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INITIATOR

1. USU Principal Author/Presenter: Capt Jennifer Connor

2. Academic Title: Resident, Advanced Education in General Dentistry Residency (AEGD-2)

3. School/Department/Center: Air Force Postgraduate Dental School (AFPDS)

4. Phone: 210-671-9822

5. Type of clearance: _x_Paper __Article __ Book ___Poster ___Presentation ___Other

6. Title: "Effects of Surface Treatments on the Mechanical Properties and Antimicrobial Activity of Desiccated Glass Ionomers"

7. Intended publication/meeting: *Operative Dentistry*

8. "Required by" date: 31 August 2014

9. Date of submission for USU approval: 28 July 2014

CHAIR OR DEPARTMENT HEAD APPROVAL

1. Name: Kraig S. Vandewalle, Director, Dental Research

2. School/Dept.: AFPDS / AEGD-2

3. Date: 28 July 2014

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Chair/Department Head Approval:___ [redacted] Date 28 July 2014

SERVICE DEAN APPROVAL

1. Name: Col Drew Fallis

2. School (if applicable): AFPDS

3. Date: 28 July 2014

4. __Higher approval clearance required (for University-, DoD- or US Gov't-level policy, communications systems or weapons issues review").

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PDC DEAN APPROVAL

1. Name:

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4. __Submitted to DoD (Health Affairs) on (date):

Or __Submitted to DoD (Public Affairs) on (date):

5. __DoD approved/cleared (as written) or __DoD approved/cleared (with changes)

6. DoD clearance/date:

7. DoD Disapproval/date:

_____

External Affairs Approval Date

Effects of Surface Treatments on the Mechanical Properties and Antimicrobial Activity of Desiccated Glass Ionomers

Jennifer Olivia Connor

APPROVED:

[redacted]

Col Kraig S. Vandewalle

[redacted]

Col Erik J. Meyers

[redacted]

Lt Col Wen Lien

30 May 2014

Date

APPROVED:

[redacted]

Col Drew W. Fallis
Dean, Air Force Postgraduate Dental School

Effect of Surface Treatments on the Mechanical Properties and Antimicrobial Activity of Desiccated Glass Ionomers

A THESIS

Presented to the Faculty of

The Air Force Postgraduate Dental School

Of the Uniformed Services University

Of the Health Sciences

In Partial Fulfillment

Of the Requirements

For the Degree of

MASTER OF SCIENCE

In Oral Biology

By

Jennifer Olivia Connor, BS, DDS

Dunn Dental Clinic
JBSA Lackland, TX
30 May 2014

Effects of Surface Treatments on the Mechanical Properties and Antimicrobial Activity of Desiccated Glass Ionomers

Jennifer Olivia Connor

APPROVED:

_____
Col Kraig S. Vandewalle

_____
Col Erik J. Meyers

_____
Lt Col Wen Lien

_____
Date

APPROVED:

_____
Col Drew W. Fallis
Dean, Air Force Postgraduate Dental School

DEDICATION

I dedicate this to my loving parents, Don and Janina. Both have been my support and backbone throughout this residency program. Dad: thank you for always being there for me with endless telephone calls home to tell you about my day, for helping me feel better during stressful or tough times, and for mentoring and guiding me throughout these years. Mom: thank you for your continued encouragement to do my best, to succeed at whatever task I've been given, and for reminding me "tomorrow is a new day." I love you both so very much and couldn't have survived this program without your love and patience. I am extremely lucky and blessed to have such wonderful parents, a daughter couldn't be more proud!

ACKNOWLEDGEMENTS

I would like to acknowledge my wonderful mentors who provided me with the advice, guidance, and assistance to complete this research project. First, Col Kraig Vandewalle is thanked and recognized for dedicating countless hours in helping me complete my experiments and providing guidance on writing my protocol and thesis. He taught me how to use the Instron and how to acquire measurements on the Knoop hardness tester as well as helping me interpret my data. His organization of deadlines and step-by-step instruction for each task allowed me to write my protocol, design two posters to present at the AADR, and complete this thesis! His passion for teaching dental materials and his wealth of knowledge with dental research is truly an inspiration!

Next, I would like to thank Lt Col Wen Lien for motivating me to select my topic the year before I started this residency and for helping me find research related to my topic. His suggestion to incorporate microbiology into this project is the reason that I pursued and completed two experiments for this thesis. I appreciate his creativity and involvement in my research and the time he dedicated in answering all my questions.

I would like to thank and acknowledge Mrs. Sally Banfield for harvesting and growing the *S. mutans*, and for calculating the CFU/mL for each of my samples. She was wonderful to work with and I appreciate all that she accomplished for this project!

Finally, I want to thank Col Erik Meyers for being on my research committee and providing advice and insight during the editing phases of my protocol and thesis. Col Meyers has done an incredible job as our program director and it has been a pleasure to work with him over the last two years. He sets the standard for what a comprehensive general dentist should strive for in terms of being a leader, an educator, and clinician!

ABSTRACT #1

Objectives: To evaluate the effect of various surface treatments on the mechanical properties of desiccated glass ionomer (GI) and resin-modified glass ionomer (RMGI) materials. **Methods**: Fifty GI (Fuji IX, GC) and fifty RMGI (Ketac Nano, 3M/ESPE) specimens were fabricated in molds and placed in five different media: casein phosphopeptide-amorphous calcium phosphate (CPP-ACP, Recaldent, GC), 0.12% chlorhexidine (CHX, Colgate PerioGard), 5% NaF (Duraflor, Medicom), cetylpyridinium chloride (CPC, Cepacol, Reckitt Benckiser), or 100% humidity (control). Beam specimens (2x2x25mm) were created for flexural strength/modulus testing (n=10). GIs were chemically cured for 6 minutes. RMGIs were light cured for 20 seconds in three over-lapping segments per side. The specimens were stored in 100% humidity in a 35ºC oven for 24 hours and then placed in air to desiccate for 24 hours. They were then stored in one of the five media for one week and tested in 3-point flexure (Instron). Flexural modulus was determined from the slope of the linear region of the load-deflection curve. Flexure fragments were used for Knoop hardness testing (Leco). A mean and standard deviation were determined per group. Data was analyzed with ANOVA/Tukey's test per group (alpha=0.025). **Results:** Significant differences were found between groups per property ($p<0.001$). For Fuji IX, treatment with NaF or CHX significantly increased the flexural strength and modulus compared to the humidity group (control). For Ketac Nano, flexural strength and modulus significantly increased with NaF, while treatment with CHX or CPC significantly reduced the flexural modulus compared to the control group. Surface hardness for both the chemical-cure GI and light-cure RMGI was not

affected by surface treatment. **Conclusions:** In general, the desiccated GI and RMGI materials retained their mechanical properties after surface treatment compared to the 100% humidity group. Sodium fluoride provided the greatest improvement in strength properties.

Table 1- Flexural strength, flexural modulus, and hardness of Fuji IX & Ketac Nano

Treatment	Fuji IX			Ketac Nano		
	Flexural Strength (MPa)	Flexural Modulus (GPa)	Hardness (Kg/mm^2)	Flexural Strength (MPa)	Flexural Modulus (GPa)	Hardness (Kg/mm^2)
Humidity (control)	14.6 (3.0) c	15.6 (3.0) b	74.9 (5.9) ab	53.9 (6.6) b	7.9 (0.7) b	28.2 (2.6) ab
CPP-ACP	13.5 (3.9) c	15.2 (3.1) b	79.6 (8.9) ab	91.3 (13.1) a	8.3 (0.4) ab	30.4 (1.4) a
NaF	38.3 (12.1) a	26.1 (3.3) a	70.4 (10.6) b	105.9 (14.7) a	8.9 (0.5) a	28.2 (2.8) ab
CHX	28.0 (5.0) b	24.5 (2.7) a	83.7 (4.1) a	50.2 (5.6) b	5.8 (0.5) c	27.3 (1.7) b
CPC	16.7 (5.9) c	17.4 (4.5) b	79.8 (6.4) ab	40.9 (12.3) b	6.1 (0.6) c	26.5 (2.0) b
Groups with the same lower case letter per column are not significantly different ($p > 0.025$)						

ABSTRACT #2

Objective: To evaluate the effect of various surface treatments on the antimicrobial activity of desiccated glass ionomer (GI) and resin-modified glass-ionomer (RMGI) materials. **Method**: Fifty GI (Fuji IX, GC) and fifty RMGI (Ketac Nano, 3M/ESPE) specimens were fabricated in (2x2x25mm) beam-shaped molds (n=10) and placed in five different media: casein phosphopeptide-amorphous calcium phosphate (CPP-ACP, Recaldent, GC), 0.12% chlorhexidine (CHX, Colgate PerioGard), 5% NaF (Duraflor, Medicom), cetylpyridinium chloride (CPC, Cepacol, Reckitt Benckiser), or 100% humidity (control). GIs were chemically cured and RMGIs were light cured. The specimens were stored in 100% humidity at 35ºC for 24 hours, placed in air to desiccate for 24 hours and then stored in one of the five media for one week. To determine antimicrobial activity, the specimens were covered with a bacterial suspension. Bacterial isolate *S. mutans* was cultured on Trypticase Soy Agar with 5% sheep blood and incubated. An inoculation suspension was prepared by harvesting growth of the organism and suspending it in sterile saline. A 1:100 dilution of the bacterial suspension was then prepared using Brain Heart Infusion broth. Each specimen was covered with the bacterial suspension and incubated for 24 hours at 35°C. After incubation, the bacterial suspension was removed and the specimens were washed. Sterile saline was added and vortex mixed. The saline solution was serially diluted 1:10 and plated on TSA plates. Plates were incubated for 3 days and CFUs/mL were calculated. Data was analyzed with Mann-Whitney U tests (alpha=0.017). **Results:** No significant difference was found between groups based on restorative material, but a significant difference was found based on

surface treatment ($p<0.012$). **Conclusion:** Surface treatment of the desiccated GI or RMGI with CHX or CPC resulted in no growth of the *S. mutans*. NaF resulted in significantly lower CFU/mL than CPP-ACP, which was significantly lower than the control group.

Table 2- CFU/mL of Fuji IX & Ketac Nano

Treatment	CFU/mL (st dev)	
	Fuji IX	Ketac Nano
Humidity (control)	2.4E+06 (3.7E+06)	6.6E+05 (4.4E+05)
CPP-ACP	7.6E+04 (7.6E+04)	1.3E+05 (2.0E+05)
NaF	6.0E+02 (6.0E+02)	1.6E+03 (8.0E+02)
CHX	No Growth	No Growth
CPC	No Growth	No Growth

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I. BACKGROUND AND LITERATURE REVIEW

A. A Brief History of Glass Ionomers

Although GIs have been around for 40 years (Nicholson, 1998), research on their unique properties is still being conducted. Since inception, incremental changes to the GI system have gradually improved its physical and clinical properties. Contemporary GIs are classified into three principal materials: conventional, resin modified, and metal-reinforced. The focus of this study involves conventional and resin modified GIs.

The conventional GI is constituted by the chemical reaction of an acid-degradable (ion-leachable) glass (powder) with an aqueous solution of a polymeric acid (liquid) (Powers, 2006; Crisp et al., 1974). The end product is the formation of polycarboxylate salts. The powder is composed of fluoroalumino-silicate glass with a formula of SiO_2—Al_2O_3—CaF_2—Na_3AlF_6—$AlPO_4$ (Powers, 2006), and the polymeric acid is approximately 40-50% concentrated anionic copolymers of acrylic, itaconic, or maleic acid (Kraft, 2002). Tartartic acid is also incorporated in the liquid as it accelerates the overall setting time and improves the handling characteristics (Crisp and Wilson, 1976). Calcium and aluminum ions liberated from the glass and polymeric acid form metallic salt bridges causing the material to set. The calcium ions that are released from the material are introduced to the tooth surface creating an adhesive bond (Powers, 2006). The acid-base setting reaction proceeds to equilibrium via three interrelated stages that include dissolution, gelation, and

precipitation.

Glass ionomers can be used as luting agents, liners, as well as temporary and permanent restorations. Particular advantages of conventional glass ionomers include biocompatibility with dental pulp, adherence to tooth structure, minimal shrinkage upon setting, low coefficient of thermal expansion, and antibacterial properties associated with continued fluoride release (Rothwell and Anstice, 1998; da Silva et al., 2007). Glass ionomers have the ability to bond chemically to enamel and dentin through an ionic interaction with calcium and phosphate ions of the tooth material (Powers, 2006). Glass ionomers can be used to bond orthodontic brackets to teeth and be used as a sealant in protecting susceptible pits and fissures (da Silva et al., 2007). Additionally, they are ideal for Atraumatic Restorative Treatment (ART), which involves incomplete caries removal followed by placement of a GI (Botelho, 2003).

Although glass ionomers have certain advantages as a restorative material, they are not without their disadvantages. These drawbacks include moisture sensitivity, low physical properties with regard to their initial mechanical strength, and low wear resistance. Teeth being restored need to be completely isolated as glass ionomers are sensitive to contact with water during setting. When the material is isolated from moisture during the procedure, the strength of the glass ionomer improves (Powers,

2006). Tensile strength values of glass ionomers are much lower than for resin composite and show brittle failure in diametral compression test (Powers, 2006). Due to their low physical properties, glass ionomers should not be used in stress-bearing areas. Their mechanical strength is insufficient which limits their use to Class III and Class V restorations (McLean, 1984; Gladys et al., 1997). Glass ionomers have inadequate wear resistance to forces from occlusion and abrade more rapidly than a resin composite (McLean, 1984). Posterior teeth requiring Class I or Class II restorations where occlusal forces and abrasion may be high, are better suited to receive amalgam or composite (Kraft, 2002).

B. A Brief History of Resin-Modified Glass Ionomers

Resin-modified glass ionomers (RMGIs) were developed approximately twenty years ago (Sidhu, 2010) to improve the mechanical properties and setting characteristics of conventional GIs. RMGIs have higher wear resistance, increased moisture resistance, higher fracture toughness, and longer working time compared to conventional GIs (Ellacuria et al., 1999; Kraft, 2002). RMGIs are used in clinical dentistry for bases, liners (Wilson 1990), core build-ups, luting agents (Nicholson, 2002) and Class V restorations (Mount, 2002) in addition to several uses in pediatric dentistry (Nicholson and Croll, 1997; Croll and Nicholson, 2002).

The ingredients of RMGIs include fluoroaluminosilicate glass and polyacrylic acid in addition to a resin monomer of 2-hydroxyethyl methacrylate (HEMA) and a photosensitive initiator system (McLean and Nicholson, 1994; McCabe, 1998). The initiator allows the RMGI to be light cured with a dental curing light that emits a wavelength of 470 nm (Nicholson and Czarnecka, 2008). Three reactions occur once the materials are mixed and exposed to a curing light. The initial setting of the material is due to resin polymerization followed by further hardening via an acid/base reaction (Mitra, 1994). The final reaction serves to chemically polymerize any unset monomer remaining (Sidhu and Watson, 1995).

Similar to conventional GIs, RMGIs can bond to tooth structure, have the ability to release fluoride to offer protection against caries, and have good esthetics (Mitra, 1991, Mount 2002). Due to the resin monomer HEMA, however, biocompatibility to pulp is much less compared to conventional GIs. Studies found that unpolymerized HEMA in the set material to be cytotoxic to the pulp since it can easily diffuse through dentin (Stanislawski et al, 1999, Guertsen et al, 2000, and do Nascimento et al., 2000). In the study done by do Nascimento et al., 2000, the resin- modified glass ionomer, Vitremer (3M ESPE), was used as a direct pulp cap material on human premolars and extracted at a later date. Upon evaluation, an inflammatory response with an observed necrotic zone was present. These teeth did not demonstrate pulp repair or dentin formation around the area of the pulp exposure. The preponderance of available evidence suggests that RMGI should not be used as a direct pulp cap

material. To date, there have been few reports of adverse effects or post-operative sensitivity when using RMGI in clinical dentistry as a restorative material (Nicholson and Czarnecka, 2008; Sidhu, 2010).

C. Modification of Conventional GIs and RMGIs

Researchers continue to strive to create clinically superior restorative materials through modification of existing materials with the quest of improving their physical properties. The ultimate goal may be to create an antibacterial restorative material by adding chlorhexidine (CHX), cetylpyridinium chloride (CPC), or fluoride (NaF), or create a material that remineralizes adjacent tooth structure by adding casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) or fluoride (NaF). Combining outside ingredients inherent to the formula of a GI or RMGI may affect powder liquid ratios and thus the ability to polymerize completely or may compromise their physical properties such as compressive or flexural strength. Furthermore, restorative materials need to withstand functional forces while being exposed to saliva and moisture at all times in the mouth. The sections below describe background information and research studies that have modified GIs and/or RMGIs by incorporating or exposing CPP-ACP, CHX, NaF, and CPC into the material and their effect on the physical properties.

D. Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP)

Amorphous calcium phosphate (ACP) has exceptional biological properties such as osteoconductivity, biodegradability, bioactivity, and absence of cytotoxicity, which result in its use in dentistry, orthopedics, and medicine (Zhao et al., 2011). ACP precipitates from highly supersaturated calcium phosphate solution and converts to crystalline phases including octacalcium phosphate or apatitic products (Zhao et al., 2011).

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been incorporated into glass ionomers as a bioactive additive since ACP is a precursor to hydroxyapatite (Zraikat et al., 2011; Zhao et al., 2011). Casein phosphopeptide (CPP) contains the sequence of phosphoseryl residues of -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- which maximizes the solubility of calcium phosphate through stabilization of ACP bound to the phosphopeptides (Reynolds, 1998; Cross et al., 2005). The CPP is added to ACP to serve the purpose of generating a high concentration gradient into the enamel subsurface lesion by the release of calcium and phosphate ions (Reynolds, 1997). In an acidic oral environment, CPP-ACP has the ability to increase release of calcium, phosphate, and fluoride ions, which inhibits demineralization and promotes remineralization of enamel (Zraikat et al., 2011). Remineralization is the process by which the crystal voids in demineralized enamel

receive a net mineral gain of calcium, phosphate, and fluoride ions (Cochrane et al., 2010).

A few studies have tested the addition of CPP-ACP to conventional GIs with variable results on working and setting time and mechanical properties depending on whether CPP-ACP was mixed into the material prior to polymerization or exposed to CPP-ACP after polymerization. Laboratory studies done by Mazzaoui et al., 2003, and Zraikat et al., 2011, incorporated CPP-ACP into the powder of the GI followed by mixing and polymerization of the material, and conducted testing. In contrast, rather than incorporating CPP-ACP into GI prior to polymerization, Abduo and Swain, 2011, exposed set GI specimens to a solution of CPP-ACP over a period of time, then conducted testing.

Mazzaoui et al., 2003, demonstrated in their study that incorporating a 1.56% w/w CCP-ACP (Recaldent, GC International, Tokyo, Japan) into a GI (Fuji IX GP, GC International, Tokyo, Japan) had a 23% increase in compressive strength, 33% increase in microtensile bond strength, and a 40-second increase in mean setting time compared to the control GI. The results of their study implies that incorporating CPP-ACP into a GI improves the physical properties of the material which can be translated to increased longevity, better health of the tooth, and reduced cost and time for the patient and doctor.

Zraikat et al., 2011, incorporated two concentrations of CPP-ACP (3% and 5% w/w) into the powder of a conventional GI (Fuji VII, GC Europe, Leuven, Belgium) and measured setting time, compressive strength, and diametral tensile strength. They found that setting time increased with increasing concentrations of CPP-ACP but remained within the range of 90-360 seconds for an auto-cured GI, which is within ISO specifications. However, the compressive and diametral tensile strength were significantly decreased. The decrease in mechanical properties is most likely due to the interference of CPP-ACP with the reaction of polyacrylic acid and glass powder as well as the alteration in the powder liquid ratio by adding the CPP-ACP into the Fuji VII powder.

Abduo and Swain, 2011, exposed the set GI (Fuji IX, GC International, Tokyo, Japan) to a solution of CPP-ACP (Dentacal, NSI, Hornsby, Australia) over a period of time. Cracks were introduced in GI specimens via deliberate desiccation and placed in different storage media to assess strength changes. Specimens were kept in air, water, and Dentacal for 21 days then subjected to biaxial flexure testing and were fractured. The results show that the specimens kept in Dentacal over a period of time demonstrated the highest mean biaxial flexure strength and lower probability of failure (high Weibull modulus), which is similar to the results of the study done by Mazzaoui et al., 2003.

E. Antibacterial agents: Chlorhexidine, Fluoride, and Cetylpyridinium chloride

Several studies have tested the addition of antibacterial agents to GIs and RMGIs to evaluate possible antibacterial effects as well as potential changes in their physical properties.

a.) Chlorhexidine

Chlorhexidine (CHX) gluconate is a topical antimicrobial mouthrinse available as a 0.12% solution in the United States and is considered the “gold standard” in its role as an antiplaque and antigingivitis agent (Lang and Brecx, 1986). CHX is effective against both gram-positive and gram-negative bacteria and acts by increasing cell membrane permeability and causing the cell membrane to rupture (Hennessy, 1977). It is known for its *substantivity*, or ability to adhere to soft and hard tissues resulting in long acting antimicrobial activity over 6 hours or more. CHX has been proven to be safe and effective; however, due to its side effects of staining, calculus formation, and taste alteration, it is better suited to be used on a short-term basis rather than long-term (American Academy of Periodontology, 1994). CHX is commonly used as a pre-procedural mouthrinse to reduce the amount of bacteria in the mouth by approximately 90% as well as to improve wound healing prior to tooth extractions and after scaling and root planing or periodontal surgery (Hermesch et al., 1998; Beiswanger et al., 1992; Ciancio, 1994).

Turkun et al., 2008, sought to examine the antibacterial affects and physical properties of adding chlorhexidine diacetate or chlorhexidine digluconate to the glass ionomer ChemFil Superior (Dentsply DeTrey, Konstanz, Germany) to potentially find a better material for the ART approach. As discussed earlier, minimal carious dentin is removed with hand instruments followed by restoration with a GI. Since hand instruments do not remove caries as well as a rotary burs, cariogenic bacteria can survive for up to 2 years under the GI restoration (Weerheijm et al.,1993, 1999). In the ART approach, residual infected dentin may remain and the GI placed might not aid in halting the caries process resulting in failure of the restoration (van Amerongen, 1996). Thus, combining an antibacterial agent with GI may assist in arresting caries and improving the life of the restoration. In the study completed by Turkun et al., 2008, chlorhexidine diacetate powder or chlorhexidine digluconate liquid was mixed with the powder of the GI to obtain 0.5%, 1.25%, and 2.5% concentrations of the respective groups. The results of their study demonstrated a long-term antimicrobial effect on *S. mutans* and *L. acidophilus*. For working and setting time, diametral tensile and biaxial flexure strength, the groups tested were similar to the ChemFil Superior control group. The 1.25% and 2.5% chlorhexidine diacetate groups demonstrated lower compressive strength values and the 0.5% and 2.5% chlorhexidine digluconate groups demonstrated lower hardness values compared to the control. They concluded that adding an antibacterial agent to a GI was beneficial in long-term antimicrobial effect against strains of *S. mutans* and *L.*

acidophilus with slight compromise of the physical properties of the material.

Additional research studies completed by Jedrychowski et al., 1983, Ribeiro and Ericson, 1991, and Takahashi Y. et al., 2006, attempted to create an antibacterial restorative material by adding CHX to GIs. These studies showed that combining CHX with GIs resulted in increased antibacterial effect *in vitro*. Unfortunately, incorporating antibacterial agents in restorative materials resulted in a decrease in the physical properties (Jedrychowski et al., 1983; Imazato, 2003; Botelho, 2004; Palmer et al., 2004). In a study, the addition of 10% and 18% chlorhexidine digluconate to the liquid of the two conventional GIs used for orthodontic band cementation did not affect mechanical properties of the materials and inhibited *S. mutans* growth (Farret et al., 2011).

A study completed by Sanders et al., 2002, examined the outcome on mechanical properties and antimicrobial activity of a 5% chlorhexidine diacetate combined with a RMGI (Photac-fil, ESPE, Norristown, PA). At 24 hours and 6-week intervals, the samples were tested for hardness, tensile strength, and erosion, and weekly for 6 weeks, anti-microbial activity was tested. The results indicate that CHX added to a RMGI slightly decreased hardness and erosion resistance yet resulted in a higher reduction in *S. mutans.* A recent study evaluated biological and mechanical behavior of a RMGI (GC Fuji Lining LC, GC Corporation, Tokyo, Japan) modified by adding 0.2%, 0.5%, 1.25%, and 2.5% chlorhexidine digluconate to the liquid while maintaining original powder liquid ratio. Their results show that incorporation of

1.25% and 2.5% chlorhexidine digluconate significantly inhibited growth of *S. mutans*. Incorporation with 2.5% chlorhexidine digluconate resulted in lower compressive strength compared to the control; however, no significant differences in mechanical properties were found for the other groups (de Castilho et al., 2013).

b.) Fluoride

Fluoride plays a significant role in dentistry and is influential in the treatment of incipient dental caries as well as prevention for future dental caries. Fluoride can be found as an active ingredient in several products such as mouthrinses, toothpastes, varnishes, gels, and glass ionomer restorative materials. Glass ionomers have the ability to release fluoride, which is beneficial for incipient enamel lesions and microleakage following placement of the restoration. Fluoride functions via three mechanisms including stimulation of remineralization, inhibition of demineralization, and antibacterial through inhibition of acid production by preventing microbial growth and metabolism (Summitt et al., 2006; Hamilton and Bowden, 1996). In concentrations greater than 200 ppm, fluoride demonstrates bactericidal activity which is greater than that released from fluoride-releasing restorative materials (Summitt et al., 2006). In a study by Yost and VanDemark 1977, NaF caused inhibition of growth rate and growth levels of *S. mutans,* especially in an acidic environment.

In laboratory studies, research has found that restorative materials capable of high fluoride release such as GIs and RMGIs translate to materials with weaker mechanical properties in comparison to compomers and composites (Cattani-Lorente et al., 1999; Xu and Burgess, 2003). Due to their ability to release fluoride, GIs and RMGIs have higher recharge capability resulting in decreased demineralization in adjacent enamel and prevention of caries (Jensen et al., 1990; Hicks et al., 2002). One study investigated the effects of mechanical properties of GIs exposed to fluoride upon recharge of the material. Results from this study demonstrated decreased compressive strengths for glass ionomers (Fuji VII, GC, Belgium and Ketac Molar, 3M ESPE, St. Paul, MN) exposed to 0.05% NaF after 28 days of incubation and 1.23% Acidulated Phosphate Fluoride (APF) gel after 42 days of incubation (Cildir and Sandalli, 2007). A review of the literature reveals the lack of additional studies evaluating mechanical properties of GIs or RMGIs exposed to fluoride solution.

c.) Cetylpyridinium chloride

Cetylpyridinium chloride (CPC) is a quarternary ammonium compound that is effective at preventing bacterial plaque accumulation and inhibiting the development of gingivitis (Ali et al., 2002). CPC is regulated by the Food and Drug Administration (FDA) and is commonly sold as a mouthrinse and throat lozenges (Al-Musallam et al., 2006). CPC has ability to destroy both gram-positive and gram-negative bacterial organisms with its bactericidal mechanism of action. In addition, it is effective against

Candida albicans (Pitcher et al., 1980). Compared to CHX, CPC has fewer unwanted side effects; however, it is not as effective against plaque and gingivitis (Dimkov et al., 2009).

CPC has been incorporated into GIs in studies completed by Botelho, 2003, 2004, 2005, and Tuzuner and Ulusu, 2010, which showed antimicrobial properties by reducing quantities of microorganisms; however, compressive strength of the GI was reduced. Additionally, Tuzuner and Ulusu, 2010, found reduced hardness values for GI (Fuji IX, GC, Tokyo, Japan) incorporated with various antibacterial agents including Cetrimide (CT), CPC, Benzalkonium chloride (BC) and CHX.

F. Susceptibility of GIs and RMGIs to desiccation

It has been well established in the literature that conventional and resin-modified glass ionomers are vulnerable to dehydration stress (Wilson and Paddon, 1993; Sidhu et al., 1997). Just as conventional GIs may undergo hygroscopic dimensional change when exposed to a moist environment, they may also contract under desiccated conditions and lose adhesion to tooth structure (Wilson and Paddon, 1993). Furthermore, the addition of resin to GIs as seen in RMGIs has not improved the susceptibility of the material to dehydration. In a study by Sidhu et al., 1997, both RMGI and conventional GI restorative materials placed in Class V preparations of extracted human mandibular third molars were subjected to dessication stress after a period of time and both materials demonstrated failure at the dentin interface. During dehydration, the strength of the material near dentin may be weakened due

to fluid movement from the dentinal tubules (Watson, 1990). Clinically, the effect of dimensional changes from dessication of the material is manifested as debonding from tooth structure (Sidhu et al., 1997).

G. Biofilm

Dental biofilm is a bacterial community comprised of well-organized and cooperating microorganisms on the surfaces of teeth (Marsh and Bradshaw, 1995; Costerton et al., 1994) whose three dimensional structure allows the flow of nutrients, metabolites and oxygen (Overman, 2000). They are responsible for many dental diseases including dental caries, periodontitis, gingivitis, and peri-implantitis; however, they are present on healthy teeth as well (Sbordone et al., 2003). Biofilm consists of various bacteria, including *Actinobacillus actinomycetemcomitans, Streptococcus mutans, Fusobacterium nucleatum, Treponema denticola, Porphyromonas gingivalis*, and *Tannerella forsythius* (Diaz et al., 2006; Scheie and Petersen, 2004; Marsh, 2005). These species are built up sequentially in layers and communicate with each other by sending out chemical signals (Armitage, 2004) facilitating the settlement of other bacteria by providing many adhesion sites, called co-adhesion or co-aggregation. Once a biofilm colony has been formed, the matrix formed over the microorganisms serves as a protective barrier and it takes 1000 times the antibacterial agents to kill a microorganism in a biofilm as it does to kill the same microorganism in a planktonic form (Costerton, 1999). It serves as a large bacterial reserve for rapid repopulation on the teeth and gingival surfaces (Demke, 2012). To minimize the development of periodontal disease, it is essential to disrupt the

colonization, proliferation and sequential layering of biofilm (Haffajee et al., 2003).

H. Biofilm formation on the surface of GIs and RMGIs

Secondary caries remains as one of the leading causes of replacement of restorations due to the colonization of bacterial biofilm at the tooth-restoration interface (Taylor and Lynch, 1992; Mjör, 1985). Shortly after cleaning the tooth surface, a proteinaceous layer known as the pellicle forms, which allows early colonizing bacteria to bind to receptor sites within the pellicle. Ability of the pellicle to form and bacteria to colonize tooth surfaces may be affected by surface properties of the enamel and/or dental restorative material (Carlén et al., 2001). Surface irregularities in enamel such as cracks and pits appear to be areas where bacteria in dental plaque adhere first according to microscopic examination (Nyvad and Fejerskov, 1987). The surface topography of the tooth and the dental restoration appear to have an effect on the formation of plaque and dental biofilms; therefore, rough surfaces are more prone to dental biofilm formation (Quirynen, 1994; Quirynen and Bollen, 1995).

Due to their chemical, physical, and biological properties, GIs and RMGIs are the materials of choice for use as liners, luting agents, and for restoring cervical areas. Upon closer examination of the conventional GI, the surfaces of the material remain relatively rough even after polishing, which may amplify the aggregation of bacterial

biofilm (Forss et al., 1991). However, when comparing the number of microorganisms on the surface of GI restorations to that of composite resin restorations, *Streptococcus mutans* levels in the dental plaque of GI restorations are lower (Svanberg et al., 1990). This observation may be as a result of fluoride release and the ability of this ion to inhibit bacterial metabolism (Ten Cate, 1999). A study completed by Pedrini et al., 2001 examined the surface of conventional GI and RMGI and the effects of dental biofilm accumulation of *S. mutans*. The results of their study are in agreement with studies exhibited by Svanberg et al., 1990 and Berg et al., 1990, which demonstrated that conventional GIs and RMGIs resulted in lower levels of *S. mutans* than composite resin. The presence of less cariogenic bacteria on these materials may be attributed to the release of fluoride (Ten Cate, 1999), which has the ability to prevent adhesion and colonization of *S. mutans* by decreasing glucan polymer production (Zameck and Tinanoff, 1987), inhibiting metabolic pathways including glycolysis (Kaufmann and Bartholmes, 1992), and tampering with the enzymatic activity associated with the cytoplasmic membrane (Hamilton, 1990).

I. Brief overview of xerostomia and the consequences on oral health and restorative dentistry

Xerostomia, the subjective feeling of intraoral dryness, is considered to be a symptom rather than a diagnosis, and is most frequently associated with salivary gland hypofunction (Fox, 2008). According to limited epidemiological studies,

approximately ten percent of the general population suffers from perpetual xerostomia (Guggenheimer and Moore, 2003; Atkinson et al., 2005). In addition, an estimated 30 percent of the population 65 years and older endures this condition (Ship et al., 2002). There are several possible etiologies for a patient experiencing xerostomia which may be the result of medications, history of radiation therapy, or diagnosis of systemic disease such as primary biliary cirrhosis, chronic active hepatitis, HIV, AIDS, bone marrow transplant, graft versus host disease, renal dialysis, anxiety and depression, diabetes type I or II, and primary or secondary Sjögren's Syndrome (SS) (Seo et al., 2009; Fox, 2008).

