THE EFFECT OF REPEATED MICROWAVE RADIATION ON THE DIMENSIONAL STABILITY OF A SPECIFIC ACRYLIC DENTURE RESIN

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Objective: The premise of this study was to determine the dimensional stability of a polymethyl methacrylate (PMMA) acrylic resin when subjected to multiple sessions of repeated microwave radiation at two different powers, 700 Watts, and 420 Watts.

Materials and Methods: Two groups each of ten standardized denture bases (N=20 in total) were fabricated using PMMA acrylic resin. Points of measurement were marked on the denture bases using a standardized template. A base measurement of each denture bases was recorded. The denture bases were randomly selected into two experimental groups. The first group of 10 denture bases were subjected to two periods of microwave radiation, 700 Watts for 3 minutes in 200 ml of room temperature deionized water. The second group of 10 denture bases were subjected to 420 Watts of microwave radiation for 3 minutes in 200 ml of room temperature deionized water. Measurements of each denture base were recorded after each period of microwave radiation. Dimensional changes were analyzed using a Student's t-test.

Results: All denture bases experienced approximately 1.0-2.0 mm of deformation in all recorded measurements after each period of microwaving. Results were very significant with all t-test having values of $p<0.05$.

Conclusion: It maybe concluded that the denture bases deformed a significant amount under experimental conditions at either 700 Watts for 3 minutes in 200 ml of water or 420 Watts for 3 minutes in 200 ml of water.

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1.0 INTRODUCTION

The introduction of acrylic resin material by Write WH in 1937 revolutionized dental prosthetics. By 1946 acrylic resin had become the most popular material for fabricating dentures. The reasons for the wide acceptance of the use of acrylic resin in the fabrication of denture bases are due to the combination of superior physical and esthetic properties, which improved upon many of the shortcomings of earlier denture base materials, such as cellulous and vulcanite rubber.[1]

Despite improved material properties, the denture bases of today still can serve as a source of infection, and a catalyst of denture stomatitis and localized oral infection for many patients. These alterations of the oral environment, creating lower pH values, decreased saliva flow, lack of mechanical cleansing by the tongue, and acting as reservoirs for microorganisms the denture bases, have been shown to be the major causative factor of denture stomatitis [2]

In denture stomatitis there is a range of severity, from asymptomatic to localized petechiae, to generalized inflammation with papillary hyperplasia. Denture wearers may complain of halitosis, bleeding and swelling, mucosal burning, xerostomia, and dysgeusia.[2] An infection from dentures are usually localized to the oral cavity particularly the palate, but in some cases infection can spread systemically. In untreated cases fungus and bacteria found on denture bases have shown to have spread through the gastrointestinal system, the pleuropulmonary

system, and cardiac system, in the form of subaccute bacterial endocardidis.[3] In extreme cases, immunocompromized subjects can develop a systemic Candida infection known as Candidaemia, which has about a 40% mortality rate.[4]

Given the possibility of localized and systemic infections, the importance of cleaning dentures should not be underestimated. Residual food and plaque can act as resources that can fuel a Candida or bacteria infection, leading to denture stomatitis and multiple papillomatosis. Jagger and Harrison have shown that a large number of people do not know how to clean their dentures satisfactorily. Denture wearers also misuse chemical cleansers which can lead to the deterioration of acrylic dentures.[5]

In addition denture wearers my not be able to adequately clean there dentures properly. Patients in long-term care facilities often are unable to brush their dentures adequately due to poor dexterity, visual acuity, dementia, and disease.[3, 6] Furthermore the denture cleansers that are used may reduce the number of microorganisms present of on the dentures, but they do not completely eliminate them and as such the dentures serve as a reserve to reinoculate the wearer.[3]

Overall opportunistic oral fungal infections have increased in denture wearers. Entrapment of microorganisms in the irregularities in denture bases and denture relining materials, overall poor oral hygiene, and systemic factors are the cause of infection.[7] Several methods of cleansing have been used to try and reduce the presence of microorganisms on dentures. The simplest is improved hygiene and not to wear dentures 24 hours a day to allow tissues to

recover. The next is the use of topical or systemic antifungal and bacterial agents. Agents such a nystatin, amphoterecin B and fluconazol, have been used effectively in the treatment of denture stomatitis. These agents may not lead to the total elimination of the microorganisms that are present. As a result recurrent infection in the denture wearing population is prevalent, and the possibility for selecting of more virulent strains of pathogens exist.[2]

To address the issues of the patient's ability, effectiveness associated with denture cleansers, antimicrobial medications, and increased pathogenicity of select microorganisms; researchers have devised a method of using water and microwave radiation to clean dentures. Dentures can be heated simply in water with a conventional microwave at a low wattage until the water boils. To date there is no exact protocol that should be uses in this method, i.e. amount of water, wattage, or duration of time. Studies suggest that the use of microwave disinfection is an effective way to treat denture stomatitis and reduce the risk of re-infection.[2, 8,9,10]

Microwave irradiation has been toted as a safe, simple, and effective low-cost disinfection method for treating dentures and treating denture stomatits.[11] In vitro studies have given conflicting results as to how microwave radiation affects the acrylic resin denture. When an acrylic resin is subjected to a temperature greater than 71C, the material enters into the range of its glass transition temperature T, and can undergo plastic deformation.[12] Whether significant deformational change takes place is a subject of controversy.[2, 13]

Machado et al state that when denture disinfection method is used, it should be effective for inactivation of microorganisms and it should not have adverse effects on the denture materials itself.[14] Hence the objective of this study, to determine if there are changes in the dimensional

stability of acrylic denture resin base when subjected to microwave radiation under standardized conditions at two varying wattages, 700 and 420 watts.

