducted to compare the effect of the different storage solutions on the maximum flexure stress (MFS) and the load at MFS of the dentin bar samples. P-values <0.05 were considered statistically significant. Data were analyzed using SPSS version 22.0 (SPSS, Chicago, IL USA).

RESULTS

A one-way ANOVA was conducted to compare the effect of various storage solutions on the dentin flexural strength of the prepared 80 dentin bar samples. There was a significant effect from the storage solution 5.25% NaOCl on the Maximum Flexure Stress (MFS), but no other storage solutions displayed a significant affect on MFS.

Post-hoc comparisons using the Tukey Honest Significant Difference (HSD) test indicated that the mean MFS for the 5.25% NaOCl solution (M = 9.90, SD = 6.27) was significantly lower than the glutaraldehyde solution (M = 27.36, SD = 10.70), the HBBS (M = 20.09, SD = 11.57), and the 0.05% thymol solution (M = 20.14, SD = 13.76). However, the glutaraldehyde, HBSS, and thymol solutions did not significantly differ from one another in either MFS or the load at MFS. These results indicate that, excluding the NaOCl solution, there is no difference in the effect on the flexural strength of dentin after three hours in HBSS, thymol and glutaraldehyde.

DISCUSSION

Research protocols have not identified the ideal storage solution for extracted teeth that are to be used for research. The effect of the storage solution on dentin and the properties of dentin are unknown. Research has used a variety of storage solutions including NaOCl, glutaraldehyde, and thymol. This study

attempted to determine if storage solutions used to disinfect and store extracted teeth could alter the properties of dentin, specifically the flexural strength.

The null hypothesis was rejected, the dentin bars stored in 3% glutaraldehyde did not have a reduced dentin flexural strength compared to our control, HBSS. The 3% glutaraldehyde samples produced a dentin flexural strength of 27.4 MPa. Marcelino's control solution, deionized water, produced a dentin flexural strength with a mean of 29.5 MPa. The flexural strength of the dentin bars stored in 0.05% thymol most closely matched our control, HBSS; however, the 20.1 MPa for both of the solutions was significantly less than what was reported by Marcelino (2014). As expected, 5.25% NaOCl solution resulted in a significantly decreased dentin flexural strength, the mean was 9.9 MPa. This is agreement with Sim (1996) and Grigoratos (2001) conclusions of a significant reduction of flexural strength.

The limitations of this study include location of dentin bar samples, duration of samples in storage solutions, sample size, and the execution of the 3-point bend test on the Instron. Outhwaite et al, in 1976, showed permeability of dentin bars closer to the pulp contained a larger diffusional surface area, leading to increased permeability. They postulated that permeability could be quite different depending on the distance from the pulp chamber. There was no delineation between what parts of the tooth the dentin bars were obtained. The teeth stored in glutaraldehyde for three hours did not show an alteration in the

flexural strength of dentin. The sample size could be a limitation of the results. If larger samples of specimens were tested, there would have been a decrease in the variance, resulting in a tighter confidence interval. The result could be potentially significant in identifying a difference between glutaraldehyde, HBSS, and thymol solutions. Due to limitation of access to equipment, the 3-point bend test was conducted on the tensile jig fixture which is not an approved flexure fixture.

CONCLUSION

Within the limitations of an *in vitro* study, it may be concluded that the dentin flexural strength is not affected by the storage solutions, HBSS, 0.05% thymol, and 3% glutaraldehyde. The dentin flexural strength is significantly reduced after just three hours of storage in a 5.25% NaOCl solution. The results of this research can be utilized for reference in future research involving the storage and testing of extracted teeth, specifically the effects on the flexural strength of dentin. Additional research is needed to evaluate the affect of these storage solutions for different amounts of time, i.e. 24 hours, 1 week, 4 weeks, 3 months, and 6 months. Traditionally extracted teeth were stored in formaldehyde/formalin in educational settings; what impact does this storage solution have on dentin. Army research may want to consider the effects of their storage medium on the teeth that are to be utilized for in vitro research.

Table 1. Maximum Flexure Stress

Statistics

		Load at Maximum Flexure Stress (N)	Maximum Flexure Stress (MPa)
N	Valid	79	79
	Missing	0	0
Mean		12.9949103	19.4923643
Median		11.8019500	17.7029200
Std. Deviation		8.27822637	12.41734088
Range		44.79191	67.18787
Minimum		.28691	.43036
Maximum		45.07882	67.61823

Table 2. Samples Per Solution

Solution

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Glutaraldehyde	20	25.3	25.3	25.3
	HBSS	20	25.3	25.3	50.6
	5.25% NAOCL	19	24.1	24.1	74.7
	0.05% Thymol	20	25.3	25.3	100.0
	Total	79	100.0	100.0	