The most common cause of xerostomia is medication-induced as a large population of elderly adults is treated by at least one medication that impairs salivary function (Ship et al., 2002). Primary SS is a chronic autoimmune connective tissue disorder affecting the salivary and lacrimal glands (keratoconjunctivitis sicca) and secondary SS is associated with other autoimmune disorders including rheumatoid arthritis and systemic lupus erythematosus. Additionally, SS is the most commonly encountered systemic condition associated with xerostomia and salivary hypofunction (Fox, 2007; Vitali et al., 2002). Nearly 100 percent of patients diagnosed with SS suffer from xerostomia (Fox, 2007) and patients who have undergone head and neck radiation therapy experience permanent xerostomia (Shiboski et al., 2007). Serous-producing salivary cells are destroyed by radiation via apoptosis resulting in atrophy and fibrosis of the salivary gland (Shiboski et al., 2007).

Lack of adequate salivary flow results in diminished buffering capacity to lubricate and protect the teeth and oral mucosa, to remove food particles and microorganisms, and to dilute acidic substances (Yip et al., 2006). A patient suffering from xerostomia and salivary dysfunction may experience catastrophic intraoral consequences to include oral discomfort, rampant and recurrent caries, increased risk of candida infections, and desiccation and decreased longevity of dental restorations (Gibson, 2007; Turner and Ship, 2007).

Management of a patient with xerostomia requires an appropriate diagnosis followed by a multidisciplinary team approach (Andrews and Griffiths, 2001; Turner and Ship, 2007; Fox, 2008). Frequent dental and radiographic examinations, patient education in oral hygiene instruction, use of topical fluoride and antimicrobrial mouth rinses and nutritional counseling of a low sugar diet are essential in the prevention of dental caries (Atkinson and Wu, 1994; Fox, 1994; Ship, 2002). Symptoms of hyposalivation may be managed by frequent intake of water, sucking or chewing on sugar-free lozenges and gum, moisturizing gels and lubricants, and saliva substitutes (Turner and Ship, 2007). A frequent sequela of xerostomia is oral candidiasis, which is usually treated with topical antifungal agents in the form of oral rinses, ointments and troches (Turner and Ship, 2007). In terms of restorative treatment for a xerostomic patient with rampant and recurrent caries, adhesive materials have been recommended such as GIs and RMGIs due to their fluoride releasing properties.

However, these materials are susceptible to acid attack and dessication within weeks of placement (Andrews and Griffiths, 2001). Severe desiccation may produce loss of adhesion or debonding from tooth structure, shrinkage, and microleakage resulting in failure of the restoration or recurrent caries (Wilson and Paddon, 1993; Watson, 1990; Sidhu et al., 1997).

II. OBJECTIVES

A. Objective Overview

Insufficient salivary flow may result in detrimental issues for a patient in terms of oral discomfort, caries, oral infection, and desiccation of dental restorations. Etiology of hyposalivation includes patients with history of radiation therapy for head and neck cancer, patients taking several medications, and individuals diagnosed with systemic disease such as Sjogren's syndrome (Tschoppe et al., 2010). Glass ionomers (GIs) and resin-modified glass ionomers (RMGIs) are excellent restorative options for a patient with xerostomia due to their fluoride releasing properties; however, they are sensitive to dimensional change when exposed to a dry environment. Severe desiccation may produce loss of adhesion or debonding from tooth structure, shrinkage, and microleakage resulting in failure of the restoration or recurrent caries (Wilson and Paddon, 1993; Watson, 1990; Sidhu et al., 1997). It has yet to be determined whether a particular type of surface treatment of desiccated GIs and RMGIs may improve physical properties of the material and offer antimicrobial protection to prevent recurrent caries in the xerostomic patient.

Secondary caries remains as one of the leading causes of replacement of restorations due to the colonization of bacterial biofilm at the tooth-restoration interface (Taylor and Lynch, 1992; Mjör, 1985). *Streptococcus mutans* is the major contributing microorganism involved in the pathogenesis of dental caries in humans (Loesche, 1986). Studies have shown that levels of *S. mutans* in dental plaque of conventional glass ionomers (GIs) and resin-modified glass ionomers (RMGIs) is lower compared to composite resin restorations (Svanberg et al., 1990; Borg et al., 1990). GI and RMGI incorporated with antimicrobial agents may assist in hindering the formation of plaque biofilm, inhibit demineralizaton and prevent secondary caries in tooth structure adjacent to the restoration. Current research has not yet examined the effects of biofilm formation of *S. mutans* on the surfaces of desiccated GIs and RMGIs incorporated with CPP-ACP, CHX, NaF, and CPC. The purpose of this study is to evaluate the effect of various surface treatments on the mechanical properties and antimicrobial activity of desiccated glass ionomer (Fuji IX) and resin-modified glass ionomer (Ketac Nano) materials.

The results of this study should give evidence to clinicians treating xerostomic patients who have existing GI or RMGI restorations or require placement of new GI or RMGI restorations whether a particular surface treatment may offer antimicrobial activity without hindering mechanical properties or perhaps improving mechanical properties of the restoration.

B. Specific Hypotheses

This study tested two specific null hypotheses as follows:

1) There is no significant difference in the mechanical properties of desiccated glass ionomer (Fuji IX) and resin-modified glass ionomer (Ketac Nano) materials treated with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), chlorhexidine (CHX), sodium fluoride (NaF) or cetylpyridinium chloride (CPC) compared to the untreated control.
2) There is no significant difference in the antimicrobial activity of desiccated glass ionomer (Fuji IX) and resin-modified glass ionomer (Ketac Nano) materials treated with CPP-ACP, CHX, NaF, or CPC compared to the untreated control.

III. MATERIALS AND METHODS

A. Experimental Design Overview

The materials which were used in this experiment are GI (Fuji IX, GC), RMGI (Ketac Nano, 3M/ESPE), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP, Recaldent, GC), 0.12% chlorhexidine (CHX, Colgate PerioGard), 5% NaF (Duraflor, Medicom), cetylpyridinium chloride (CPC, Cepacol, Reckitt Benckiser), or 100% humidity (control) (See Table 3 and 4).

For mechanical property testing, a total of 5 groups were created per restorative material (see Table 5). Ten specimens were prepared per group resulting in 100 total specimens. Fuji IX (groups #1-5) and Ketac Nano (groups #6-10) were stored in 100% humidity for 24 hours followed by desiccation in air for 24 hours and then placed into the following storage media: 1) 100% humidity, 2) casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), 3) 0.12% chlorhexidine (CHX), 4) 5% NaF, or 5) cetylpyridinium chloride (CPC). After 1 week, flexural strength and flexural modulus were obtained for each specimen. Fragments from the flexure test were returned to each storage media and three measurements using Knoop hardness testing were determined the next day.

For antimicrobial activity testing, the number of groups and types of specimens were similar to mechanical testing. Fuji IX (groups #1-5) and Ketac Nano (groups #6-10) were stored in 100% humidity for 24 hours followed by desiccation in air for 24 hours and then placed into the same storage media as before. After 1 week, each specimen was covered with 2.0 mL of prepared bacterial suspension and incubated for 24 hours in an aerobic environment. The samples were washed with sterile saline followed by plating on TSA II plates, which were incubated in an aerobic environment. After 3 days, the numbers of CFU on the plates were counted and CFU/mL recovered was calculated.

B. Experimental Design

Flexural Strength / Modulus

To ensure uniformity of fabrication and to minimize interoperator differences, one provider created all samples. To prepare each specimen for flexural testing, a (2 mm x 2 mm x 25 mm) aluminum mold (Sabri, Downers Grove, IL) was lightly lubricated with a silicone spray (WD-40, WD-40 Company, San Diego, CA) and then placed on a Mylar strip. One hundred specimens were divided into 50 rectangular specimens of GI (Fuji IX) and 50 rectangular specimens of RMGI (Ketac Nano). The specimens were fabricated by inserting the restorative material into the mold (n=10). The top surface of the mold was covered with a second Mylar strip and glass slide to ensure that the end of the specimen was flat and parallel to the opposite surface of the mold. GIs were chemically cured for 6 minutes and RMGIs were light cured so that one side of the specimen was exposed to a light polymerization unit (Coltolux LED, Coltene/Whaledent Inc., Cuyahoga Falls, OH) for 20 seconds each in three separate overlapping increments. Next, the mold was turned, and the opposite side of the specimen was exposed to the light in a similar manner. The adequacy of the light unit's intensity was assessed immediately prior to specimen preparation using a radiometer (LED Radiometer, SDS/Kerr, Orange, CA). All specimens were stored in 100% humidity in a 37º C oven (Model 20 GC, Quincy Lab, Chicago, IL) for 24 hours as the majority of the acid-base reaction takes approximately 1 day (Wong 1985, Stamboulis 2006). At 24 hours, all specimens were removed from 100% humidity

and left in air for 24 hours for desiccation to occur and allow the formation of craze lines (Abduo and Swain, 2011). Once desiccation was complete, each of the five different storage media received 10 specimens of GI and 10 specimens of RMGI. The first group was returned to 100% humidity, the second group was placed in a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)-containing solution, the third group was placed in 5% NaF, the fourth group was placed in a 0.12% solution of Chlorhexidine, and the fifth group was placed in a 0.05% cetylpyridinium chloride solution (Cepacol, Reckitt Benckiser Inc., Parsippany, NJ). The specimens were stored in a laboratory oven at 37º C.

Table 3- Restorative materials

Material	Type	Manufacturer	Liquid/Resin	Filler
Fuji IX	Conventional Glass Ionomer	Fuji IX, GC America, Alsip, IL	Polyacrylic acid No resin	Fluoroalumino-silicate glass
Ketac Nano Quick-Mix Capsule	Resin Modified Glass Ionomer (Nano-Ionomer)	3M/ESPE St. Paul, MN	Polyacrylic acid PEGDMA, BISGMA HEMA TEGDMA	Fluoroalumino-silicate glass Nanofiller (Silica and Zirconia) Nanoclusters (Silica and Zirconia)

Table 4- Surface treatment materials

Material	Manufacturer
100% humidity in 37º C oven	Model 20 GC, Quincy Lab, Chicago, IL
CPP-ACP (Casein phosphopeptide-amorphous calcium phosphate)	Recaldent, GC America, Alsip, IL
5% NaF (Sodium fluoride)	Duraflor, Medicom, Augusta, GA
0.12% CHX (Chlorhexidine)	Colgate PerioGard, New York, NY
0.05% CPC Cetylpyridinium chloride solution	Cepacol, Reckitt Benckiser Inc., Parsippany, NJ

Table 5- Study Groupings

Restorative Material	Surface Treatment	Group #
Fuji IX	100% humidity	1
	CPP-ACP	2
	5% NaF	3
	0.12% CHX	4
	0.05% CPC	5
Ketac Nano	100% humidity	6
	CPP-ACP	7
	5% NaF	8
	0.12% CHX	9
	0.05% CPC	10

After 1 week, each specimen was tested using a Universal Testing Machine (Model 5543, Instron, Canton, MA, USA) at a crosshead speed of 0.25 mm/min. Each specimen was placed on a three-point bending test device, which is constructed with a 20 mm span length between the supporting rods, and the central load is applied with a head diameter of 2 mm. The flexural strength was obtained using the expression:

$$\sigma_{FS} = \frac{3Fl}{2bd^2}$$

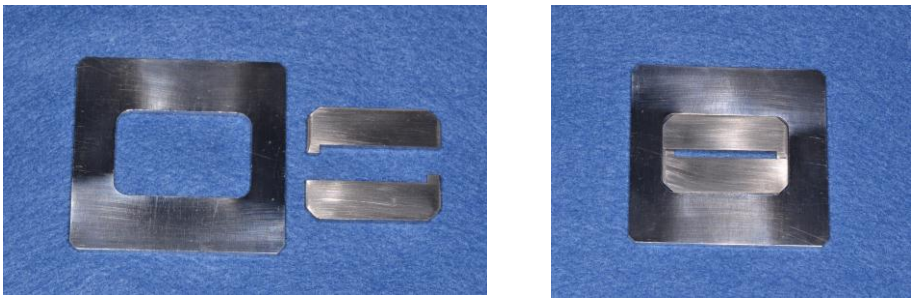
where *F* is the loading force at the fracture point, *l* is the length of the support span (20 mm), *b* is the width, and *d* is the depth. Width and depth measurements were made using an electronic digital caliper (GA182, Grobet Vigor, Carlstadt, NJ). The mean flexural strength and standard deviation were calculated for specimens from each of the two restorative materials that were stored in five different media over a 1-week span. Flexural modulus was determined from the slope of the linear region of the load-deflection curve using the analytical software (Instron).

Microhardness

Fragments (50 GI specimens and 50 RMGI specimens) from the flexural strength test were used for the Knoop hardness test (LM 300AT, Leco, St. Joseph, MI). Prior

Figure 1- Preparation of Fuji IX for Groups #1-5

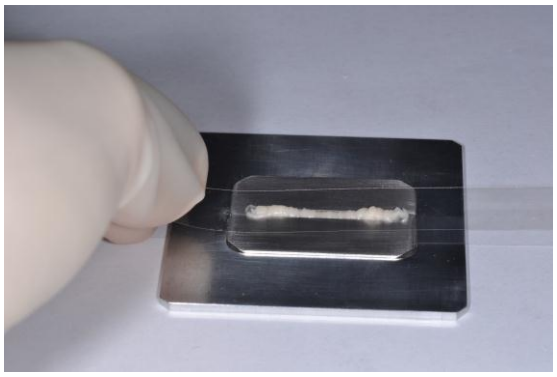
A- 2 x 2 x 25 mm aluminum mold



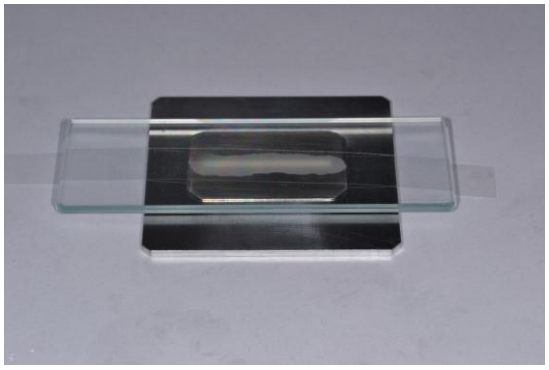
B- Trituration of Fuji IX for 10 sec per manufacturer's instructions

C- Mylar strip underneath mold, dispensing Fuji IX into the mold

D- Placing mylar strip over unpolymerized Fuji IX



E- Flattening the surface with a glass slide



F- View of the final prepared surface

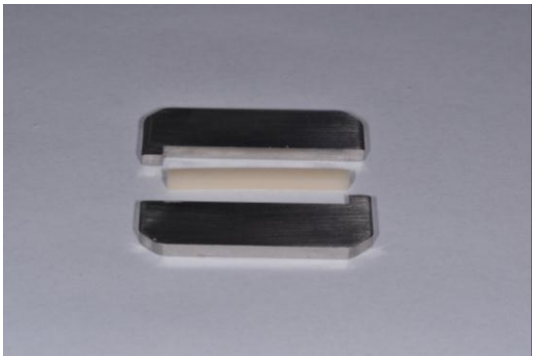
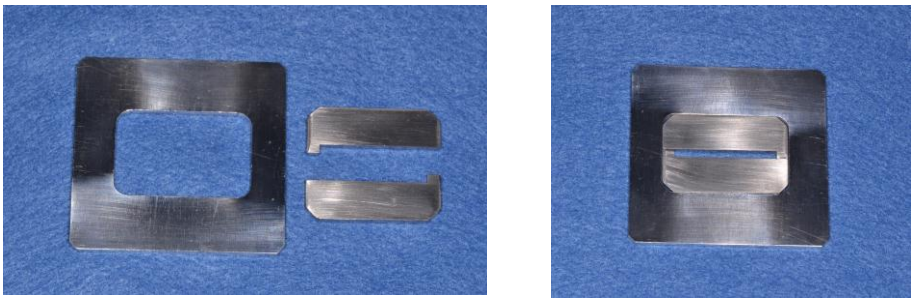


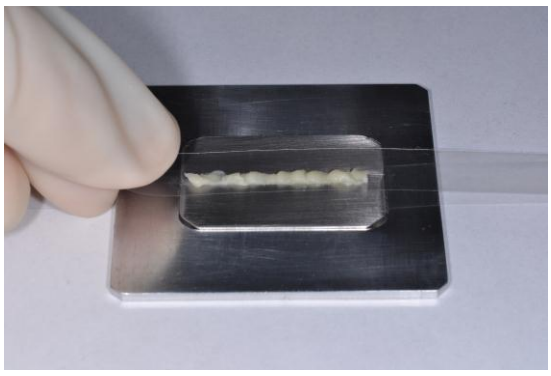
Figure 2- Preparation of Ketac Nano for Groups #6-10