1.1 CERTAIN PATHOGENS ASSOCIATED WITH DENTURES

Many pathogens, oral and systemic, have been associated with dentures. These pathogens can be found on dentures. Studies have demonstrated that oral pathogens such as Candida spp., Staphylococcus spp., Streptococcus spp., Lactobacillus spp., Pseudomonas spp., Enterobacter spp., and Actunimyces spp. can be found in biofilms on dentures. These pathogens have been associated with such disease as caries, mucosal inflammation and periodontal disease, urinary tract infections, conjunctivitis, pneumonia and meningitis, an addition to systemic infections.[15] In addition, concern has been raised because there can be constant swallowing and aspiration of microorganisms associated with the biofilm on dentures. These microorganisms can have significant effect on the general health of denture wearers, in particular those that are elderly or immunocompromised.[9]

In a study by Fouche et al, they were able to recover C. albicans from all participants, and a case of C. tropicalis. In addition to Candida spp, Fouche et al. recovered Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus salivarius, Streptococcus bovis, Streptococus mitis, Streptococus mutans, Streptococcus pneumonia, Streptococcus salivarius, Streptococcus sanguis I and II, Micrococcus luteus, Micrococcus varians, Staphylococcus epidermidis, Haemophilus influenza and Neisseria meningitides, Neisseria (Branhamella) catarrhalis, Staphylococcus albus (epidermidis), streptococci, and pneumococci. The main species of candida includes C. albicans and C. glabrata as the main infective agents, also C. tropicalis, C. pseudotropicalis, and C. parapsilosis.[16]

The above listing of microorganisms is not a definitive list of pathogens present on dentures by any means, but demonstrates that there are indeed pathogenic organisms of great concern that harbored on and contained with a denture. The concern for it is not for just the denture wearer but also the public as many of these pathogens can lead to communicable diseases

1.2 PATHOGENICITY OF CANDIDA COLONIZATION

Denture stomatitis and associated lesions are most commonly linked with candida infection and oral cadidosis. Other factors such as denture hygiene, trauma, bacterial infection, systemic disease, deficiency of the immune system, permeability of acrylic resin, and denture lining materials can also play a role in the development of such lesions. The physical manifestation of these lesions is caused by the host reaction and is due to opportunistic infection. T-lympnocytes produce cytokines which activate the inflammation pathway, causing an influx of polymorphonuclear neutrophils which limit the spread of infection. For candida infections to progress there must first be a breach in the stratum corneum layer of the tissue, and lack of immune factors such as complement-dependent chemotactic factors, and neutrophils. Underlying factors that may allow for the proliferation of cadidosis include: malnutrition, including iron, folate and vitamin B12 deficiencies, hypoendocrine states, hypothyroidism, Addison's disease, diabetes mellitus, blood dyscrasias such as leukaemia, agranulocytosis, HIV, thymic aplasia, irradiation xerostomia, drug therapy, and Sjogren's syndrome. In general, candida favors 3 groups of people, the very young, very old, and females who are pregnant.[16]

Divisions of candida infection include acute pseudomembranous candidosis (thrush), acute atrophic candidosis (from steroids and antibiotics), chronic hyperplastic candidosis (candida plaques/ leukoplakia), and chronic atrophic candidosis/candida-associated denture stomatitis.[16] Atrophic and hyperplastic forms are most common, and are more often found in women.[17]

There have been several suggested classifications systems for denture stomatitis. The most cited is a classification system devised by Newton in 1962. The system that divides candida associated denture stomatitis infections into three types based on their clinical appearance. Type I is the initial stage of infection defined by the presence of localized pin-point hyperemia. Type II, the most common type, is defined by having diffuse erythema and edema of denture baring areas of the palatal mucosa. The affected area will often extent to the margins of the prosthesis, and there may be accompanying angular chelitis. This condition is usually not painful. Type III is defined by a hyperplastic reaction, known as papillary hyperplasia, the presence of both nodular lesions and atrophic areas found on the palatal mucosa.[16]

There are a multifactorial set of conditions that can predispose a denture wearer to oral Candida infection. These conditions include, diabetes, kidney infections, xerostomia, oral trauma in addition to the candida's pathogenicity. Candida infection triggers both cell mediated immunity and humoral immune response, with the cell mediated immunity protecting against candida infection. Salerno et al. site Pathogenetic theory as to how Candida triggers a host response to degrade tissue through the induction of IgA1 production and amino-peptidases, hyaluronidases, chondroitinases, and neuraminidases. These proteins degrade the oral epithelium, leading to an increase inflammatory exudate that favor yeast proliferation and bacterial colonization.[18] Denture wearers who are susceptible to denture stomatitis have also been found to be deficient in migration inhibition factor and may have overactive suppressor t-cells or T-lymphocyte defects.[16] Results from a study by Golecka et al found that patient with immunosuppression, such as patient who undergo organ transplantation, were more frequently

to have C. albicans infections and denture stomatitis.[19] The use of corticosteroids can predispose patients to Candida and bacterial infections through the reduction of interleukin-2 production, and other medication used as immunosuppressants can reduce T-cell function and cause changes in macrophages and neutrophils, even causing possible neutropenia under long-term use.[20]