Table 3. Average Maximum Flexure Stress

Descriptive

		N	Mean	Std. Deviation	Std. Error
Load at Maximum Flexure Stress (N)	Glutaraldehyde	20	18.2418145	7.13616796	1.59569566
	HBSS	20	13.3925420	7.71310751	1.72470327
	5.25% NAOCL	19	6.5993926	4.18293780	.95963175
	0.05% Thymol	20	13.4261160	9.17022132	2.05052383
	Total	79	12.9949103	8.27822637	.93137323
Maximum Flexure Stress (MPa)	Glutaraldehyde	20	27.3627225	10.70425150	2.39354340
	HBSS	20	20.0888130	11.56966294	2.58705528
	5.25% NAOCL	19	9.8990863	6.27440750	1.43944780
	0.05% Thymol	20	20.1391715	13.75533330	3.07578603
	Total	79	19.4923643	12.41734088	1.39706000

Table 4. 95% Confidence Interval for Maximum Flexure Stress

Descriptives

		95% Confidence Interval for Mean		Minimum	Maximum
		Lower Bound	Upper Bound		
Load at Maximum Flexure Stress (N)	Glutaraldehyde	14.9019851	21.5816439	10.53481	32.91898
	HBSS	9.7826966	17.0023874	5.07973	37.97853
	5.25% NAOCL	4.5832811	8.6155041	.28691	14.66929
	0.05% Thymol	9.1343203	17.7179117	3.08730	45.07882
	Total	11.1406886	14.8491319	.28691	45.07882
Maximum Flexure Stress (MPa)	Glutaraldehyde	22.3529786	32.3724664	15.80221	49.37848
	HBSS	14.6740441	25.5035819	7.61960	56.96780
	5.25% NAOCL	6.8749187	12.9232539	.43036	22.00393
	0.05% Thymol	13.7014773	26.5768657	4.63094	67.61823
	Total	16.7110315	22.2736971	.43036	67.61823

Table 5. Test of Homogeneity of Variances

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Load at Maximum Flexure Stress (N)	1.070	3	75	.367
Maximum Flexure Stress (MPa)	1.070	3	75	.367

Table 6. ANOVA

ANOVA

		Sum of Squares	df	Mean Square	F
Load at Maximum Flexure Stress (N)	Between Groups	1334.631	3	444.877	8.319
	Within Groups	4010.633	75	53.475	
	Total	5345.264	78		
Maximum Flexure Stress (MPa)	Between Groups	3002.922	3	1000.974	8.319
	Within Groups	9023.926	75	120.319	
	Total	12026.848	78		

Table 7. ANOVA Significance

ANOVA

		Sig.
Load at Maximum Flexure Stress (N)	Between Groups	.000
	Within Groups	
	Total	
Maximum Flexure Stress (MPa)	Between Groups	.000
	Within Groups	
	Total	

Table 8. Tukey HSD Load at Maximum Flexure Stress

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Solution	(J) Solution	Mean Difference (I-J)	Std. Error	Sig.
Load at Maximum Flexure Stress (N)	Glutaraldehyde	HBSS	4.84927250	2.31246857	.163
		5.25% NAOCL	11.64242187*	2.34269820	.000
		0.05% Thymol	4.81569850	2.31246857	.168
	HBSS	Glutaraldehyde	-4.84927250	2.31246857	.163
		5.25% NAOCL	6.79314937*	2.34269820	.025
		0.05% Thymol	-.03357400	2.31246857	1.000
	5.25% NAOCL	Glutaraldehyde	-11.64242187*	2.34269820	.000
		HBSS	-6.79314937*	2.34269820	.025
		0.05% Thymol	-6.82672337*	2.34269820	.024
	0.05% Thymol	Glutaraldehyde	-4.81569850	2.31246857	.168
		HBSS	.03357400	2.31246857	1.000
		5.25% NAOCL	6.82672337*	2.34269820	.024
Maximum Flexure Stress (MPa)	Glutaraldehyde	HBSS	7.27390950	3.46870312	.163
		5.25% NAOCL	17.46363618*	3.51404757	.000
		0.05% Thymol	7.22355100	3.46870312	.168
	HBSS	Glutaraldehyde	-7.27390950	3.46870312	.163
		5.25% NAOCL	10.18972668*	3.51404757	.025
		0.05% Thymol	-.05035850	3.46870312	1.000
	5.25% NAOCL	Glutaraldehyde	-17.46363618*	3.51404757	.000
		HBSS	-10.18972668*	3.51404757	.025
		0.05% Thymol	-10.24008518*	3.51404757	.024
	0.05% Thymol	Glutaraldehyde	-7.22355100	3.46870312	.168
		HBSS	.05035850	3.46870312	1.000
		5.25% NAOCL	10.24008518*	3.51404757	.024

*. The mean difference is significant at the 0.05 level.