A- 2 x 2 x 25 mm aluminum mold

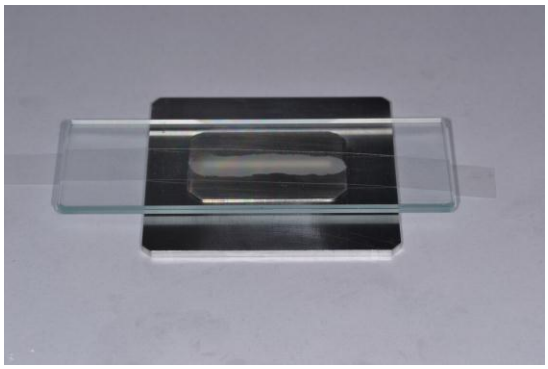


B- Mylar strip underneath mold, dispensing Ketac Nano into mold

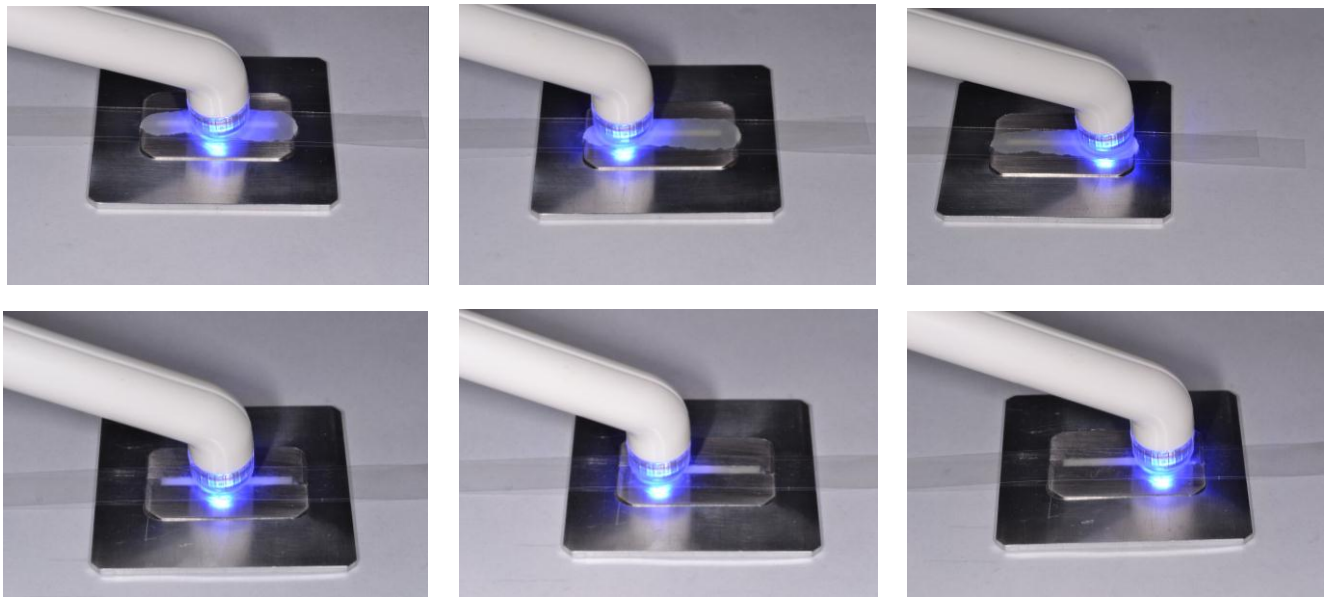
C- Placing mylar strip over unpolymerized Ketac Nano



D- Flattening the surface with a glass slide



E- Light curing 3 locations on top and bottom side of mold, 20 seconds each



F- View of the final prepared surface

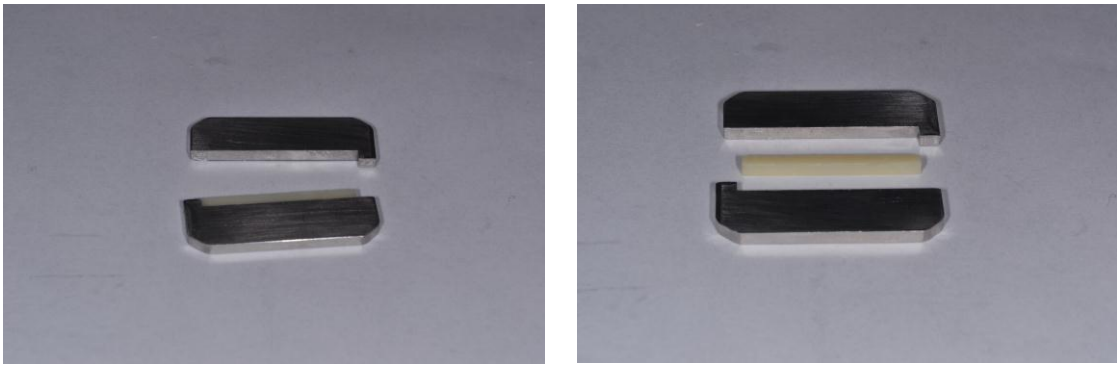
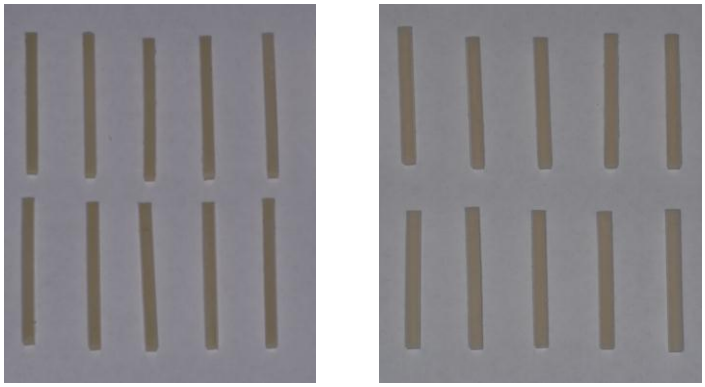


Figure 3- Placement of Fuji IX and Ketac Nano specimens into 5 different storage media

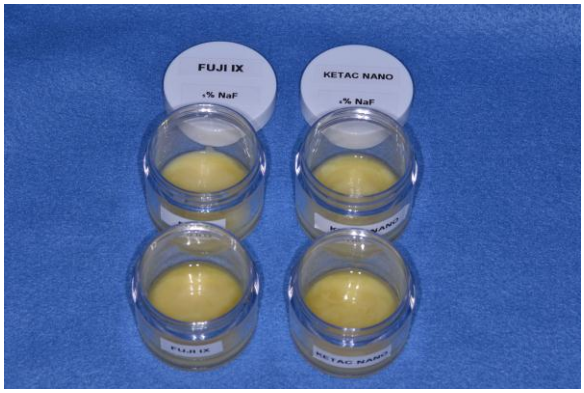
A- View of the prepared Fuji IX and Ketac Nano specimens (10 per group)



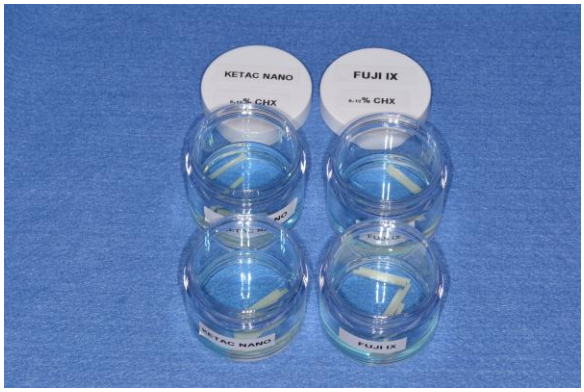
B- Specimens in containers for humidity group

C- Specimens in containers for CPP-ACP group

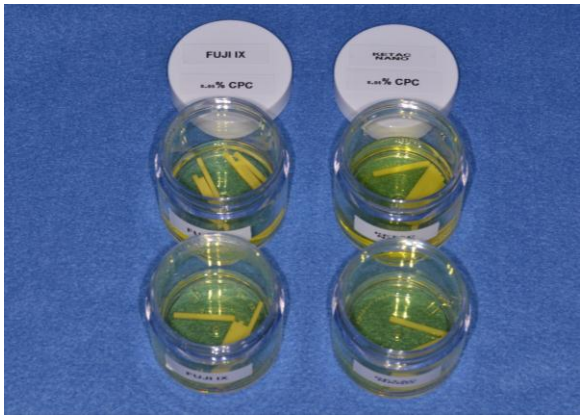
D- Specimens in containers for 5% NaF group



E- Specimens in containers for 0.12% CHX group



F- Specimens in containers for 0.05% CPC group

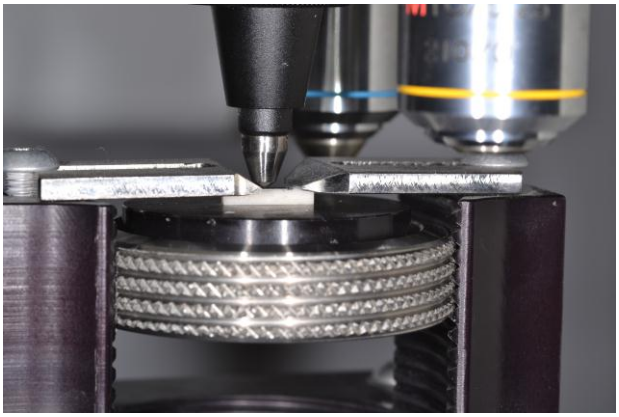


G- Specimens stored in 100% humidity in a 37º C oven

Figure 4- Flexural strength and Knoop hardness test

A- Flexural strength testing on Instron

B- Knoop hardness testing



to testing, fragments from the flexural strength test were returned to the designated storage material it was previously stored and microhardness testing was performed the next day. Knoop hardness testing was performed with a 200 gram load and a 10 second dwell time. Three hardness measurements were made for each specimen and averaged. The mean Knoop Hardness Number and standard deviation were calculated from each of the two restorative materials that were stored in five different media over a 1-week span.

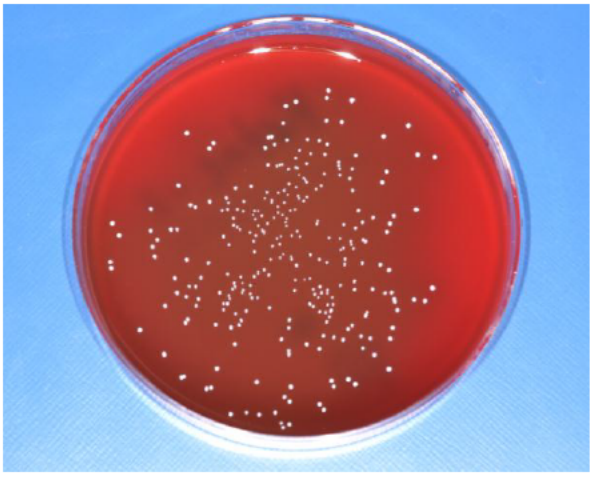
Inhibition of *S. mutans* by GI and RMGI

Specimens for bacterial count were prepared in the same manner as for flexural strength testing. A total of 50 rectangular specimens of GI (Fuji IX) and 50 rectangular specimens of RMGI (Ketac Nano) were fabricated. All specimens were stored in 100% humidity in a 37º C oven for 24 hours followed by desiccation in air for 24 hours and placed in the same five different storage media for one week as discussed above (n=10).

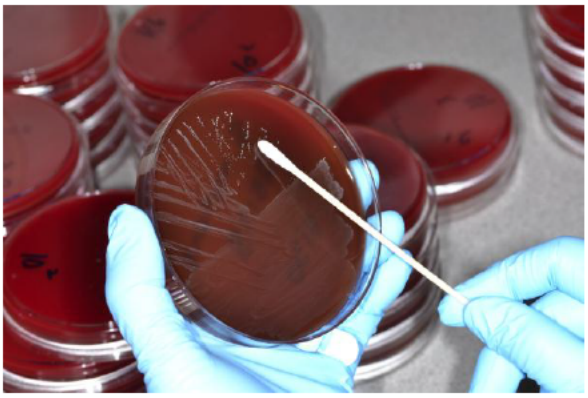
Bacterial isolate *S. mutans* (ATCC 25175) was cultured on Trypticase Soy Agar with 5% sheep blood (TSAII, BBL™221239/221262) and incubated at 35 ± 2°C, 5% CO_2 in an aerobic environment. An inoculation suspension of *S. mutans* was prepared by harvesting growth of the organism from TSA II and suspending it in sterile saline to a

Figure 5- Preparation for bacterial count for Groups #1-10 by microbiologist

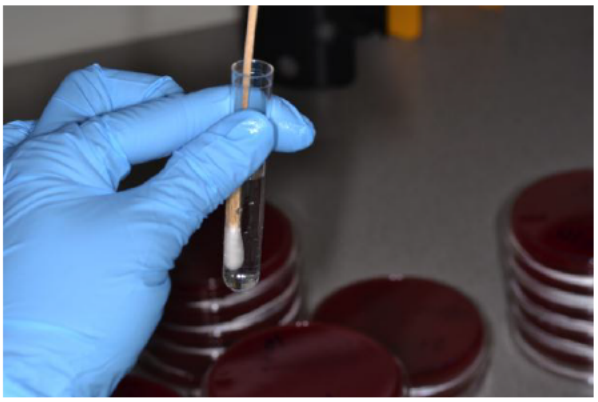
A- Culture of *Streptococcus mutans*



B- Harvest growth of *S. mutans*



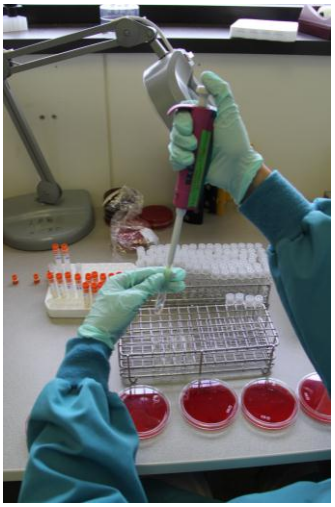
C- Suspended in sterile saline



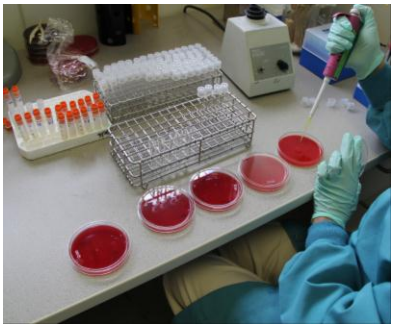
D- Spreading bacterial dilution on TSA II™ plate using L-rod spreader

E- Mixing the bacterial suspension before serial dilution using vortex mixer

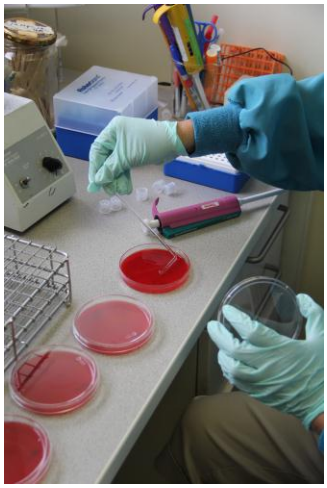
F- Performing 1:100 serial dilution



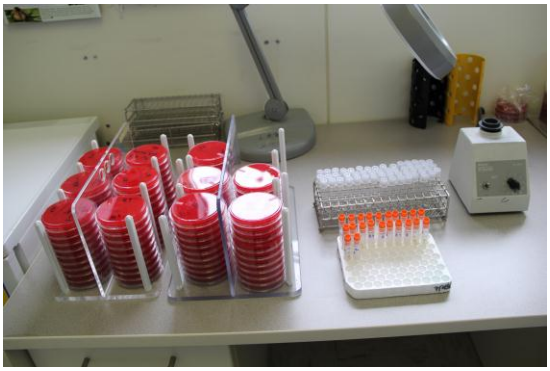
G- Dispensing 100 µl of serially diluted bacterial suspension to TSA II™ plate



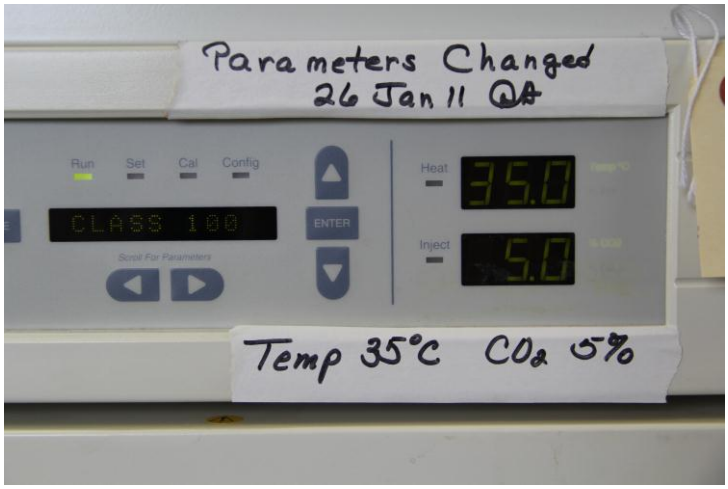
H- Spreading bacterial dilution on TSA II™ plate using L-rod spreader