Besides host immune factors the phenotypic expression of Candida acts as its own virulence factor inspite of host immune response. Yeast cells are unicellular, eukaryotic organisms that multiply by budding. Cells are separated by septum, and when linked together form a hypha. C. albicans forms true hyphae, whereas C. glabrata, also highly associated with denture stomatitis, has peudohyphae . Adhesion enhanced by transformation from blastoconia to hypha stage and extracellular enzymes including proteinases and phonolipases.[17] Candida spps form soft cream-colored colonies in a pH range of 2.5-7.5 and a temperature range of 20-38 C, and are primarily located on dorsum of the tongue, oral mucosa and within the plaque on teeth.[18,21]

Candida colonization is usually found on the palatal mucosa under complete and partial dentures. It is unusual to find the condition under prosthetics that cover the mandibular mucosa. Associated conditions related to denture stomatitis include angular cheilitis, atrophic glossitis, acute pseudo-membranous candidosis, and chronic hyperplastic candidosis. Patients may exhibit mucosal bleeding, swelling, burning sensation, halitosis, xerostomia, and unpleasant taste.[16] The denture-palatal interface offers a unique ecological niche for Candida colonization due to its relatively anaerobic and acidic environment.[22]

Studies of van Reenen and Budtz-Jorgensen conclude that denture stomatitis is associated with the growth of candida on the denture rather than on the palatal mucosa.[16] Salerno et al. concluded that the permeability of resin is the main factor in colonization, which allows for, a binding of Candida to the host, and effective hyphae penetration.[18] Studies have shown that saliva promotes the adheasion of C albicans to denture acrylic. Salivary pellicle contains a-amylase, high-molecular-weight mucans, lysozymes, ans s-IgA which may serve as receptors for the adheasion of Candida.[23] Overall, saliva reduces adhesion in oral cavity, but mucin, statherins, and other proteins within saliva can act as receptors for mannoproteins, fibrogen, fibronectin, and laminin, present on the surface of C. albicans. Pusateri, Monaco, and Egerton found that when acrylic resin disk were first exposed to saliva for 30 minutes prior to inoculation of C. albicans, the result was significantly greater growth on the disks that were not exposed to saliva prior to inoculation.[23]

Candida adheres directly to the surface, or by means of a layer of denture plaque, to the surface of the denture. Submersion to saliva decreases surface roughness and surface free energy of acrylic resins, explaining studies where resin samples were submerged in saliva first, had less adherence of Candida overall. Saliva shows a cleansing effect, also contains lysozymes, histatin, lactoferrin, calprotectin, and IgA., though also contains mucins, statherin, and proline-rich-proteins which absorb C. albicans and facilitate its adherence. Low molecular weight proteins and there amount are correlated to the amount of Candida present.[7]

Though saliva offers some defense against infection, the roughness and porosity of denture bases can provide a secure substrate for candida. Van Reenen demonstrated in vitro with

fluorescent dye that C. albicans penetrated acrylic resin, and that the unpolished surface, intaglio surface of a denture had greater permeability.[16] Materials with the roughest surfaces usually demonstrate the highest yeast counts. Surface irregularities can serve as reserves for yeast because of surface area for retention, protection fro sheer forces in the mouth and during cleaning.[7] Porosities, roughness, free energy, cell surface mannoprotein and hydrophobicity are factors responsible of Candida adherence to acrylic denture bases.[22] Kumamoto cites that surface attachment causes C. albicans cells to enter into a special physiological state where they are highly resistant to antifungal drugs and express drug efflux determinants CDR1, CDR2, and MDR1.[24]

Surface free energy is one of the main factors related to the development of denture related candidosis.[7] Defined as the interaction between the forces of cohesion and adhesion, predicts whether or not wetting occurs. There is a linear relationship between contact angle measurements on various substratum and C. albicans. Higher surface energy leads to increased hydrophobicity and higher adhesion of microorganisms, though other factors such as cell surface proteins, extracellular matrix, diet, salivary composition and secretion rates, and antibody tiers are all factors in plaque formation, and have confounded data showing a direct correlation between contact angle and Candida colinization68 Acrylic adhesion of Candida to the acrylic surface it is controlled by attractive London-van der Walls forces and electrostatic forces.[25]

Lastly, with regards to Candida and dentures, Fernandes et al. have noted that there has recently been a shift towards non-albicans species, from C. albicans towards C. glabrata as

currently being the main species of yeast involved in denture stomatitis. C glabrata is a fungal pathogen, which exhibits superior adhesion to acrylic dentures when compared to C. albicans, and is now noted as being responsible for 15% of mucosla and systemic cadidosis. Also, there a suggested synergistic relationship between the 2 species.[26]