Table 9. Tukey HSD 95% Confidence Interval

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Solution	(J) Solution	95% Confidence Interval	
			Lower Bound	Upper Bound
Load at Maximum Flexure Stress (N)	Glutaraldehyde	HBSS	-1.2269229	10.9254679
		5.25% NAOCL	5.4867957	17.7980481
		0.05% Thymol	-1.2604969	10.8918939
	HBSS	Glutaraldehyde	-10.9254679	1.2269229
		5.25% NAOCL	.6375232	12.9487756
		0.05% Thymol	-6.1097694	6.0426214
	5.25% NAOCL	Glutaraldehyde	-17.7980481	-5.4867957
		HBSS	-12.9487756	-.6375232
		0.05% Thymol	-12.9823496	-.6710972
	0.05% Thymol	Glutaraldehyde	-10.8918939	1.2604969
		HBSS	-6.0426214	6.1097694
		5.25% NAOCL	.6710972	12.9823496
Maximum Flexure Stress (MPa)	Glutaraldehyde	HBSS	-1.8403844	16.3882034
		5.25% NAOCL	8.2301961	26.6970762
		0.05% Thymol	-1.8907429	16.3378449
	HBSS	Glutaraldehyde	-16.3882034	1.8403844
		5.25% NAOCL	.9562866	19.4231667
		0.05% Thymol	-9.1646524	9.0639354
	5.25% NAOCL	Glutaraldehyde	-26.6970762	-8.2301961
		HBSS	-19.4231667	-.9562866
		0.05% Thymol	-19.4735252	-1.0066451
	0.05% Thymol	Glutaraldehyde	-16.3378449	1.8907429
		HBSS	-9.0639354	9.1646524
		5.25% NAOCL	1.0066451	19.4735252

Table 10. Tukey HSD, Load at Maximum Flexure Stress (N)

Load at Maximum Flexure Stress (N)

Tukey HSD[a,b]

Solution	N	Subset for alpha = 0.05	
		1	2
5.25% NAOCL	19	6.5993926	
HBSS	20		13.3925420
0.05% Thymol	20		13.4261160
Glutaraldehyde	20		18.2418145
Sig.		1.000	.168

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 19.740.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 11. Tukey HSD, Load at Maximum Flexure Stress (MPa)

Load at Maximum Flexure Stress (MPa)

Tukey HSD[a,b]

Solution	N	Subset for alpha = 0.05	
		1	2
5.25% NAOCL	19	9.8990863	
HBSS	20		20.0888130
0.05% Thymol	20		20.1391715
Glutaraldehyde	20		27.3627225
Sig.		1.000	.168

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 19.740.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Figure 1. Tukey HSD Mean Maximum Flexure Stress