I- Materials: TSA II™ plates, samples soaking in *Strep mutans* suspension, sterile saline, and vortex mixer

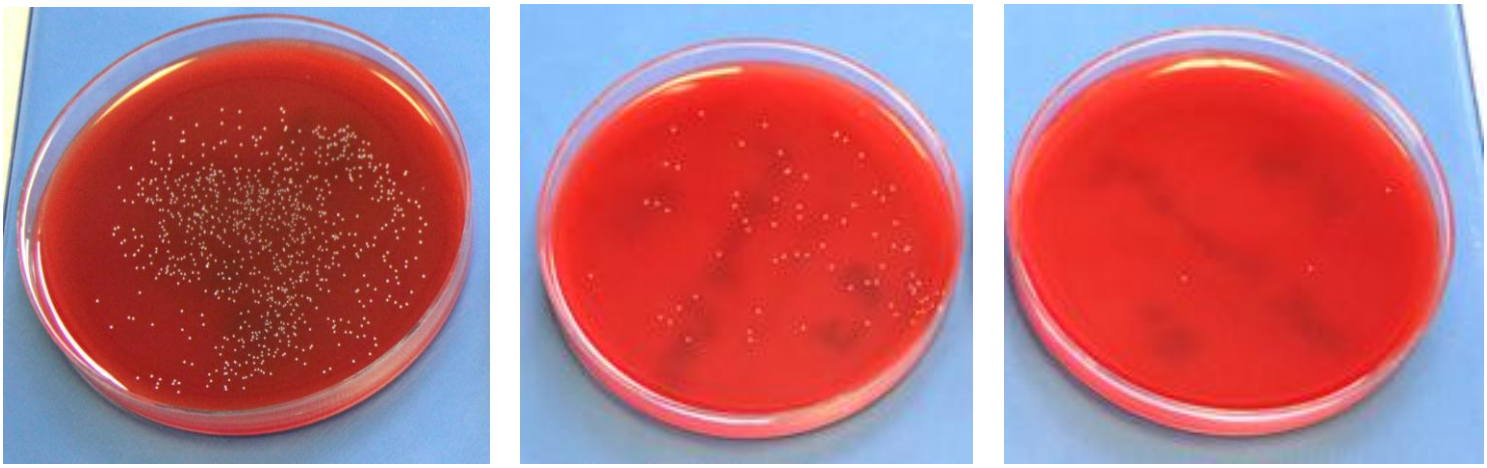


J- Thermo Forma Incubator & Parameters: Temp: 35 ± 2°C, 5% CO2

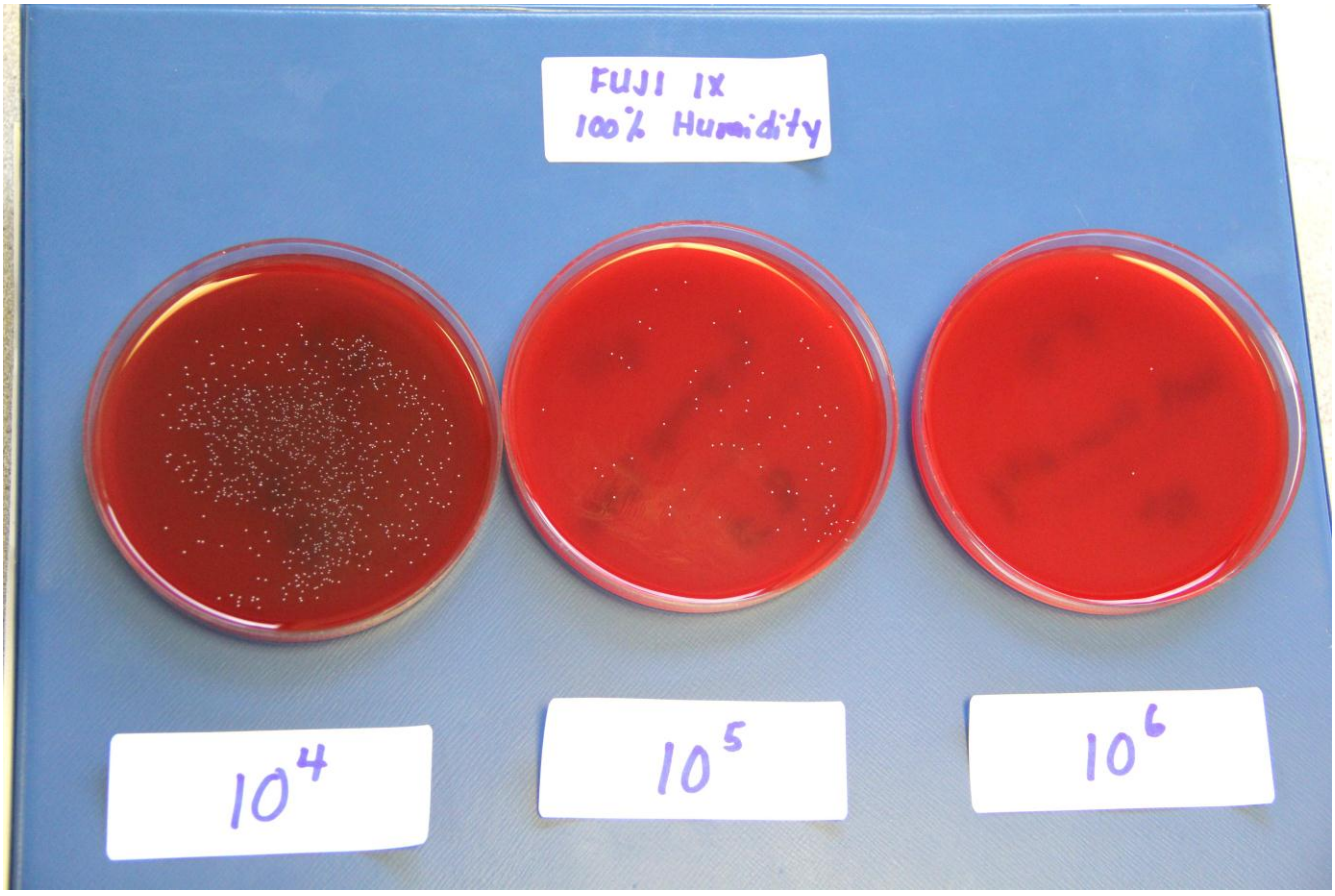


K- TSA II™ plates in the incubator

L- Growth of *Strep mutans* on TSA II™ plates, dilution factors 10^4 - 10^6



M- Example: Fuji IX, 100% Humidity-growth on TSA II™ plates, dilution factors 10^4 - 10^6 (not shown 10^2 – 10^3 plates, CFU too numerous to count)



turbidity equal to a 0.5 McFarland turbidity standard (approx. 1.5 X 10^8 CFU/mL). A 1:100 dilution of the bacterial suspension was then prepared using Brain Heart Infusion broth (Bacto 237400). Each sample was transferred from its designated storage media with sterile forceps and placed in a vial with 2.0 mL of the bacterial suspension. Samples were incubated for 24 hours at 35 ± 2°C, 5% CO2 in an aerobic environment. After incubation, the bacterial suspension was removed and the samples were washed with 2 mL sterile water x 3. After the final wash, 2 mL of sterile saline was added to each sample and the samples were vigorously vortex mixed for 2 minutes. The saline solution was serially diluted 1:10 and plated on TSA II plates (10^2 - 10^6). Plates were incubated at 35 ± 2°C, 5% CO2 in an aerobic environment for 3 days. The numbers of CFUs on the plates were counted. Lastly, CFU/mL recovered was calculated.

C. Statistical Management of Data

Mechanical Testing

A mean and standard deviation was determined per group. A two-way Analysis of Variance (ANOVA) was used to evaluate the effects of material type (2-levels) or storage media (5-levels) on mechanical properties of the desiccated glass ionomers (alpha = 0.05). Significant differences were found between groups based on material type ($p < 0.001$) or storage media ($p < 0.001$); however, there were significant interactions ($p < 0.001$). The data were further analyzed with one-way ANOVAs with Tukey's post hoc test per glass ionomer material. A Bonferroni correction was

applied because multiple comparisons were done between groups (alpha = 0.025). See Appendix B.

The sample size of 10 per group provided 80% power to detect the following small effect sizes: 0.258 (or approximately 0.51 standard deviation difference) between means for the main effect of material type, and 0.334 (or approximately 0.67 standard deviation difference) among means for the main effect of storage media and for the interaction term, when testing with a two-factor ANOVA at the alpha level of 0.05 (NCSS PASS 2002).

Antibacterial Testing

A mean and standard deviation was determined per group. Due to the non-normal distribution and large variability, the data was analyzed with a Kruskall Wallis and Mann-Whitney U non-parametric statistical tests per glass ionomer material. Significant differences were found between groups ($p < 0.012$). A Bonferroni correction was applied because multiple comparisons were done between groups (alpha = 0.017).

IV. RESULTS

The statistical analysis was reviewed and approved by the clinical research administrator, Clinical Research Division, JBSA Lackland, TX.

Mechanical Testing

Significant differences were found between groups per property ($p<0.001$). For Fuji IX, treatment with NaF or CHX significantly increased the flexural strength and modulus compared to the humidity group (control). For Ketac Nano, flexural strength and modulus significantly increased with NaF, while treatment with CHX or CPC significantly reduced the flexural modulus compared to the control group. Surface hardness for both the chemical-cure GI and light-cure RMGI was not affected by surface treatment.

Antibacterial Testing

No significant difference was found between groups based on restorative material, but a significant difference was found based on surface treatment ($p<0.012$). Surface treatment of the desiccated GI or RMGI with CHX or CPC resulted in no growth of the *S. mutans*. NaF resulted in significantly lower CFU/mL than CPP-ACP, which was significantly lower than the control group.

V. DISCUSSION

A. Significant differences in mechanical properties of desiccated GI and RMGI exposed to surface treatments

The first null hypothesis was rejected in this study. It stated that there would be no significant difference in the mechanical properties of desiccated glass ionomer (Fuji

IX) and resin-modified glass ionomer (Ketac Nano) materials treated with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), chlorhexidine (CHX), sodium fluoride (NaF) or cetylpyridinium chloride (CPC). This study, however, showed a significant difference between treatment groups per mechanical property. There was no significant difference noted for surface hardness of dessicated GI or RMGI after any surface treatment.

As discussed earlier during the literature review section, depending on whether CPP-ACP was mixed into the conventional GI prior to polymerization or exposed to CPP-ACP after polymerization, mechanical properties of the material were affected differently. Studies by Mazzaoui et al., 2003 and Zraikat et al., 2011 mixed CPP-ACP into the unset conventional GI and evaluated mechanical properties. While Mazzaoui et al., 2003 found an increase in compressive strength and microtensile bond strength of a conventional GI, Zraikat et al., 2011 determined that compressive and diametral tensile strength were decreased. Abduo and Swain, 2011 exposed set GI specimens to a solution of CPP-ACP over a period of time after desiccation for 24 hours, then conducted mechanical testing. In their study, specimens kept in Dentacal over a period of time demonstrated the highest mean biaxial flexure strength and lower probability of failure (high Weibull modulus), which is similar to the results of the study done by Mazzaoui et al., 2003. While Abduo and Swain, 2011 discovered an increase in mechanical properties of the desiccated conventional GI exposed to CPP-ACP, this present study found that treatment of

dessicated Fuji IX with CPP-ACP had no significant difference in flexural strength and flexural modulus compared to the humidity group (control). The difference in results of the mechanical properties may be attributed to the utilization of the three point bending test in this study versus the use of the mean biaxial flexure strength in their study. In addition, Abduo and Swain, 2011 exposed desiccated GIs to CPP-ACP solution over 21 days, whereas in this study, desiccated GIs were exposed to CPP-ACP for one week. It is possible that storage times may have an effect on mechanical properties. No formal published articles could be found that evaluated the addition of CPP-ACP to a desiccated RMGI. In this study, the treatment of desiccated Ketac Nano with CPP-ACP had a significant increase in flexural strength and modulus compared to the control group.

Studies done by Tuzuner and Ulusu, 2010, Turkun et al., 2008, Jedrychowski et al., 1983, Ribeiro and Ericson, 1991 and Takahashi Y. et al, 2006 showed that combining CHX with GIs resulted in an increased antibacterial effect; however, it resulted in a decrease in the physical properties. These studies found an overall decrease in physical properties of conventional GIs modified by adding CHX while this present study found that for dessicated Fuji IX, surface treatment with CHX significantly increased the flexural strength and modulus compared to the control group. In each of the studies mentioned, CHX was added to the powder or liquid of a conventional GI, mixed, and allowed to polymerize prior to mechanical testing, while this present study exposed set GI to CHX solution. This may explain why they found

decreased physical properties while our study found increased physical properties. Two studies describe that the mechanical properties of GIs are affected by the mixing ratio of the powder and liquid (Botelho, 2003 and Billington et al., 1990). Perhaps the addition of CHX to the powder or liquid of a conventional GI may have a negative effect on the mechanical strength, whereas exposing the GI to CHX after polymerization may have a positive effect. CHX was incorporated into a RMGI in research completed by Sanders et al., 2002. The results demonstrated slightly decreased mechanical properties over time yet higher reduction in *S. mutans.* de Castilho et al., 2013 found that a RMGI incorporated with 2.5% chlorhexidine digluconate resulted in lower compressive strength compared to the control; however, no significant differences in mechanical properties were found for the other groups modified by adding 0.2%, 0.5%, 1.25% chlorhexidine digluconate to the liquid portion of the RMGI. Therefore, a lower concentration of CHX added to a RMGI does not affect mechanical properties. The two studies mentioned found an overall decrease in mechanical properties of RMGI modified with CHX, which is consistent with the results of this present study. No formal published articles could be found that evaluated the addition of CHX to a desiccated GI or RMGI. In this study, treatment of Ketac Nano with CHX significantly reduced flexural modulus compared to the control group.

In laboratory studies, research has found that a restorative materials capable of high fluoride release such as GIs and RMGIs translate to materials with weaker

mechanical properties in comparison to compomers and composites (Cattani-Lorente et al., 1999; Xu and Burgess, 2003). One study investigated the effects of mechanical properties of GIs exposed to fluoride upon recharge of the material. Results from a study completed by Cildir and Sandalli, 2007 demonstrated decreased compressive strengths for glass ionomers exposed to NaF, while in this present study, treatment with NaF significantly increased the flexural strength and modulus of dessicated Fuji IX compared to the control group. Their study evaluated compressive strength with Fuji VII and Ketac Molar whereas this present study inspected flexural strength with Fuji IX. Perhaps the different types of mechanical testing and materials used may explain the differences observed between their study and this study. No formal published articles could be found that evaluated the addition of NaF to a desiccated GI or RMGI. In this present study, treatment with NaF significantly increased flexural strength and modulus for dessicated Ketac Nano.

CPC has been incorporated into GIs in studies completed by Botelho, 2003, 2004, 2005 and Tuzuner and Ulusu, 2010, which showed increase in antimicrobial properties yet decreased compressive strength of GIs. Tuzuner and Ulusu, 2010 found reduced hardness values when CPC was added to a conventional GI. While the studies mentioned found decreased mechanical properties of conventional GIs incorporated with CPC, this present study demonstrated no significant difference in flexural strength or modulus compared to the control group when desiccated Fuji IX

was treated with CPC. In each of the studies mentioned, CPC was added to the powder or liquid of a conventional GI, mixed, and allowed to polymerize prior to mechanical testing, while this present study exposed set GI to CHX solution. Perhaps the addition of CPC to the powder or liquid of a conventional GI may have a negative effect on the physical properties due to alteration in powder liquid ratios, whereas exposing the GI to CPC after polymerization did not have an influence on the strength properties. No formal published articles could be found that evaluated the addition of CPC to a desiccated GI or RMGI. In this present study, a significant reduction was found in flexural modulus of desiccated Ketac Nano treated with CPC compared to the control group.

B. **Significant differences in antimicrobial properties of desiccated GI and RMGI exposed to surface treatments**

The second null hypothesis was also rejected. It stated that there would be no significant difference in the antimicrobial activity of desiccated glass ionomer (Fuji IX) and resin-modified glass ionomer (Ketac Nano) materials treated with CPP-ACP, CHX, NaF, or CPC against the dental biofilm formation of *Streptococcus mutans.* This study showed a significant difference was found based on surface treatment.

In an acidic environment, CPP-ACP offers anticariogenic activity via the ability to increase release of calcium, phosphate, and fluoride ions, which inhibits demineralization and promotes remineralization of enamel (Zraikat et al., 2011). No

formal published articles could be found that evaluated the antimicrobial properties of surface treatment of desiccated GI and RMGI with CPP-ACP. In this present study, surface treatment of dessicated GI or RMGI resulted in a slight decrease of *S. mutans* growth compared to the humidity group (control).