1.3 BIOFILM STRUCTURE AND FORMATION

Biofilms are unique in that they allow for protected growth of microorganisms in a hostile environment. Films allow for circulation of nutrients and communication between cells. A biofilm may have different areas or heterogeneities within the film where various cells types can exist as groups. Within the biofilm itself, there can be even different genetic expression of cells the cells themselves. A biofilm can be thought of as a living analogous to the tissue of an organ. They develop preferentially on inert surfaces, dead tissue. Biofilms can also be found on living tissue, and medical devices.[27]

Donlan and Costerton define a mature biofilm as a community of microorganisms irreversibly attached to a surface, containing an exopolymeric matrix and exhibiting distinctive phenotypic properties. As such C. albicans is a dimorphic yeast and as such can exist in two different forms based upon the environmental condition in which it is subjected. For example studies by Baillie and Dougless, and Kuhn et al, demonstrate that when denture resin material is used as a substrate on which to incubate C. albicans, there was a layer of cells binding to the substrate, then the above layer exist filamentous cells in the hyphal form that are surrounded by an extensive expolymeric matrix.[24] This is of significance in that the hyphal form of candida is the most pathologic form that the yeast exist.

Colonization of yeast or oral bacteria consists of initial attachment, followed by proliferation and biofilm transformation. The formation of a biofilm enhances yeast resistance to host defense and antimicrobial agents. There is poor penetration of antibiotics into biofilms. Antibiotic therapy has proven to be most effective against planktonic cells but can fail to kill the sessile cells within the biofilm.

The result is recurring symptoms even after several cycles of antibiotic therapy.[27] Roughness of polymers and hydrophobicity of yeast cells is a possible explanation to the prevalence of Candida found on acrylic dentures.[28] In general, yeast cells have high potential to adhere to dental resin, and are known to be extremely resistant to many disinfectant measures. Adhesion of microorganisms to denture surfaces is accomplished through the use of a polymeric matrix around colonies.[4] Mechanical cleansing and chemical disinfection have been recommended methods to reduce yeast bio-burden.[4, 29]

The matrix itself is not an impenetrable barrier to the diffusion of antifungal drugs, rather it helps to protect the cells from dislodgement, and creates a nurturing environment that promotes growth. From what is currently understood, actual antifungal resistance is based on a cell's phenotype. Ramage et al. disrupted biofilms and resuspended the organisms, only to find that the antifungal resistance that the organisms exhibited in the biofilm was that same when they exist in a planktonic form, demonstrating that the antifungal resistance is related to phenotype and not protection from the exopolymeric matrix. What is not understood is what mechanisms allow for increased phenotypic resistance once the cells have been part of a biofilm. They demonstrated that C. albicans cells growing in a biofilm were 100-fold more

resistant to fluconazole, and 20 to 30-fold more resistant to amphotericin B when compared to platonkic cells. Antifungal resistance is at some point established once cells become part of a biofilm. Speculation is that this resistance is triggered through contract-dependent gene expression. Also shown through these studies, C. albicans is the most pathogenic organism of the Candida group, and produced the most extensive biofilm structure.[24]

Ramage et al found also that flow is the key factor in how a C. albicans biofilm will develop. Biofilms that develop under flowing rather than static conditions are more tenacious, having greater exopolymeric matrixes. The result is that biofilms that develop in flowing conditions such as in the circulatory system are harder to resolve compared to those that develop under more stagnant conditions such as under a maxillary denture.[24]

Microorganisms interact with each other by using end products of one another or communicating through cell signaling molecules. Quorum sensing is one such phenomenon in which a genetic response in one species is triggered when small molecules are released in sufficient concentration from another species. This was demonstrated in as study of Hogna Vik and Kolter in which molecules released in sufficient quantity in a bacteria-candida biofilm triggered candida to differentiate into the hyphae stage.[7]

1.4 PREVALENCE OF DENTURE STOMATITS

It has been noted that denture stomatitis is the most common form of oral candidal infection with incidence of about 65% of denture wears affected.[4,30] Studies have shown that oral candidiasis is present in about 60-72% of denture wearers. C. albicans is the most common species responsible for about 70% of infections.[8,29] Ribeiro et al examined the microorganisms present on the dentures of 90 individual denture wears with C. albicans being the microorganism most frequently found, on 65.5%, followed by Streptococcus Mutans 53.3%, and Staphlococcus aureus 34.4% of dentures respectively.[31]

Denture stomatitis can be non-symptomatic, or it may present with mucosal bleeding, swelling, burning, halitosis, unpleasant taste, and xerostomia. Newton classified that all three types can exist simultaneously.[32] Acrylic dentures are the predisposing factor for oral candidosis, and act as reservoirs for infection. High salivary yeast counts are much more common in complete denture wearers then dentate individuals. Olsen found that that yeasts were present in 78-100% of patients with denture-induced stomatitis, in contrast to only 30-60% of the dentate non denture wearing population. In a study of 110 edentulous patients, denture stomatitis in Type II diabetics was much higher than non-diabetics 57.3% vs. 30%.[33] Also higher was burning mouth, xerostomia, angular cheilitis, and glossitis.