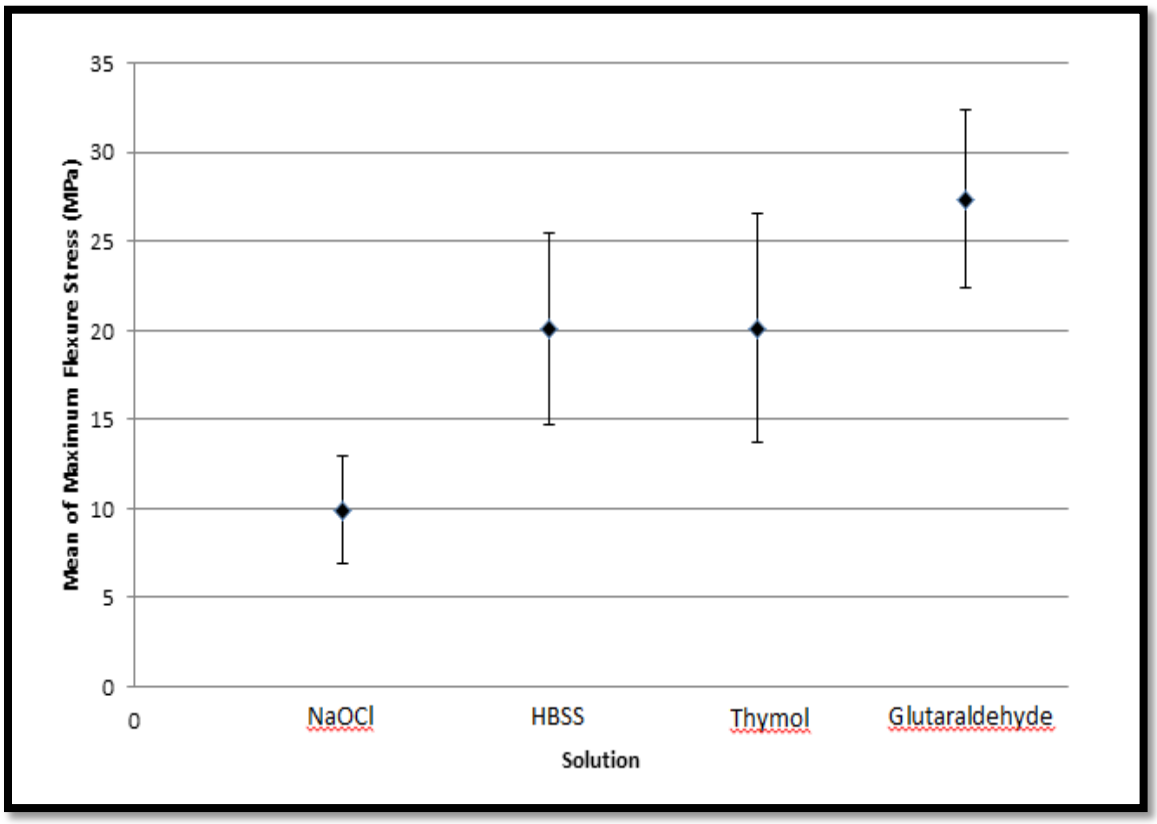


Figure 2. Buehler IsoMet Low Speed Saw

Figure 3. Specimen preparation on the Buehler IsoMet Low Speed Saw

Figure 4. Specimen preparation – Close-up

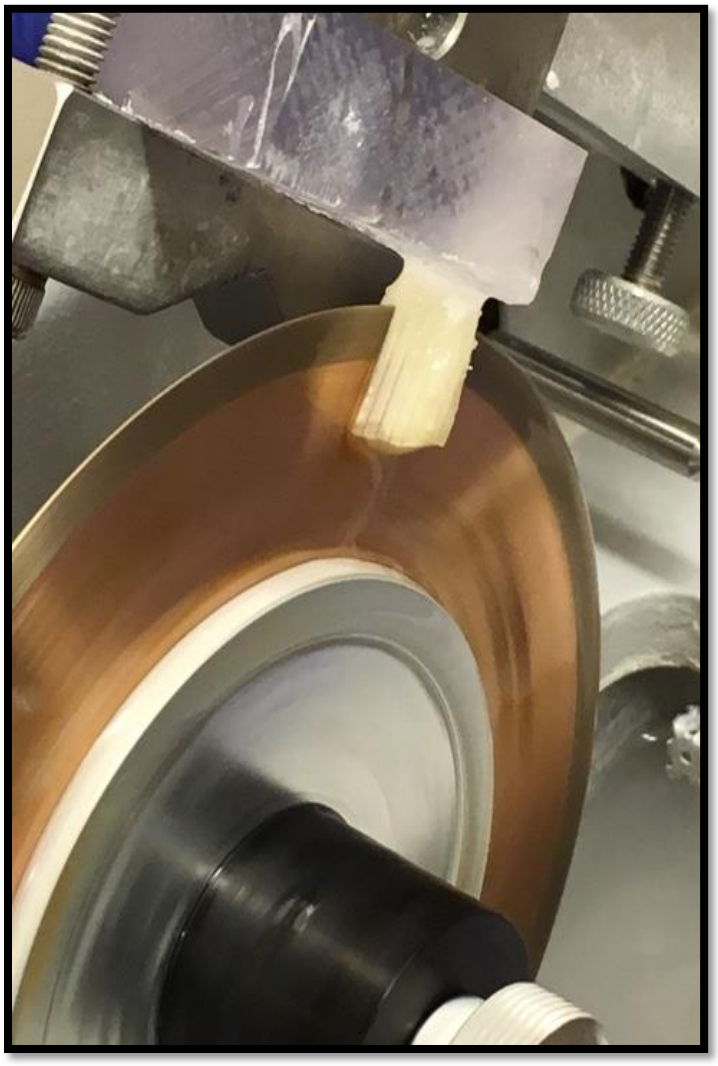


Figure 5. Prepared dentin bar specimen: Width 1.03 mm

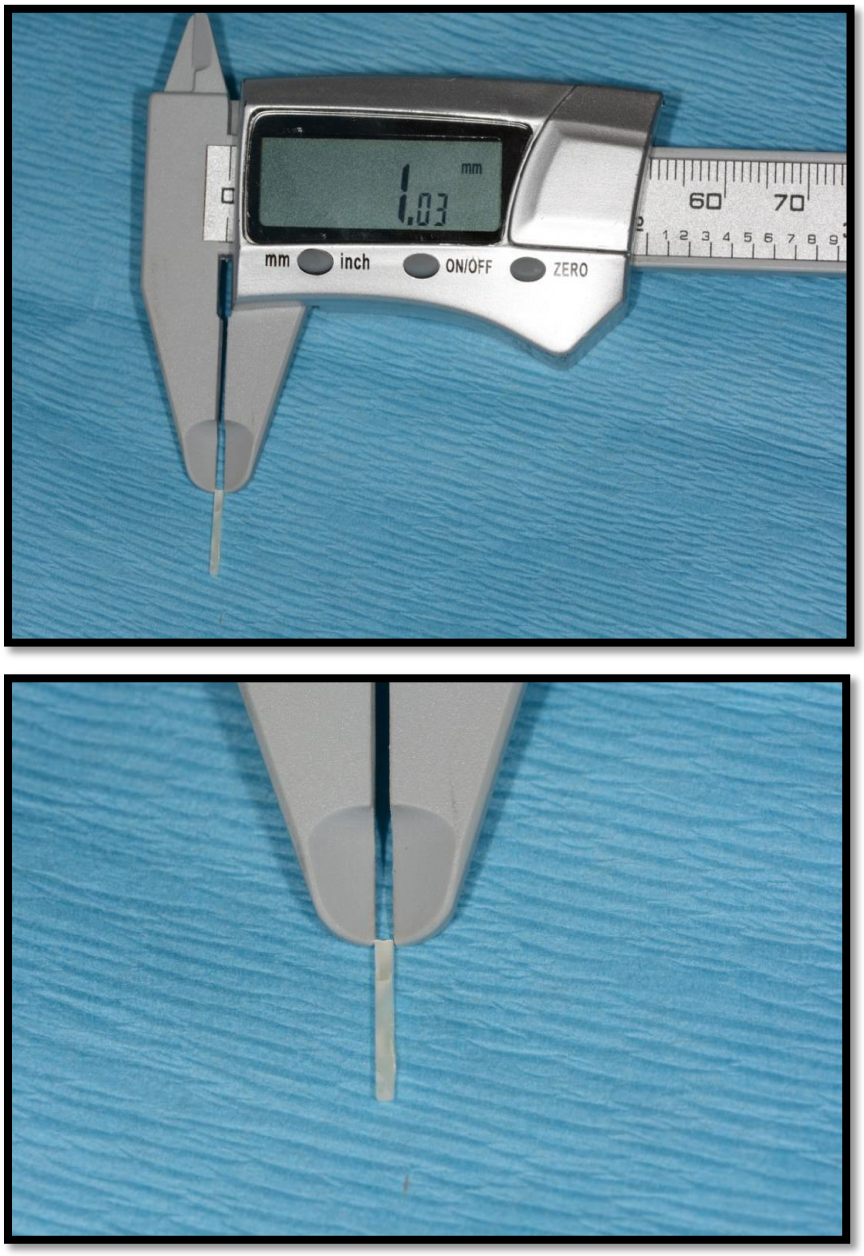


Figure 6. Prepared dentin bar specimen: Length 9.94 mm