Fluoride offers anticariogenic properties through three mechanisms including the stimulation of remineralization, inhibition of demineralization, and inhibition of acid production by preventing microbial growth and metabolism (Summitt et al., 2006; Hamilton and Bowden, 1996). In a study by Yost and VanDemark 1977, NaF caused inhibition of growth rate and growth levels of *S. mutans*, which is consistent with the results of this study in terms of antimicrobial effects from surface treatment with NaF. No formal published articles could be found that evaluated the antimicrobial properties of surface treatment of desiccated GI or RMGI with NaF. Surface treatment of NaF of dessicated GI or RMGI resulted in significantly lower CFU/mL than CPP-ACP, which was significantly lower than the control group.

CHX is effective against both gram-positive and gram-negative bacteria and acts by increasing cell membrane permeability and causes the cell membrane to rupture (Hennessy, 1977). When chlorhexidine diacetate powder or chlorhexidine digluconate liquid was mixed with the powder of the GI, Turkun et al., 2008 found a long-term antimicrobial effect on *S. mutans* and *L. acidophilus.* Studies by Jedrychowski et al., 1983, Ribeiro and Ericson, 1991, and Takahashi Y. et al., 2006

combined CHX with GI and the results showed increased antibacterial effect *in vitro*. In a study by Farret et al., 2011, *S. mutans* growth was inhibited after 10% and 18% chlorhexidine gluconate was added to two conventional GIs. Sanders et al., 2002 added CHX to a RMGI and discovered a higher reduction in *S. mutans* compared to controls. No formal published articles could be found that evaluated the antimicrobial properties of surface treatment of desiccated GI or RMGI with NaF. The results of this present study demonstrate that surface treatment of the desiccated GI or RMGI with CHX resulted in no growth of *S. mutans*, which is similar to the results of the studies mentioned.

CPC has the ability to destroy both gram-positive and gram-negative bacterial organisms with its bactericidal mechanism of action (Pitcher et al., 1980). It is a quaternary ammonium compound that is effective at preventing bacterial plaque accumulation and inhibiting the development of gingivitis (Ali et al., 2002). CPC incorporated into GIs demonstrated antimicrobial properties by reducing quantities of microorganisms in studies done by Botelho, 2003, 2004, 2005, and Tuzuner and Ulusu, 2010. No formal published articles could be found that evaluated the antimicrobial properties of surface treatment of desiccated RMGI with CPC. The results of this present study demonstrate that surface treatment of the desiccated GI or RMGI with CPC resulted in no growth of *S. mutans*, which is similar to the results of the studies mentioned.

Patients may present with xerostomia due to various etiologic factors including history of radiation therapy for head and neck cancer, daily regimen of several medications, and systemic disease such as Sjogren's syndrome (Tschoppe et al., 2010). Preventative measures offered by the dental provider are imperative to manage the increased risk of caries in these patients due to lack of the natural cleansing of saliva. As the pH buffering capacity and ion for remineralization are no longer present, the oral environment tends to favor cariogenic bacteria such as *S. mutans, lactobacillus,* and *actinomyces* (Brown et al, 1975). Therefore, these patients are predisposed to a more aggressive and extensive rate of caries (Meraw and Reeve, 1998; Guggenheimer and Moore, 2003). For patients with history of irradiation, it has been suggested that fluoride gel placed in custom fabricated flexible trays be worn for a minimum of 5-10 mins once daily after toothbrushing and flossing. Patients are informed to not brush, rinse, eat or drink for 30 mins following fluoride application (Chai et al., 2006). In addition, two studies found that recurrent caries was prevented around restorations such as GI, RMGI, and composite when fluoride was used in xerostomic patients (McComb et al., 2002; Haveman et al., 2003).

The results of this study indicate that surface treatment of desiccated GI or RMGI with 5% NaF provides a combination of antimicrobial properties in the prevention of dental caries as well as providing the greatest improvement in strength properties of both desiccated materials. The other surface treatments evaluated in this study

demonstrated antimicrobial properties, yet did not simultaneously improve the strength of both desiccated materials. Therefore, dental providers may suggest that their xerostomic patients wear custom fabricated trays with 5% NaF to maximize contact time with the tooth and restoration in the prevention of caries and improvement in strength properties of their existing GI and RMGI restorations. Future studies may examine different types/concentrations of fluoride and its mechanical and antibacterial effects on GIs and RMGIs or other restorations such as composite or amalgam in the xerostomic patient.

VI. CONCLUSION

In general, the desiccated GI and RMGI materials retained their mechanical properties after surface treatment compared to the 100% humidity group. Sodium fluoride provided the greatest improvement in strength properties.

Surface treatment of the desiccated GI or RMGI with CHX or CPC resulted in no growth of the *S. mutans*. NaF resulted in significantly lower CFU/mL than CPP-ACP, which was significantly lower than the control group.

Therefore, the recommendations that can be given based on this study's results are that desiccated GIs and RMGIs exposed to 5% NaF: 1) enhanced the mechanical properties of both materials and, 2) provided inhibition of growth of *S. mutans.*

Appendix A- Raw Data by Group

Legend

Treatment Groups:

Fuji IX: conventional glass ionomer

Ketac Nano: resin modified glass ionomer

Surface treatment:

Humidity: 100% humidity in 35ºC oven

CPP: Casein phosphopeptide-amorphous calcium phosphate

NaF: 5% Sodium fluoride

CHX: 0.12% Chlorhexidine

Cepacol: 0.05% Cetylpyridinium chloride solution

Flexural strength and modulus:

N: Newtons

w: width

h: height

MPa: Mega Pascals

Mod: flexural modulus

Bacterial count:

CFU/mL: Colony Forming Units/milliliter

Flexural Strength and Modulus Fuji IX

Humidity

	N	w	h	MPa	Mod
1	4.18	2.01	2.11	14.01	15.27
2	4.46	2.08	2.16	13.79	17.00
3	3.98	2.14	2.09	12.77	16.41
4	5.12	2.08	2.14	16.13	17.12
5	4.79	2.10	2.11	15.37	16.98
6	5.54	2.07	2.10	18.21	16.84
7	3.02	2.04	2.08	10.27	11.67
8	6.31	2.16	2.09	20.06	20.69
9	3.49	2.14	2.12	10.89	12.43
10	4.59	2.17	2.06	14.95	11.22
mean	4.55			14.64	15.56
st dev	0.97			3.03	2.96

CPP

	N	w	h	MPa	Mod
1	4.82	2.11	2.11	15.39	16.68
2	6.27	2.08	2.09	20.70	18.95
3	4.86	2.05	2.06	16.76	15.76
4	3.68	2.08	2.11	11.92	14.80
5	4.44	2.09	2.02	15.62	20.15
6	4.12	2.06	2.01	14.85	12.84
7	2.43	2.12	2.09	7.87	12.76
8	3.91	2.08	2.14	12.31	17.40
9	3.00	2.04	2.09	10.10	12.61
10	2.39	2.09	2.12	9.54	10.41
mean	3.99			13.51	15.24
st dev	1.20			3.88	3.11

NaF

	N	w	h	MPa	Mod
1	12.61	2.02	2.05	44.56	28.73
2	7.69	1.91	2.08	27.92	20.54
3	13.14	1.98	2.05	47.37	30.32
4	10.04	2.00	2.10	34.15	28.62
5	16.18	1.99	2.11	54.79	25.60
6	15.22	2.09	2.09	50.01	27.98
7	13.98	2.00	2.10	47.55	25.97
8	7.05	2.01	2.02	25.79	26.78
9	5.23	1.98	2.05	18.86	20.62
10	9.47	1.99	2.13	31.47	25.47
mean	11.06			38.25	26.06
st dev	3.71			12.12	3.28

CHX

	N	w	h	MPa	Mod
1	11.33	2.11	2.10	36.53	29.61
2	7.65	2.02	2.10	25.76	22.81
3	9.33	2.10	2.02	32.66	23.76
4	8.69	2.13	2.11	27.49	24.86
5	8.15	2.06	2.09	27.17	23.34
6	9.94	2.13	2.06	32.99	26.85
7	7.94	2.04	2.05	27.78	26.34
8	6.50	2.00	2.06	22.98	21.26
9	7.83	2.01	2.07	27.27	25.66
10	6.09	2.04	2.16	19.20	20.68
mean	8.35			27.98	24.52
st dev	1.56			5.04	2.71

Cepacol

	N	w	h	MPa	Mod
1	2.64	2.12	2.11	8.39	11.17
2	4.38	2.05	2.08	14.82	13.30
3	5.34	2.18	2.06	17.32	16.30
4	3.34	2.13	1.98	12.00	13.78
5	6.57	2.11	2.16	20.02	18.17
6	9.87	2.08	2.15	30.80	26.67
7	4.60	2.06	2.14	14.63	20.01
8	4.78	2.10	2.09	15.63	14.98
9	4.59	2.09	2.10	14.94	18.98
10	5.55	2.13	2.04	18.78	20.72
mean	5.17			16.73	17.41
st dev	1.98			5.94	4.50

Flexural Strength and Modulus Ketac Nano

Humidity

	N	w	h	MPa	Mod
1	18.06	2.17	2.21	51.12	8.54
2	21.04	2.09	2.24	60.19	8.44
3	15.51	2.16	2.08	49.79	8.40
4	19.09	2.19	2.08	60.44	7.27
5	19.51	2.16	2.11	60.86	8.78
6	21.23	2.15	2.16	63.49	7.52
7	17.19	2.09	2.21	50.52	8.11
8	16.10	2.18	2.13	48.84	7.86
9	16.39	2.17	2.14	49.48	6.33
10	14.81	2.22	2.12	44.53	7.72
mean	17.89			53.93	7.90
st dev	2.27			6.60	0.73

CPP

	N	w	h	MPa	Mod
1	30.51	2.07	2.11	99.32	8.14
2	26.71	2.07	2.12	86.13	8.46
3	32.23	2.08	2.09	106.42	8.54
4	21.27	2.1	2.2	62.78	7.96
5	31.76	2.08	2.11	102.89	8.72
6	30.11	2.17	2.1	94.39	8.15
7	24.17	2.14	2.05	80.63	7.68
8	30.09	2.04	2.07	103.27	8.08
9	28.26	2.04	2.17	88.26	9.14
10	22.05	2.18	2.07	88.52	7.92
mean	27.72			91.26	8.28
st dev	3.99			13.13	0.43

NaF

	N	w	h	MPa	Mod
1	35.70	2.03	2.13	116.29	8.34
2	32.70	2.02	2.15	105.06	8.60
3	32.99	2.05	2.10	109.47	8.35
4	30.31	2.04	2.14	97.33	8.94
5	27.52	2.01	2.07	95.86	8.65
6	22.18	2.10	2.10	71.85	8.72
7	35.42	2.03	2.15	113.24	8.93
8	37.57	2.04	2.15	119.52	9.23
9	34.03	2.09	2.11	109.72	8.98
10	36.80	2.01	2.13	121.06	9.92
mean	32.52			105.94	8.87
st dev	4.73			14.68	0.47

CHX

	N	w	h	MPa	Mod
1	14.54	2.16	2.07	47.13	5.28
2	19.13	2.06	2.17	59.16	6.24
3	16.42	2.09	2.17	50.05	6.50
4	18.10	2.18	2.09	57.02	5.94
5	11.92	2.11	2.07	39.55	5.16
6	16.25	2.07	2.18	49.56	5.63
7	14.93	2.12	2.11	47.45	5.26
8	17.62	2.08	2.16	54.47	5.77
9	16.31	2.22	2.10	49.98	5.90
10	16.46	2.20	2.18	47.23	6.40
mean	16.17			50.16	5.81
st dev	2.03			5.62	0.48

Cepacol

	N	w	h	MPa	Mod
1	20.75	2.11	2.12	65.64	5.40
2	14.77	2.10	2.17	44.81	5.65
3	12.67	2.08	2.17	38.81	5.78
4	10.04	2.10	2.17	30.46	6.02
5	10.78	2.06	2.21	32.14	5.23
6	12.42	2.07	2.18	37.88	6.14
7	7.65	2.10	2.18	23.00	5.89
8	16.78	2.09	2.22	48.87	6.73
9	12.61	2.17	2.22	35.37	7.35
10	15.80	2.06	2.10	52.18	6.35
mean	13.43			40.92	6.05
st dev	3.75			12.33	0.63

Hardness Fuji IX

	Humidity			
1	65.00	66.70	62.80	64.83
2	63.30	71.80	73.00	69.37
3	73.50	75.10	80.20	76.27
4	80.40	61.20	70.60	70.73
5	82.60	78.90	67.30	76.27
6	75.00	66.90	79.50	73.80
7	72.50	77.30	80.90	76.90
8	74.90	79.90	82.60	79.13
9	88.20	83.00	89.10	86.77
10	75.80	78.50	71.50	75.27
			avg	74.93
			st dev	5.94

	CPP			
1	90.10	85.40	100.50	92.00
2	87.90	84.40	77.00	83.10
3	56.30	67.60	61.00	61.63
4	81.00	80.90	73.80	78.57
5	70.70	85.70	81.80	79.40
6	79.40	87.40	95.80	87.53
7	73.60	71.70	68.20	71.17
8	87.10	79.80	80.30	82.40
9	80.90	68.00	72.20	73.70
10	82.60	83.20	92.70	86.17
			avg	79.57
			st dev	8.88

	NaF			
1	51.50	52.20	42.20	48.63
2	75.50	68.20	70.30	71.33
3	70.80	56.70	56.10	61.20
4	53.40	76.60	75.60	68.53
5	78.60	76.90	77.20	77.57
6	90.50	88.40	82.40	87.10
7	65.00	76.70	93.70	78.47
8	96.80	61.30	64.30	74.13
9	66.80	84.50	64.90	72.07
10	58.50	62.00	72.80	64.43
			avg	70.35
			st dev	10.60

	CHX			
1	86.80	88.70	92.90	89.47
2	87.50	83.60	82.70	84.60
3	90.80	80.80	94.40	88.67
4	77.80	75.10	80.40	77.77
5	80.30	88.80	82.50	83.87
6	88.80	83.30	85.40	85.83
7	74.60	89.40	81.20	81.73
8	75.20	83.20	83.50	80.63
9	87.40	82.40	90.00	86.60
10	74.40	79.90	79.30	77.87
			avg	83.70
			st dev	4.14

	Cepacol			
1	71.70	78.40	77.20	75.77
2	79.20	81.90	76.90	79.33
3	87.50	88.80	89.70	88.67
4	73.80	61.00	67.10	67.30
5	89.70	87.10	79.20	85.33
6	76.50	79.70	82.50	79.57
7	88.00	88.90	78.90	85.27
8	79.80	72.80	69.70	74.10
9	84.40	84.40	82.90	83.90
10	69.50	83.60	82.70	78.60
			avg	79.78
			st dev	6.35

Hardness Ketac Nano

	Humidity			
1	21.50	22.90	21.40	21.93
2	28.90	28.30	27.90	28.37
3	28.70	29.60	33.10	30.47
4	26.40	29.80	26.80	27.67
5	32.50	28.40	29.90	30.27
6	26.30	28.20	30.60	28.37
7	25.80	32.60	31.00	29.80
8	30.90	30.60	31.10	30.87
9	26.00	25.90	28.90	26.93
10	26.30	26.40	29.80	27.50
			avg	28.22
			st dev	2.60

	CPP			
1	29.30	31.10	31.20	30.53
2	29.80	32.00	29.00	30.27
3	31.10	31.40	33.20	31.90
4	32.40	30.80	29.40	30.87
5	30.60	31.50	29.60	30.57
6	30.00	30.20	30.40	30.20
7	28.80	28.00	28.00	28.27
8	28.00	29.60	28.30	28.63
9	33.40	33.00	33.00	33.13
10	28.90	30.90	30.40	30.07
			avg	30.44
			st dev	1.41

	NaF			
1	31.70	32.30	35.60	33.20
2	24.50	27.30	30.80	27.53
3	30.30	30.60	35.20	32.03
4	25.50	27.40	27.30	26.73
5	32.00	25.00	24.90	27.30
6	24.80	24.90	27.10	25.60
7	22.90	21.30	28.90	24.37
8	27.70	32.10	31.70	30.50
9	26.10	25.90	29.90	27.30
10	24.90	28.90	29.20	27.67
			avg	28.22
			st dev	2.81

	CHX			
1	29.10	28.20	25.30	27.53
2	25.40	24.90	26.30	25.53
3	27.60	26.90	28.20	27.57
4	31.20	27.40	29.60	29.40
5	25.10	26.80	27.60	26.50
6	28.10	28.90	28.50	28.50
7	30.30	30.70	29.30	30.10
8	25.50	24.10	27.20	25.60
9	25.70	25.60	25.90	25.73
10	25.70	26.80	25.50	26.00
			avg	27.25
			st dev	1.65