There is a higher prevalence of denture stomatitis after the sixth decade of life due to an increased number of complete and partial denture wearers. There is a predominance of females vs. males who have denture stomatitis. This is possibly attributed to hormonal changes especially after menopause.

The age of denture is a factor also. Study by Zomorodian et al found that only 25% of denture wearers whose denture was less than a year old experienced denture stomatitis, whereas >84% of denture wearers whose denture was over 5 years old had denture stomatits.[11]

2.0 A BRIEF HISTORY OF DENTURE DISINFECTION

A traditional method for disinfection and cleansing a denture is through brushing with soap and or a dentifrice. Patients at times can lack the visual acuity and manual dexterity needed to remove the biofilm present.[2, 4] As a result, several other methods of disinfection have been recommended and developed, chemical solutions such as, sodium hypochlorite, glutaraldehyde, and chlorine dioxide. Drawbacks of these solutions include discoloration/bleaching from sodium hypochlorate, cyctotoxicity from glutaraldehyde, and corrosive effects of chlorine dioxide.[14, 34]

Using a sodium hypochlorite soak has historically been the major method of disinfecting dentures. Ghalichebaf, Graser and Zander 1982 tested four agents for removing plaque and found that the agents with the highest sodium hypochlorite content were most effective. Rudd 1984 demonstrated a disinfectant effect when a 5.25% sodium hypochlorite solution was used. Furthermore Moore, Smith and Kenny 1984 found that hypochlorites could be used to eliminate denture plaque after only short term exposures. In 1992 Basson, Quick and Thomas reported that a solution of 4.0% Milton (a mixture of sodium hypochlorite 0.04% plus sodium chloride 0.66%) was highly effective[13] Another protocol using a bleaching solution proposed by Pavarina et al. is to scrub the dentures first with 4% chlorhexidine and then immerse the dentures in a 3.78% sodium perborate solution at 50 deg C for 10 minutes.[14, 35]

In a study by Vieira et al when comparing the efficacy of decontamination of PMMA denture resin using 2 alkaline peroxides for 3 and 15 min, 0.5% sodium hypochlorite for 10 min, and distilled water as a control for 15 min, it was found that Sodium hypochlorite was the only treatment that removed al viable cells from the denture surface, thereby completely eliminating C albicans, and C. glabrata.[6]

Lin et al. found that chlorine dioxide, Alcide LD, was a more effective disinfectant then 5.25% sodium hypochlorite and iodofive. Although chlorine dioxide was the most effective in the study, it still did not fully disinfect the denture resin tested.[36] Study by Jose et al, demonstrated that denture cleansers such as Boots Smile, EDTA and sodium perborate, Medical Interporous, EDTA and sodium biocarbonate, Steradent, Tetraacetylethylenediamine solution and carbonate peroxide, sodium hypochlorite (1.5%), and sodium hydroxide (1.7%) proved to be effective in disinfection and removal of C. albicans, yet these cleansers left a residual layer which could cause regrowth and colonization.[37]

C. glabrata may exhibit higher adherence to denture surfaces and greater acquired antifungal drug resistance. Adhesion of Candida depends initially of denture surface roughness and may be indicative of pathologic potential. Recently, Ferreira et al found that C. glabrata had greater counts compared to C. albicans when denture resin samples were exposed to either a commercial enzymatic cleanser solution, or a 0.5% NaOCl solution. There study found that only the 0.5% NaOCl solution was effective at disinfecting samples inoculated with either C. glabrata or C. albicans.[38]

Other chemical soaks included alcohol-base disinfectants and chlorhexidine gluconate. Alcohol-based disinfectants have been found to reduce the flexural strength of non-crosslinked denture base acrylic resin, whereas staining is a major side effect by chlorhexidine. Chlorhexidine is a cationic chlorophenyl bisbiguanided which binds to negatively charged surfaced. It has a broad spectrum of antimicrobial activity, including Candida spp. Chlorhexidine has been proved to be more effective than nystatin or amphotericin B in killing adherent cells on denture bases.[23] Pavaria et al found that when they disinfected dentures either using a 10 minute soak in 4% chlorhexidine gluconate, 1% sodium hypochlorite, Biocide (iodophor), or Amosan (alkaline peroxide), that 4% chlorhexidine gluconate, 1% sodium hypochlorite, and Amosan solutions were effective in reducing the amount of microorganisms, whereas the Biocide solution showed positive growth.[35]

McCourtie, MacFarlane and Samaranayake found that a chlorhexidine 2% for 30 minutes was effective at reducing adherence of C. albicans on denture acrylic by 85% Organisms with greater capacity of adherence to denture resin were killed more easily with lower concentrations of chlorhexidine. They suggested that soaking dentures for even 10 minutes in chlorhexidine should be effective enough to reduce levels of C. albicans for 70-100% based upon their findings.[39] In a second study McCourtie, MacFarlane, and Samaranayake found that when acrylic strips were coated with either saliva or serum solution, chlorhexidine gluconate 2% reduced adherence of candida between 19-86%, including C. albicans, C. tropicalis, and C. glabrata. They found that the inhibition of yeast adherence continued up until 19 days after exposure to the chlorhexidine.[40] Chlorhexidine gluconate has been shown to

cause discoloration of artificial teeth. Sodium hypochlorite associate with bleaching of denture base, corrosion of metal frameworks, and changes in flexural strength and hardness of the resin bases. Chlorhexidine leads to antiseptic resistance of infective organisms over time. Also quotes Consani et al 2007 study, no harmful effects in the adaptation of denture basses to supporting tissues.[41]