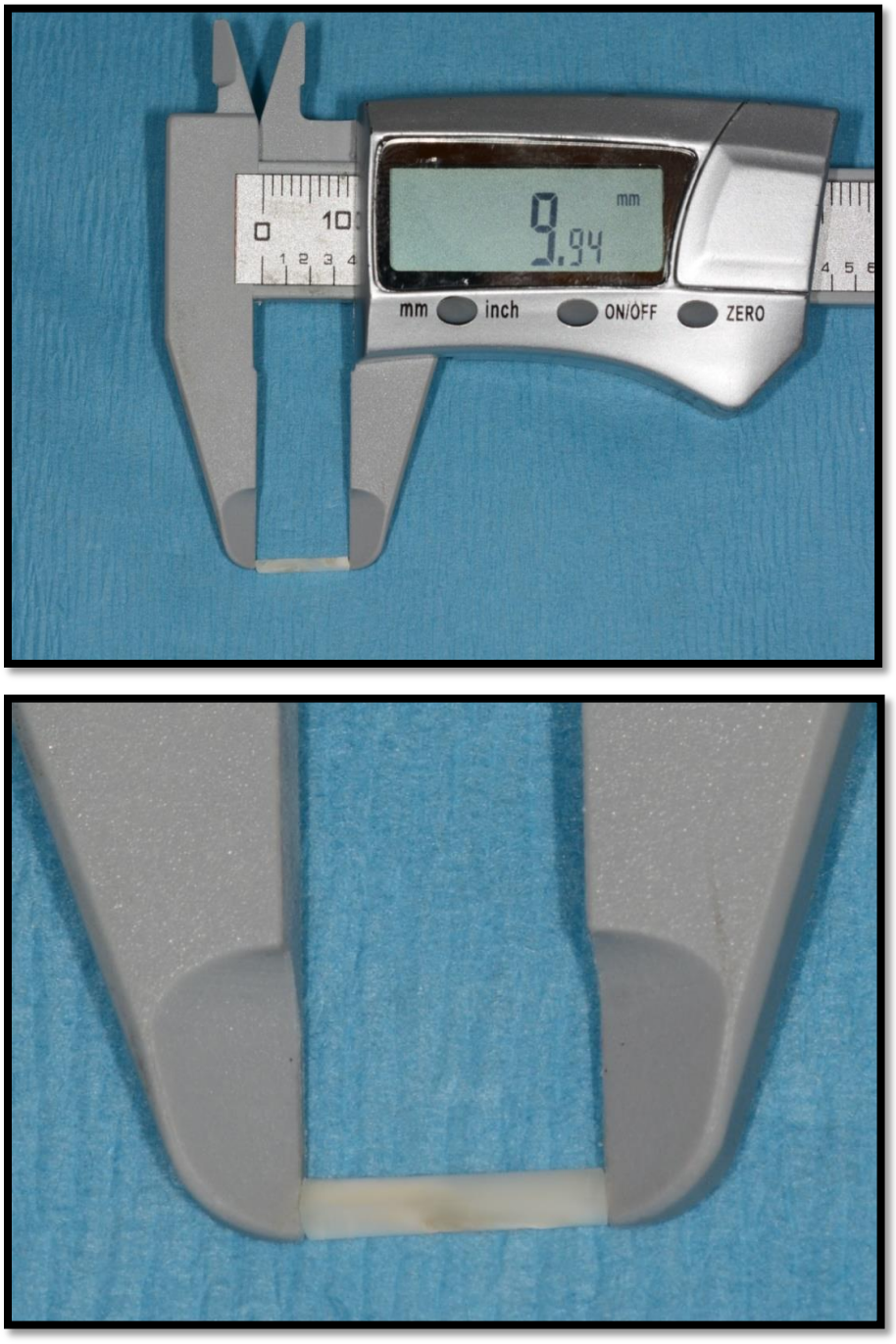


Figure 7. Inston 5943 Single Column Table Top System

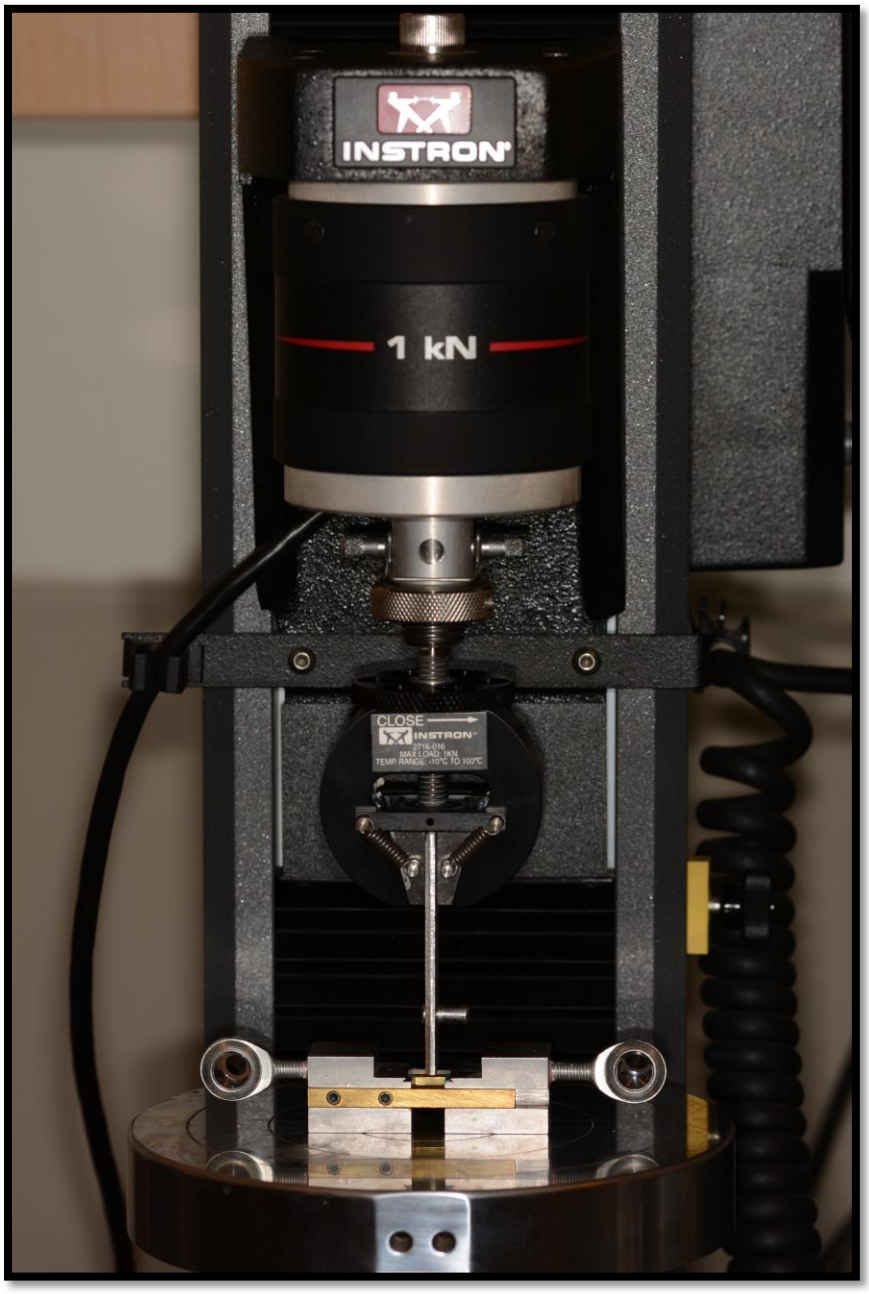
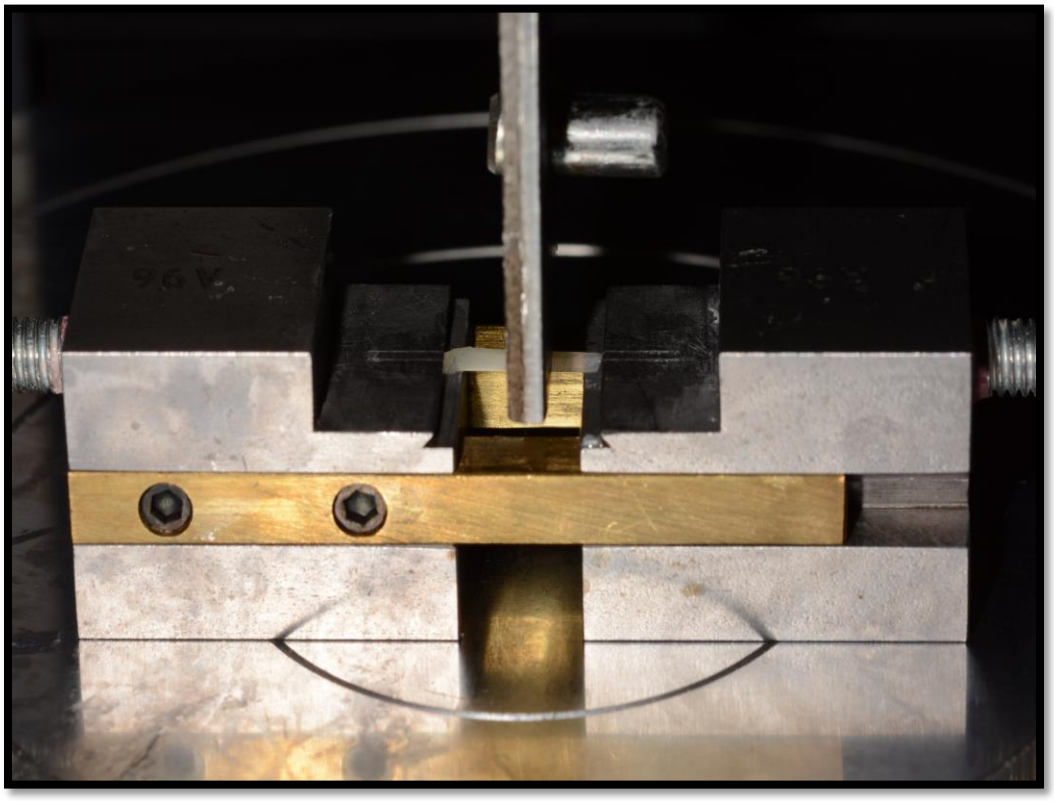


Figure 8. Dentin bar prior to three-point bend test on Inston 5943 Single Column Table Top System



REFERENCES

American Association of Endodontists. Endodontics Colleagues for Excellence: Root canal irrigants and disinfectants. 2011 Winter.

American Society for Testing and Materials. D790M-86 IN:Annual Book of ASTM Standards. 1989: 08.01,Plastics(1), C177-D1600.

Aydin B, Pamir T, Baltaci A, Orman M, Turk T. Effect of storage solutions on microhardness of crown enamel and dentin. *Euro J Dent*; 2015 Apr-Jun;9(2): 262-6.

Booth, A. Sterilization of Medical Devices. CRC Press. 1998: 8.

CDC. Guidelines for infection control in dental health-care settings. *Morbidity and Mortality Weekly Report*; 2003;52(RR-17): 1-76.

Chandki, R. A comparison of different methods for disinfection or sterilization of extracted human teeth to be used for dental education purposes. *World J of Dent* 2013; 4(1): 29-31.