	Cepacol			
1	29.20	28.00	27.10	28.10
2	25.30	28.70	25.60	26.53
3	25.30	26.30	27.70	26.43
4	25.00	27.10	25.60	25.90
5	24.80	23.00	25.50	24.43
6	24.20	23.10	26.60	24.63
7	25.60	24.40	24.50	24.83
8	23.40	24.20	27.90	25.17
9	30.50	29.10	31.40	30.33
10	29.20	28.50	28.50	28.73
			avg	26.51
			st dev	1.97

CFU/mL of Fuji IX and Ketac Nano

Specimens	CFU/mL	
Ketac100%Hum 1	580000	
Ketac100%Hum 2	470000	
Ketac100%Hum 3	490000	
Ketac100%Hum 4	610000	
Ketac100%Hum 5	300000	
Ketac100%Hum 6	130000	
Ketac100%Hum 7	410000	
Ketac100%Hum 8	770000	
Ketac100%Hum 9	1200000	
Ketac100%Hum 10	1700000	
Ave Total	666000	439527
Specimen	CFU/mL	
Fuji 100% Humid 1	4500000	
Fuji 100% Humid 2	11000	
Fuji 100% Humid 3	7400000	
Fuji 100% Humid 4	270000	
Fuji 100% Humid 5	18000	
Fuji 100% Humid 6	400000	
Fuji 100% Humid 7	2500	
Fuji 100% Humid 8	200	
Fuji 100% Humid 9	11000000	
Fuji 100% Humid 10	200	
Ave Total	2360190	3748845
Ketac-CPP-ACP 1	650000	
Ketac-CPP-ACP 2	53000	
Ketac-CPP-ACP 3	100000	
Ketac-CPP-ACP 4	410000	
Ketac-CPP-ACP 5	7000	
Ketac-CPP-ACP 6	12000	
Ketac-CPP-ACP 7	12000	
Ketac-CPP-ACP 8	15000	
Ketac-CPP-ACP 9	49000	
Ketac-CPP-ACP 10	14000	
Ave Total	132200	207798
Fuji-CCP-ACP 1	290000	
Fuji-CCP-ACP 2	88000	
Fuji-CCP-ACP 3	81000	
Fuji-CCP-ACP 4	94000	
Fuji-CCP-ACP 5	57000	
Fuji-CCP-ACP 6	24000	
Fuji-CCP-ACP 7	29000	
Fuji-CCP-ACP 8	46000	
Fuji-CCP-ACP 9	39000	
Fuji-CCP-ACP 10	8400	
Ave Total	75640	76420

Specimen	CFU/mL	
Ketac NaF 1	2800	
Ketac NaF 2	1900	
Ketac NaF 3	1900	
Ketac NaF 4	1600	
Ketac NaF 5	1700	
Ketac NaF 6	300	
Ketac NaF 7	2800	
Ketac NaF 8	2000	
Ketac NaF 9	1100	
Ketac NaF 10	300	
Ave Total	1640	827
Fuji NaF 1	100	
Fuji NaF 2	100	
Fuji NaF 3	300	
Fuji NaF 4	300	
Fuji NaF 5	600	
Fuji NaF 6	400	
Fuji NaF 7	1000	
Fuji NaF 8	1100	
Fuji NaF 9	100	
Fuji NaF 10	2000	
Ave Total	600	578
Specimen	CFU/mL	
Fuji CHX 1		
Fuji CHX 2		NG
Fuji CHX 3		NG
Fuji CHX 4		NG
Fuji CHX 5		NG
Fuji CHX 6		NG
Fuji CHX 7		NG
Fuji CHX 8		NG
Fuji CHX 9		NG
Fuji CHX 10		NG
Ave Total		
Ketac CHX 1		NG
Ketac CHX 2		NG
Ketac CHX 3		NG
Ketac CHX 4		NG
Ketac CHX 5		NG
Ketac CHX 6		NG
Ketac CHX 7		NG
Ketac CHX 8		NG
Ketac CHX 9		NG
Ketac CHX 10		NG
Ave Total		

Ave Total		
Fuji CPC 1		NG
Fuji CPC 2		NG
Fuji CPC 3		NG
Fuji CPC 4		NG
Fuji CPC 5		NG
Fuji CPC 6		NG
Fuji CPC 7		NG
Fuji CPC 8		NG
Fuji CPC 9		NG
Fuji CPC 10		NG
Specimen	**CFU/mL**	
Ketac CPC 1		NG
Ketac CPC 2		NG
Ketac CPC 3		NG
Ketac CPC 4		NG
Ketac CPC 5		NG
Ketac CPC 6		NG
Ketac CPC 7		NG
Ketac CPC 8		NG
Ketac CPC 9		NG
Ketac CPC 10		NG
Ave Total		

Appendix B- Statistical Analysis

2-way ANOVA

Flexural Strength

Tests of Between-Subjects Effects
Dependent Variable: MPA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	90231.443(a)	9	10025.716	118.400	.000
Intercept	205496.302	1	205496.302	2426.825	.000
COND	23992.362	4	5998.091	70.835	.000
GROUP	53402.126	1	53402.126	630.657	.000
COND * GROUP	12836.954	4	3209.239	37.900	.000
Error	7620.930	90	84.677		
Total	303348.675	100			
Corrected Total	97852.373	99			

a R Squared = .922 (Adjusted R Squared = .914)

Homogeneous Subsets

MPA
Tukey HSD

COND	N	Subset 1	Subset 2	Subset 3	Subset 4
Cep	20	28.8245			
humid	20	34.2855	34.2855		
CHX	20		39.0715		
CPP	20			52.3835	
NaF	20				72.0935
Sig.		.337	.473	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 84.677.

a Uses Harmonic Mean Sample Size = 20.000.

b Alpha = .05.

2-way ANOVA

Flexural Modulus

Tests of Between-Subjects Effects
Dependent Variable: MODULUS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4964.338(a)	9	551.593	94.367	.000
Intercept	18412.047	1	18412.047	3149.947	.000
COND	555.117	4	138.779	23.742	.000
GROUP	3829.506	1	3829.506	655.155	.000
COND * GROUP	579.715	4	144.929	24.795	.000
Error	526.067	90	5.845		
Total	23902.453	100			
Corrected Total	5490.406	99			

a R Squared = .904 (Adjusted R Squared = .895)

Homogeneous Subsets

MODULUS
Tukey HSD

COND	N	Subset		
		1	**2**	**3**
humid	20	11.7300		
Cep	20	11.7310		
CPP	20	11.7575		
CHX	20		15.1625	
NaF	20			17.4645
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 5.845.

a Uses Harmonic Mean Sample Size = 20.000.

b Alpha = .05.

1-way ANOVA

Fuji IX Flexural Strength

Tests of Between-Subjects Effects Dependent Variable: MPA					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	4534.984(a)	4	1133.746	24.454	.000
Intercept	24692.642	1	24692.642	532.599	.000
COND	4534.984	4	1133.746	24.454	.000
Error	2086.316	45	46.363		
Total	31313.942	50			
Corrected Total	6621.300	49			
a R Squared = .685 (Adjusted R Squared = .657)					

Homogeneous Subsets

MPA Tukey HSD				
	N	**Subset**		
COND		**1**	**2**	**3**
CPP	10	13.5060		
humid	10	14.6450		
Cep	10	16.7330		
CHX	10		27.9830	
NaF	10			38.2470
Sig.		.826	1.000	1.000
Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 46.363.				
a Uses Harmonic Mean Sample Size = 10.000.				
b Alpha = .025.				

1-way ANOVA

Fuji IX Modulus

Tests of Between-Subjects Effects Dependent Variable: MODULUS					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	1059.701(a)	4	264.925	23.288	.000
Intercept	19517.743	1	19517.743	1715.662	.000
COND	1059.701	4	264.925	23.288	.000
Error	511.930	45	11.376		
Total	21089.373	50			
Corrected Total	1571.631	49			
a R Squared = .674 (Adjusted R Squared = .645)					

Homogeneous Subsets

MODULUS Tukey HSD			
	N	**Subset**	
COND		**1**	**2**
CPP	10	15.2360	
humid	10	15.5630	
Cep	10	17.4080	
CHX	10		24.5170
NaF	10		26.0630
Sig.		.606	.843
Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 11.376.			
a Uses Harmonic Mean Sample Size = 10.000.			
b Alpha = .025.			

1-way ANOVA

Ketac Nano Flexural Strength

Tests of Between-Subjects Effects
Dependent Variable: MPA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	32294.332(a)	4	8073.583	65.643	.000
Intercept	234205.786	1	234205.786	1904.245	.000
COND	32294.332	4	8073.583	65.643	.000
Error	5534.614	45	122.991		
Total	272034.733	50			
Corrected Total	37828.946	49			

a R Squared = .854 (Adjusted R Squared = .841)

Homogeneous Subsets

MPA
Tukey HSD

COND	N	Subset 1	Subset 2
Cep	10	40.9160	
CHX	10	50.1600	
humid	10	53.9260	
CPP	10		91.2610
NaF	10		105.9400
Sig.		.083	.037

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 122.991.

a Uses Harmonic Mean Sample Size = 10.000.

b Alpha = .025.

1-way ANOVA

Ketac Nano Modulus

Tests of Between-Subjects Effects
Dependent Variable: MODULUS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	75.131(a)	4	18.783	59.785	.000
Intercept	2723.810	1	2723.810	8669.806	.000
COND	75.131	4	18.783	59.785	.000
Error	14.138	45	.314		
Total	2813.080	50			
Corrected Total	89.269	49			

a R Squared = .842 (Adjusted R Squared = .828)

Homogeneous Subsets

MODULUS
Tukey HSD

COND	N	Subset		
		1	2	3
CHX	10	5.8080		
Cep	10	6.0540		
humid	10		7.8970	
CPP	10		8.2790	8.2790
NaF	10			8.8660
Sig.		.862	.553	.151

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .314.

a Uses Harmonic Mean Sample Size = 10.000.

b Alpha = .025.

1-way ANOVA

Fuji IX Hardness

Tests of Between-Subjects Effects Dependent Variable: KHN					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	1056.034(a)	4	264.008	4.651	.003
Intercept	301608.144	1	301608.144	5312.848	.000
COND	1056.034	4	264.008	4.651	.003
Error	2554.631	45	56.770		
Total	305218.810	50			
Corrected Total	3610.665	49			
a R Squared = .292 (Adjusted R Squared = .230)					

Homogeneous Subsets

KHN Tukey HSD			
	N	**Subset**	
COND		**1**	**2**
NaF	10	70.3460	
humid	10	74.9340	74.9340
CPP	10	79.5670	79.5670
Cep	10	79.7840	79.7840
CHX	10		83.7040
Sig.		.055	.087
Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 56.770.			
a Uses Harmonic Mean Sample Size = 10.000.			
b Alpha = .025.			

1-way ANOVA

Ketac Nano Hardness

Tests of Between-Subjects Effects Dependent Variable: KHN					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	87.833(a)	4	21.958	4.726	.003
Intercept	39558.657	1	39558.657	8514.643	.000
COND	87.833	4	21.958	4.726	.003
Error	209.068	45	4.646		
Total	39855.558	50			
Corrected Total	296.901	49			
a R Squared = .296 (Adjusted R Squared = .233)					

Homogeneous Subsets

KHN Tukey HSD			
	N	**Subset**	
COND		**1**	**2**
Cep	10	26.5080	
CHX	10	27.2460	
humid	10	28.2180	28.2180
NaF	10	28.2230	28.2230
CPP	10		30.4440
Sig.		.398	.161
Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square(Error) = 4.646.			
a Uses Harmonic Mean Sample Size = 10.000.			
b Alpha = .025.			

Literature Cited

Abduo J, Swain M. Self-reparability of glass-ionomer cements: an in vitro investigation. Eur J Oral Sci 2011;119:187-191.

Ali J, Khar R, Ahuja A, Kaba R. Buccoadhesive erodible disk for the treatment of oro-dental infections: design and characterization, Int. J. Pharm 2002;283:93–103.

Al-Musallam TA, Evans CA, Drummond JL, Matasa C, Wu CD. Antimicrobial properties of an orthodontic adhesive combined with cetylpyridium chloride. Am J Orthod Dentofacial Orthop 2006;129:245-51.

Altschul, S., Gish, W., Miller, W., Myers, E. & Lipman, D. Basic local alignment search tool. J Molec Biol 1990;215(3):403–410.

American Academy of Periodontology: Chemical agents for control of plaque and gingivitis. Committee on Research, Science and Therapy. Position Paper, Chicago, 1994, The Academy.

Andrews N, Griffiths C. Dental complications of head and neck radiotherapy: part 2. Aust Dent J 2001; 46:(3):174-182.

Araujo JC, Teran FC, Oliveira RA, Nour EA, Montenegro MA, Campos JR, Vazoller RF. Comparison of hexamethyldisilazane and critical point drying treatments for SEM analysis of anaerobic biofilms and granular sludge. J Electron Microsc 2003;52:429-33.

Armitage GC. Basic features of biofilms-why are they such difficult therapeutic targets? Ann R Australas Coll Dent Surg 2004;17:30-4.

Atkinson JC, Wu A. Salivary gland dysfunction: causes, symptoms, treatment. JADA 1994;125(4):409–16.

Atkinson JC, Grisius M, Massey W. Salivary hypofunction and xerostomia: diagnosis and treatment. Dent Clin North Am. 2005; 49(2): 309-26.

Baehni PC, Takeuchi Y. Anti-plaque agents in the prevention of biofilm-associated oral diseases. Oral Dis 2003;9:23-9.

Beiswanger BB, Mallat ME, Jackson RD et al: Clinical effects of a 0.12% chlorhexidine rinse as an adjunct to scaling and root planing, J Clin Dent 1992;3:33–38.

Berg JH, Farrell JE, Brown LR. Class II glass ionomer/silver cermet restorations and their effect on interproximal growth of mutans streptococci. Pediatr Dent 1990;12(1):20-23.

Billington RW, Williams JA, Pearson GJ. Variation in powder/liquid ratio of a restorative glass-ionomer cement used in dental practice. Br Dent J 1990;169:164–167.

Borges BCD, Borges JdS, de Araujo LSN, Machado CT, dos Santos AJS, Pinheiro IVdA. Update on nonsurgical, ultraconservative approaches to treat effectively non-cavitated caries lesions in permanent teeth. Eur J Dent 2011;5(2):229-236.

Botelho MG. Inhibitory effects on selected oral bacteria of antibacterial agents incorporated in a glass ionomer cement. Caries Res 2003;37:108-14.

Botelho MG. Compressive strength of glass ionomer cements with dental antibacterial agents. SADJ 2004;59:51-3.

Botelho MG. The antimicrobial activity of a dentin conditioner combined with antibacterial agents. Oper Dent 2005;30:75-82.

Brown LR, Dreizen S, Handler S, Johnston DA. Effect of radiation-induced xerostomia on human oral microflora. J Dent Res 1975;54:740–50.

Carlén A, Nikdel K, Wennerberg A, Holmberg K, Olsson J. Surface characteristics and in vitro biofilm formation on glass ionomer and composite resin. Biomater 2001;22:481-487.

Cattani-Lorente MA, Dupuis V, Moya F, Payan J, Meyer JM. Comparative study of the physical properties of a polyacid-modified composite resin and a resin-modified glass ionomer cement. Dent Mater 1999;15:21-32.

Ciancio S: Expanded and future uses of mouthrinses, J Am Dent Assoc 1994;125(Suppl 2):29S–32S.

Cildir SK, Sandalli N. Compressive strength, surface roughness, fluoride release and recharge of four new fluoride-releasing fissure sealants. J Dent Mater 2007;26(3)335-341.

Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New Approaches to Enhanced Remineralization of Tooth Enamel. J Dent 2010;89(11):1187-1197.

Costerton JW, Lewandowski Z, DeBeer D, et al. Biofilms, the customized microniche. J Bacteriology 1994;176: 2137-2142.

Crisp S, Pringuer M.A., Wardleworth D, Wilson A.D. Reactions in Glass Ionomer Cements: II. An Infrared Spectroscopic Study. J Dent Res 1974;53:1414

Crisp S, Wilson A.D. Reactions in Glass Ionomer Cements: V. Effect of Incorporating Tartaric Acid in the Cement Liquid. J Dent Res 1976;55:1023

Croll TP, Nicholson JW. Glass-ionomer cements in pediatric dentistry: review of the literature. Pediatr Dent 2002;24:423-9.