One way to target C. albicans is to removed Efg10, which is a regulator of hyphal morphogenesis. Prevents hyphal form in biofilms.[24] The use of antifungal denture materials has been proposed to control Candida induced denture stomatitis. Dentures are impregnated with medications such as miconazole or chlorhexidine digluconate in high antifungal concentrations that can be delivered to the underlying mucosa reducing concentrations of viable Candida and bacteria.[42]

In a study by Cao et al, they demonstrated that the use of antifungal medications in denture material could serve an effective means of delivering medication directly to the effected site for prolonged periods of time (weeks to months). In their study miconazole or chlorhexidine degluconate were covalently bound to PMAA-resin disk during curing. The advantage is not only long delivery, but also rechargability.[42]

Treatments for candida infections included Triazole drugs such as fluconazole and itraconazole, with resistance emerging after long-term treatment.[8] Other treatments include the use of other topical against such as nystatin, chlorhexidine, and miconazole. Though these treatments can be effective, they do not treat the prosthesis itself, which serves as reservoir for

continued infection.[4, 8] Salerno et al. suggest simply to remove the dentures for 2 weeks during duration of therapy, and not to use nystatin and chlorhexidine at same time, as they negate the effects of one another.[18]

Nystatin is another antifungal commonly used for treatment of C. albicans. Has a high sugar content which can contribute to caries especially in patient who have diabetes, and xerostomia. Mycelex troches are another alternative but are expensive and must be used 5 times a day, so there is failure with patient compliance. Nizoral and Diflucan have been linked to liver problems, and liver enzymes should be monitored. Chlorhexidine 24 days overnight eliminates C albicans. Germicdal solutions such as benzoic acid, alkaline hypochlorite have been effective, but are not completely rinsed off and can be absorbed by the acrylic.[43] The substrate used in the study was Lucitone 199!

Budz-Jorgensen, Holmstrup, and Krogh determined in their study at fluconazole is a safe and well-tolerated antimycotic drug, but even they noticed that the drug was not completely effective in resolving Candida infection under maxillary dentures. At the time they contributed it to drug concentration, but since then fluconazole has proven to be one of the less potent medications in treating oral Candida infections.[44] Ellepola and Samaranayke examined sub-lethal dosages of antifungals, nystatin, amphotericin B, %-fluorocytosine, ketoconazole and fluconazole, on the premise that these antifungals are diluted in the oral environment. They found that of the 7 types of C abicans tested, they could be controlled with minimal exposure to antifungals, only 4-8 treatments at minimal dosage with specimens being rinsed between applications. The Candida was not eliminated but could be reduced.[33] Similarly Dorocka-

Bokowska, Konopka and Duzgunes found that sub-lethal dosages of polyenes, amphotericin B, nystatin, and natamycin were used that there was a marked decrease of yeast adherence, most notably for amphotericin B with 50-60% reduction of adherence for free floating yeast, and 2-10% of adherent yeast.[19]

A study by Marcos-Arias et al examined the antifungal activity of essential oils. They found that of the oils tested, carvacrol, eugenol, geraniol, linalool, and terpinen-4-ol were active against oral Candida isolates, including isolates resistant to fluconazole. The essential oils may be a promising alternative current medications used topically in the treatment of oral candidiasis.[45]

Another recent treatment is the possibility of using histatin 5 which is secreted by the major salivary glands in humans. In a study comparing histatin 5 to fluconazole, Konopka et al found that histatin 5 exhibits antifungal activity on C albicans and C. glabrata biofilms on denture acrylic, whereas biofilm associated candida is highly resistant to fluconazole with on 20% inhibition of C albicans, and 30% inhibition for C. glabrata respectively.[46]

Dentures produce ecologic changes in the oral mucosa that facilitate the proliferation and colonization of microorganisms in particular yeast 56 Denture stomatitis has been shown to be treated with phototherapy, and it is an effective method for reducing Candida spp. on dentures.[22,47] In a study by Mimi et al they were able to resolve denture stomatitis in 4 of 5 patients using phototherapy consisting of application of Photogem followed by illumination with a blue LED light (455 nm at 37.5 J/cm2).[47]

3.0 PROPERTIES OF ACRYLIC DENTURE RESIN RELATING TO MICROWAVE IRRADATION

Most prosthetic acrylic resins consist of polymethyl methacrylate (PMMA) with additional copolymers like polybutylmethacrylate or butadiene styrene. Crosslinking agents such as glycol dimethacrylate, rubber, or fibers are added to improve toughness, impact resistance and to prevent crack propagation[6].