Goodis HE, Marshall GW, White JM, Gee L, Hornberger B, Marshall SJ. Storage effects on dentin permeability and shear bond strengths. *Dent Mater*; 1993; Mar;9: 79-84.

Grigoratos, D. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. *Int'l Endo J* 2001; 34: 113-9.

Heymann HO, Swift EJ, Ritter AV. Sturdevant's Art and Science of Operative Dentistry. 6th ed. 2013.

Hope CK, Griffiths DA, Prior DM. Finding an alternative to formalin for sterilization of extracted teeth for teaching purposes. *Journal of Dental Education*; 2013;77(1): 68-71.

Jameson MW, Tidmarsh BG, Hood JAA. Effect of storage media on subsequent water loss and regain by human and bovine dentine and on mechanical properties of human dentine *in vitro*. Arch Oral Biol; 1994;39: 759-67.

Johnson WT, Noblett WC. Cleaning and Shaping in Endodontics: Principles and Practice. 4th ed. Saunders, Philadelphia, PA, 2009.

Kishen A, Asundi A. Experimental investigation on the role of water in the mechanical behavior of structural dentine. *J Biomed Mater Res A*; 2005;73: 192-200.

Kivanc BH, Fracture resistance of thin-walled roots restored with different post systems. *International Endodontic Journal;* 2009; 42: 997-1003.

Marcelino APM, Bruniera JF, Rached-Junior FA, Silva SRC, Messias DC. Impact of chemical agents for surface treatments on microhardness and flexural strength of root dentin. *Braz Oral Res*. 2014 Nov-Dec;28(6): 491-6.

Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine-mechanical, chemical and structural evaluation. *Int Endod J*. 2007; 40(10): 786-93.

Marshall Junior GW, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. *J Dent*; 1997; Nov;25(6): 441-58.

Mobarak, EH. (Mobarak, El-Badrawy, Pashley, & Jamjoom, 2010). *J Prosthet Dent* 2010; 104: 92-7.

Muhleman HR. Storage medium and enamel hardness. *Helv Odontol Acta*; 1964:8: 112-7.

Outhwaite WC, Livingston MJ, Pashley DH. Effects of changes in surface area, thickness, temperature and post-extraction time on human dentine permeability. *Arch Oral Biol*; 1976; 21: 599-603.

Pashley, DH. Dentin permeability: Effects of cavity varnishes and bases. *J Prosthet Dent* 1985; 53(4): 511-516.

Plotino G, Grande NM, Bedini R, Pameijer CH, Somma F. Flexural properties of endodontic posts and human root dentin. *Dent Mater* 2007; 23: 1129-35.

Reeder, OW. Dentin permeability: determinants of hydraulic conductance. *J Dent Res* 1978; 57(2): 187-93.

Perdigao J, Reis A, Loguercio AD, Dentin adhesion and MMPs: a comprehensive review. *J Esthet Restor Dent*; 2007; May-Jun;32(3): 261-5.

Shababang S. Treatment options: Apexogenesis and Apexification. *JOE*; 2013; 39(3S): S26-29.

Shafiei, F. Fracture resistance of Endodontically treated maxillary premolars restored by silorane based composite with or withour fiber or nano-ionomer . *J Adv Prosthodont*; 2014; 6(3): 200:6 .

Stappert CF. Fracture resistance of different partial coverage ceramic molar restorations. *J Am Dent Assoc;* 2006: 137(4): 514-22..

Strawn, SE. Spectroscopic changes in human dentine exposed to various storage solutions-short term. *J Dent* 1996; 24: 417-23.

Tate, WH. Disinfection of human teeth for educational purposes. *J Dent Ed* 1991; 55(9): 583-5.

Tesch W, Eidelman N, Roschger P, Goldenberg F, Klaushofer K, Fratzl P. Grade microstructure and mechanical properties of human crown dentin. *Calcif Tissue Int*; 2001;69: 147-57.

Toledano M, Osorio R, Albaladejo A, Aguilera FS, Osorio E. Differential effect of *in vitro* degradation on resin-dentin bonds produced by self-etch versus total-etch adhesives. *J Biomed Mater Res A*; 2006 Apr;77(1):128-35.

Urbani G, Cavalleri G, Gotte P. Disinfection and sterilization in dentistry. Use of potentiated glutaraldehyde (DIBA-GLAXO) in the water systems of dentistry units: analysis of microbiological activity, physic-chemical compatibility and residues in washing water. *Clin Trials J*; 1990;27(1): 20-9.

Wemes JC, Arends J. The hardness of bovine dentine after glutaraldehyde treatment. *Oral Surg Oral Med Oral Pathol*; 1984:58: 722-4.

World Health Organization. *WHO List of Essential Medicines*. 19th. 2015. Retrieved 28 January 2017.