Cross KJ, Huq NL, Palamara JE, Perich JW, Reynolds EC. Physiochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. J Biol Chem 2005;280:15362-15369.

da Silva R.C., Zuanon A.C.C., Spolidorio D.M.P., Campos J.A.D.B. Antibacterial activity of four glass ionomer cements used in atraumatic restorative treatment. J Mater Sci 2007;18:1859-1862.

de Castilho ARF, Duque C, de Cassia Negrini T, Sacono NT, de Paula AB, de Souza Costa CA, Palomari Spolidorio DM, Puppin-Rontani RM. In vitro and in vivo investigation of the biological and mechanical behaviour of resin-modified glass-ionomer cement containing chlorhexidine. J Dent 2013;41(2):155-163.

do Nascimento AB, Fontana UF, Teixeira HM, Costa CA. Biocompatibility of a resin-modified glass-ionomer cement applied as pulp capping in human teeth. Am J Dent 2000;13:28-34.

Demke R. Plaque inhibition: the science and application of oral rinses. Dent Today 2012;31(2):96-101.

Diaz PI, Chalmers NI, Rickard AH, et al. Molecular characterization of subject-specific oral microflora during initial colonization of enamel. Appl Environ Microbiol 2006;72:2837-2848.

Dimkov A, Nicholson JW, Gjorgievska E. The possibility of incorporating antimicrobial components into glass-ionomer cements. Contributions, Sec. Biol. Med. Sci. 2009:219-237.

Dunn WJ. Dental Emergency Rates at Two Expeditionary Medical Support Facilities Supporting Operations Enduring and Iraqi Freedom. Mil Med 2004; 169, 7:510.

Ellacuria J, Triana R, Soler I, Minguez N, Prado J, Cearra P, Garcia-Godoy F. Development of glass ionomers. Ital J of Paediatr Dent 1999;2:59-66.

Farret MM, de Lima EM, Mota EG, Oshima HMS, Barth V, de Oliveira SD. Can we add chlorhexidine into glass ionomer cements for band cementation? Angle Orthod 2011;81:496-502.

Forgie AH, Pine CM, Pitts NB. Restoration removal with and without the aid of magnification. J Oral Rehab 2001;28:309.

Forss H, Seppä L, Alakuijala P. Plaque accumulation on glass ionomer filling materials. Proc Finn Dent Soc 1991;87(3):343-350.

Fox PC. Management of dry mouth. Dent Clin North Am 1997;41(4):863–76.

Fox PC. Autoimmune diseases and Sjögren's syndrome: an autoimmune exocrinopathy. Ann N Y Acad Sci 2007;1098:15–21.

Fox PC. Xerostomia: Recognition and Management. Suppl to Access 2008;1-7.

Gibson G. Identifying and treating xerostomia in restorative patients. J Esthet and Restor Dent 1998;10(5):253-264.

Gladys S, Van Meerbeek B, Braem M, Lambrechts P, Vanherle G. Comparative physic-mechanical characterization of new hybrid restorative materials with conventional glass-ionomer and resin composite restorative materials. J Dent Res 1997;76(4):883-894.

Guertsen W. Biocompatibility of resin-modified filling materials. Crit Rev Oral Biol Med 2000;11:333-55.

Guggenheimer J, Moore PA. Xerostomia: etiology, recognition and treatment. J Am Dent Assoc 2003; 134(1):61-69.

Haffajee AD, Arguello EI, Ximenez-Fyvie LA, et al. Controlling the plaque biofilm. Int Dent J. 2003;53(suppl 3):191-199.

Hamilton IR. Biochemical effects of fluoride on oral bacteria. J Dent Res 1990;69(special issue):660-667.

Hamilton IR, Bowden GHW. Fluoride effects on oral bacteria. In: Fejerskov O, Ekstrand J, Burt BA, editors. Fluoride in dentistry. Copenhagen: Munksgaard; 1996. p. 230–51.

Hennessy T. Antibacterial properties of Hibitane. J Clin Periodontol 1977;4:36-48.

Haveman CW, Summitt JB, Burgess JO, Carlson K. Three restorative materials and topical fluoride gel used in xerostomic patients: a clinical comparison. J Am Dent Assoc 2003;34:177–84.

Hermesch CB, Hilton TJ, Biesbrock AR et al: Perioperative use of 0.12% chlorhexidine gluconate for the prevention of alveolar osteitis: efficacy and risk factor analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:381–387.

Hicks J, Garcia-Godoy F, Donly K, Flaitz C. Fluoride-releasing restorative materials and secondary caries. Dent Clinic North Am 2002;46:247-276.

Hunter AR, Treasure ET, Hunter AJ. Increases in cavity volume associated with removal of class 2 amalgam and composite restorations. Oper Dent 1995;20:2-6.

Imazato S. Antibacterial properties of resin composites and dentin bonding systems. Dent Mater 2003;19:449-57.

Jedrychowski J, Caputo A, Kerper S. Antibacterial and mechanical properties of restorative materials combined with chlorhexidines. J Oral Rehabil 1983;10:373-81.

Jensen ME, Wefel JS, Triolo PT, Hammesfahr PD. Effects of a fluoride-releasing fissure sealant on artificial enamel caries. Am J Dent 1990;3:75-78.

Kaufmann M, Bartholmes P. Purification, characterization and inhibition by fluoride of enolase from Streptococcus mutans. Caries Res 1992;26(2):110-116.

Kraft L. Calcium Aluminate Based Cement as Dental Restorative Materials. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 2002;775.

Kohler B, Birkhed D, Olsson S. Acid production by human strains of *Streptococcus mutans* and *Streptococcus sobrinus.* Caries Res 1995;29:402-6.

Lang NP, Brecx MC: Chlorhexidine digluconate—an agent for chemical plaque control and prevention of gingival inflammation, J Periodont Res 1986;21(suppl):74–89.

Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 1986;50(4):353-380.

Marsh PD. Dental plaque: biological significance of a biofilm and community life-style. J Clin Periodontol 2005;32(suppl 6):7-15.

Marsh PD, Bradshaw DJ. Dental plaque as biofilm. J Industr Microbiol 1995;15:169-175.

Mazzaoui SA, Burrow MF, Tyas MJ, Dashper SG, Eakins D, Reynolds EC. Incorporation of casein phosphopeptide–amorphous calcium phosphate into a glass ionomer cement. J Dent Res 2003;82:914–8.

McCabe JF. Resin-modified glass-ionomers. Biomater 1998;19:521-7.

McComb D, Erickson RL, Maxymiw WG, Wood RE. A clinical comparison of glass ionomer, resin-modified glass ionomer and resin composite restorations in the treatment of cervical caries in xerostomic head and neck radiation patients. Oper Dent 2002;27:430–7.

McLean J.W. Alternatives to amalgam alloys: Part I. Br Dent J 1984;157:432-433.

McLean JW, Nicholson JW, Wilson AD. Proposed nomenclature for glass-ionomer cements and related materials. Quintessence Int 1994;25:587-9.

Meraw SJ, Reeve CM. Dental considerations and treatment of the oncology patient receiving radiation therapy. J Am Dent Assoc 1998;129:201–5.

Mitra SB. Adhesion to dentine and physical properties of a light-cured glass ionomer liner/base. J Dent Res 1991;70:72-4.

Mitra SB. In vitro fluoride release from a light cured glass ionomer liner/base. J Dent Res 1991;70:75-8.

Mitra S. Curing reactions of glass-ionomer materials. In: Hunt PR, ed. Glass-ionomers: The next generation. Proceedings of the 2nd International Symposium on Glass ionomers. Philadelphia, 1994:13-23.

Mjör, IA. Frequency of secondary caries at various anatomical locations. Oper Dent 1985;10(3):88-92

Mount GJ. Color atlas of glass ionomer cement. 3rd ed. London: Dunitz; 2002.

Neu TR, Lawrence JR. Development and structure of microbial stream biofilms as studied by confocal laser scanning microscopy. FEMS. Microbiol Ecol 1997;24:11-25.

Nicholson JW. The chemistry of medical and dental materials. Cambridge: RSC; 2002.

Nicholson JW, Croll TP. Glass-ionomer cements in restorative dentistry. Quintessence Int 1997;28:705-14.

Nicholson JW, Czarnecka B. The biocompatibility of resin-modified glass-ionomer cements for dentistry. Dent Mater 2008; 24:1702-1708.

Nyvad B, Fejerskov O. Scanning electron microscopy of early microbial colonization of human enamel and root surfaces in vivo. Scand J Dent Res 1987;95:287-96.

Overman PR, Biofilm: a new view of plaque. J Contemp Dent Pract 2000;15;1(3):18-29.

Pachuta SM, Meiers JC. Dentin surface treatment and glass ionomer microleakage. Am J Dent 1995;8:187-190.

Palmer G, Jones FH, Billington RW, Pearson GJ. Chlorhexidine release from an experimental glass ionomer cement. Biomater 2004; 25:5423-31.

Pedrini D, Gaetti-Jardim Júnior E, Coelho de Vasconcelos A. Retention of oral microorganisms on conventional and resin-modified glass-ionomer cements. Pesqui Odontol Bras 2001;15(3):196-200.

Pitcher GR, Newman HN and Strahan JD. Access to subgingival plaque by disclosing agents using mouth-rinsing and direct irrigation. J. Clin. Periodontol 1980;300–305.

Powers, John M. Powers. Craig's Restorative Dental Materials, 12th Edition. Mosby, 2006.20.3.1

Quirynen M. The clinical meaning of the surface roughness and the surface free energy of intra-oral hard substrata on the microbiology of the supra- and subgingival plaque: results from in vitro and in vivo experiments. J Dent (Suppl 1) 1994;22:S13-6.

Quirynen M, Bollen CML. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. J Clin Periodontol 1995;22:1-14.

Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dent 1998;18:8–16.

Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76:1587–95.

Ribeiro J, Ericson D. In vitro antibacterial effect of chlorhexidine added to glass ionomer cements. Scand J Dent Res 1991;99:533-40.

Robinson C, Shore RC, Bonass WA, Brookes WA, Brookes SJ, Boteva E, Kirkham J. Identification of human serum albumin in human caries lesions of enamel: the role of putative inhibitors of remineralization. Caries Res 1998;32:193-9.

Rothwell M, Anstice H.M., Pearson G.J. The uptake and release of fluoride by ion-leaching cements after exposure to toothpaste. J Dent 1998;26:591-597.

Rozen R, Bachrach G, Bronshteyn M, Gedalia I, Steinberg D. The role of fructans on dental biofilm formation by *Streptococcus sobrinus, Streptococcus mutans, Streptococcus gordonii,* and *Actinomyces viscosus.* FEMS Microbiol Lett 2001;195:205-10.

Sanders BJ, Gregory RL, Moore K, Avery DR. Antibacterial and physical properties of resin modified glass-ionomers combined with chlorhexidine. J Oral Rehabil 2002;29(6):553-558.

Sbordone L and Bortolaia C. Oral microbial biofilms and plaque-related diseases: microbial communities and their role in the shift from oral health to disease. Clin Oral Investig. 2003;7(4):181-8.

Scheie AA, Petersen FC. The biofilm concept: consequences for future prophylaxis of oral diseases? Crit Rev Oral Biol Med 2004;15:4-12.

Seo DG, Kim J, Lee CY, Park SH. Diagnosis of Sjögren's Syndrome from a xerostomia case accompanied by multiple dental caries. Oper Dent 2009;34(3):359-364.

Shiboski CH, Hodgson TA, Ship JA, Schiodt M. Management of salivary hypofunction during and after radiotherapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103(supplement 1):S66–S73.

Ship JA. Diagnosing, managing, and preventing salivary gland disorders. Oral Dis 2002;8(2):77–89.

Ship JA, Pillemer SR, Baum BJ. Xerostomia and the geriatric patient. J Am Geriatr Soc 2002;50(3):535–43.

Sidhu SK. Clinical evaluations of resin-modified glass-ionomer restorations. Dent Mater 2010;26:7-12.

Sidhu SK, Watson TF. Resin-modified glass ionomer materials. A status report for the American Journal of Dentistry. Am J Dent 1995;8:59-67.

Sidhu SK, Sherriff M, Watson TF. The effects of maturity and dehydration shrinkage on resin-modified glass-ionomer restorations. J Dent Res 1997;76(8):1495-1501.

Stamboulis A, Matsuya S, Hill RG, Law RV, Udoh K, Nakagawa M, Matsuya Y. MAS-NMR spectroscopy studies in the setting reaction of glass ionomer cements. J Dent 2006;34:574-581.

Stanislawski L, Daniau X, Lautie A, Goldberg M. Factors responsible for pulp cytotoxicity induced by resin-modified glass ionomer cements. J Biomed Mater Res; Appl Biomater 1999;48:277-88.

Steinberg D. Studying plaque biofilms on various dental surfaces. In: An YH, Friedman RJ, editors. Handbook of bacterial adhesion principles methods and application. Totowa, NJ: Humana Press; 2000;353-70.

Stepanovic, S., Cirkovic, I., Ranin, L., Svabic-Vlahovic, M. Biofilm formation by Salmonella spp. and Listeria monocytogenes on plastic surface. Lett App Microbiol 2004;38:428–432.

Summitt JB, Robbins JW, Hilton TJ, Schwartz RS. Fundamentals of Operative Dentistry. 3rd ed. Illinois: Quintessence Publishing Co, Inc; 2006:413, 539.

Svanberg M, Mjör IA, Orstavik D. Mutans streptococci in plaque from margins of amalgam, composite, and glass-ionomer restorations. J Dent Res 1990;69(3):861-864

Takahashi Y, Imazato S, Kaneshiro AV, Ebisu S, Frencken JE, Tay FR. Antibacterial effects and physical properties of glass ionomer cements containing chlorhexidine for the ART approach. Dent Mater 2006; 22:647-52.

Taylor MJ, Lynch E. Microleakage. J Dent 1992;20(1):3-10.

Ten Cate JM. Current concepts on the mechanism of action of fluoride. Acta Odontol Scand 1999;57(6)325-329

Toledano M, Osorio R, Osorio E, Fuentes V, Prati C, Garcia-Godoy F. Sorption and solubility of resin based restorative dental materials. J Dent 2003;31:43-50.

Turkun LS, Turkun M, Ertugrul F, Ates M, Brugger S. Long-term antimicrobial effects and physical properties of a chlorhexidine-containing glass ionomer cement. J Esthet Restor Dent 2008;20:(1):29-44.

Turner MD, Ship JA. Dry mouth and its effects on the oral health of elderly people. J Am Dent Assoc 2007;138:15S-20S.

Tuzuner T, Ulusu T. Effect of antibacterial agents on the surface hardness of a conventional glass-ionomer cement. J. Appl Oral Sci 2012 Feb;20(1):45-9.

Twetman S, Lindqvist L. Effect of salivary lysozyme on glucose incorporation and acid production in *Streptococcus mutans.* Caries Res 1985;19:414-21.

Tyas MJ. Placement and replacement of restorations by selected practitioners– a review. Australian Dent J 2005;50:(2):81-89.

van Amerongen WE. Dental caries under glass ionomer restorations. J Public Health Dent 1996;56:150-4.

Vitali C, Bombardieri S, Jonsson R; European Study Group on Classification Criteria for Sjögren's Syndrome, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61(6):554–8.

Watson TF. A confocal microscopic study of some factors affecting the adaptation of a light-cured glass ionomer to tooth tissue. J Dent Res 1990;69:1531-1538.

Wilson AD, Paddon JM. Dimensional changes occurring in a glass-ionomer cement.Am J Dent 1993;6:280-282.

Weerheijm KL, de Soet JJ, van Amerongen WE, de Graaff J. The effect of glass ionomer cement on carious dentine: an in vivo study. Caries Res 1993;27:417-23.

Weerheijm KL, Kruelen CM, de Soet JJ, et al. Bacterial counts in carious dentine under restorations: 20 year in vivo effects. Caries Res 1999;33:130-4.

Wiater A, Choma A. Szczodrak J. Insoluble glucans synthesized by cariogenic streptococci: a structural study. J Basic Microbiol 1999;39:265-73.

Wilson AD. Resin modified glass ionomer cements. Int J Prosthodont 1990;3:425-9.

Wong TC, Bryant RW. Glass ionomer cements: dispensing and strength. Aust Dent J 1985; 30:336-340.

Xu X, Burgess JO. Compressive strength, fluoride release and recharge of fluoride-releasing materials. Biomater 2003;24:2451-2461.

Yip KHK, Smales RJ, Kaidonis JA. Tooth Erosion Prevention and Treatment. India: Jaypee Brothers Medical Publishers, Ltd; 2006:38.

Yost KG, VanDemark PJ. Growth inhibition of *Streptococcus mutans* and *Leuconostoc mesenteroides* by sodium fluoride and ionic tin. Applied and Environ Microbiol 1978;35(5):920-924.

Zameck RL, Tinanoff N. Effects of NaF and SnF2 on growth, acid and glucan production of several oral streptococci. Arch Oral Biol 1987;32(11):807-810.

Zhao J, Liu Y, Sun W, Zhang H. Amorphous calcium phosphate and its application in dentistry. Chem Cent J 2011; 5(40): 1-7.

Zraikat H.A., Palamara J.E.A., Messer H.H., Burrow M.F., Reynolds E.C. The incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass ionomer cement. Dent Mater 2011; 27: 235-243.