Denture fracture has been attributed to either impact or fatigue failure. According to Kelly, factors influencing fatigue strength include frenum notches, surface irregularities, and foreign body inclusions. Porosities and residual monomer content have been shown as important factors influencing flexural fatigue strength. The processing technique use to polymerize the denture base resin has also been found to be an important factor, in that it can induce stress into the denture base during processing.[1]

The underlying causes for denture fractures may be difficult to determine due to the number of variables including denture function, processing, and handling., porosity residual monomer, cracks, and poor adaptation of the prosthesis.[48] Fractures in the maxillary denture base occur along the midline, and can be related to poorly balance occlusion, problems in design and production, poor strength of repair materials, and inherent stress in the base itself. Outside fractures occurs usually as impact accidents, such as when the denture dislodges from the mouth, as for example, during coughing. Internal fracture occur from excessive bite force and

an improper occlusal plane, high frenal attachments, lack of balance occlusion, poor fit, and limitations of the denture material. Midline fracture is the result of flexural fatigue caused by cyclic deformation of the base, as flexure of the maxillary denture base most often occurs along the midline.[49]

Ultimate flexural strength is important as it reflects a materials potential to resist failure under a flexural load. Dentures require high flexural strength, as alveolar resorption is a gradual irregular process that leaves the tissue-borne prostheses unevenly supported. Denture materials should also have a high proportional limit to resist plastic deformation. Fatigue resistance is also desirable to avoid fracture from repeated masticatory loads.[50]

In subjecting dentures to postpolymerization microwave irradiation has been found that this irradiation can be an effective method for increasing the flexural strength of dentures and denture liners through reducing the residual monomer content by further polymerization at free radical sites. Microwave energy has also been used for polymerization, having the advantage of reduced time for curing, a smaller time for obtaining the plastic phase, a larger homogeneity of the mixture, resulting in improved mechanical strength and excellent adaptation. It has been reported that microwave polymerization involves heating the resin monomer only, not the polymer, which allows a restively low processing temp around the resin, resulting in little residual monomer and good dimensional accuracy. Microwave irradiation of autopolymerizing acrylic resin after polymerization decreases the residual monomer by 25%, with an overall increase in flexural strength, impact strength and glass transition temperature

of the material. There is a higher degree of polymeric conversion and a minimal level of residual monomer leading to reduced toxicity.[51]

Yet there are deleterious effects that can be noted from microwave postpolymerization irradiation. Seo et al. noted dimensional changes could be found in the acrylic in both intact and relined denture bases. Pavarina et al found decreased flexural strength of a hard chair side acrylic resin reline material. Campanha et al noted a decreased Vickers hardness of acrylic denture teeth.[35, 52, and 53]

When using postpolymerization irradiation to heat acrylic resin water to boiling, Machado et al found that specimens of Lucitone experienced a breakdown of their surface layer. The resultant effect was microcrazing and loss of integrity, though in their study they did not find hardness was affected by the irradiation.[54]

4.0 PROCESSING OF ACRYLIC DENTURE RESIN

Processing is important in that if residual monomer remains it can affect the mechanical properties of a resin denture base, by acting as a plasticizer. Residual monomer can leach out of the denture base and is a potential irritant of the oral mucosa, eliciting irritation, inflammation, and allergic response.[52] Residual monomer after heat processing should be very low. If standard procedures are followed for the processing of Lucitone 199 denture resin, residual monomer should be less than 2%. It is suggested that a terminal boil of 1 hour is used to produce the maximum amount of monomer conversion. 30,31. If a further protocol is used where the denture bases are stored in water for at least 24 hours prior to testing, the residual monomer should be reduced even more, resulting in an more stable denture base. Studies by Yunus et al and Polyzois et al found that the effect of microwave treatment post-polymerization was favorable in improving strength of acrylic resin.[52, 55, 56]

Residual monomer is known to have a plasticizing effect and reduces the polymer interchain forces. In such cases, deformation occurs more easily under loading. Residual monomer can be reduced by further polymerization, though the diffusion of unreacted monomer molecules out of the resin. These are both temperature dependent.[14] Lower deformation values also happen after resin is stored in water for a time and excess monomer is allowed to leach out.[9, 14]

Residual monomer can be directly related to the flexural fatigue strength, Banerjee et al in their study found that, water-bath polymerization yielded the lowest values of flexural strength, supporting the hypothesis of Kelly, Reitz, and Declark, that the residual monomer

content and porosities in denture base resin can lead to imperfection and the formation of stress propagation, making the resin base brittle.[1] A study by Blagojevic and Murphy used water bath polymerization with a 3 hour terminal boil producing superior properties, indentation hardness, impact strength, and Young's modulus of elasticity. Water bath curing with long terminal boil suggest low residual monomer and a higher overall glass transition temperature.[57] Microorganisms initially adhere to denture surface and subsequently penetrate into dentures via a complex of pores and tracks that are formed during the release of gases during polymerization.[58] In a study by Kalla, Rao, and Kumar where they examined adherence of C. albicans on 4 types of PMMA denture base resins, heat cured, light cured, and self cured, the heat cured Lucitone 199 demonstrated the lowest adherence of C. albicans. It was hypothesized that irregular topography, related to porosity and length of curing time, lead to greater adherence of C. albicans.[59]

Residual monomer is found dependent on curing conditions.[60] Blagojevic and Murphy for that water-bath polymerization with a long curing cycle and a 3-hour terminal boil produced superior properties. They also noted that consistently superior results were produced by simply following the method of use recommended by the material manufacturer. It was found that using a water-bath enhanced the degree of polymerization resulting in a lower level of residual monomer and an increased glass transition temperature, T(g). In particular if a 3-hour boil was accomplished.[57] Curing method influences the amount of residual monomer in acrylic resin. Bayraktar, Guvener, and Uresin using liquid chromatography, that when comparing heat, autopolymerized, and microwave energy curing methods, that the lowest amount or residual

momomer was found using heat curing with a log-term terminal boil and then stored in distilled water for at least 1 day. As for autopolymerized and microwave cured resin, levels of residual monomer were higher than heat cured acrylic with terminal boil until a month in storage in water. They were stored at 37 deg C and not 23 deg C.[61]

Heating during chemical and microwave disinfection may enhance further polymerization and release of residual monomer.16 Dogan et al showed that a longer curing period at 100 C decreases the level of residual monomer. Harrison et al. showed that the highest level of residual monomer was found when the material was the weakest.[51] Shrinkage rather then expansion might be expected because of release or residual monomer also found in Seo et al., and further changes may occur from release of stress in the resin.[10]

The terminal boil is very important in reducing residual monomer. Urban et al. found that even when a short polymerization cycle is done, that if a terminal boil is done after that, residual monomer was found to be less than that of the long cycle for Lucitone 550 acrylic resin.[52, 62] Harrison and Huggett 1992, evaluated the effect of polymerization cycle on residual monomer and fount that terminal boils dramatically decreased the amount of residual monomer.[63]

Acrylic denture bases change dimension, before, during, and after processing. Size, shape, and thickness can influence the dimensional changes of denture bases Denture base adaptation is affected by linear shrinkage of acrylic resin, dimensional changes that occur during and after processing, and by the posterior palatal border opening, dependent on the size and shape of

the palate.[64, 65] Slow processing is recommended to avoid high residual stress generated by thermal expansion differences between the plaster mold and denture base. Duymas and Yanikoglu found that acrylic resin samples that have undergone slow curing at a lower temperature and had less thickness had the least amount of change in dimension and weight due to water sorption.[65]

The amount of dimensional change in denture resin can be influenced by, different polymerization techniques and thickness. Techniques can include the types of material used, resin, stone, method of packing, type of processing, microwaved, heat cured, use of a terminal boil, and method of cooling and deflasking. Variation in base thickness can lead to differences in internal stress within the denture base, which can change a dentures adaptation and stability. A study by Miessi et al. examined dimensional stability of denture resin bases. They found that the dimensional change of denture base to stone cast was influenced by the storage period, and the greatest amount of change was found on the palate region for all groups. Furthermore all resin bases had dimensional change. Denture bases polymerized by conventional methods, heat cured over microwaved, presented the greatest change overall in the palate region.[66] Patil et al. found that microwave heating is independent of thermal conductivity, in that it uses dielectric heating which raises the temperature rapidly, the inside and outside of the material are equally heated. In their study additional heating radiation was found to be beneficial in the heat cured resins in improving dimensional accuracy of the denture base.[51]

According to Anusavice, whenever dimensional alterations are inhibited, as when dentures are flasked and processed, internal stresses are introduced. During reheating, internal stresses may relax as the material approaches its glass transition temperature. The temperature range for distortion of polymethyl methacrylate ranges from 71-91 deg C.[12] The glass transition temperature, T(g), is the temperature at which larger polymer chains are able to move rather freely within the polymeric mass. The result is that the material transforms from a brittle solid to a rubbery solid. Dependent plasticizers, and moisture content of the material, an increase in polymerization can lead to an increase in T(g) (Phoenix et al).[10,67,68] The T(g) of PMMA is approximately 100.4 deg C and hence boiling water could adversely affect denture bases exposed to such heat, produced with microwave irradiation. Hence, a combination of the lowest power for the least amount of time is recommended.[4] Final conversion of methyl methacrylate with an ethyleneglycol dimethacrylate cross-linking agent decreases with the increasing content of the cross-linking agent. Ridged polymer structure hinders conversion of MMA, even though glass transition temperature is reached further conversion of MMA does not take place.[51]

Microwave polymerization technique was introduce by Nishii et al, and in several studies have shown that microwaved denture bases have comparable physical and mechanical properties to conventionally processed denture bases. Improved properties of microwaved denture bases include reduced porosity, and better dimensional accuracy.[1] Ganzarolli et al. examined the internal fit of 3 heat cured acrylic denture bases, 2 conventionally flasked, and one injection molded (Classico, Onda Cryl, and PalaXpress). They used the same method of

measurement as Foat, Rodregues, and Cury, and found that after 30 days of storage in deionized water there was better adaption to the metal model. The best adaption was found to be from the injection molded acrylic.[67]

Consani et al state that maxillary complete denture base adaptation can be improved through microwave disinfection using a traditional flask closure method. They compared traditional flask closure and restriction closure that uses 2 additional iron plates on each side of the flask to maintain pressure after release of the denture press. They measured gap between the acrylic base plate and the cast on which it was prepared using an optical micrometer. They found that denture base adaptation was improved after microwave disinfection in the region just distal to the canines and the posterior palatal seal. From their study they found that acrylic resin after microwaving has a better fit.[64]

5.0 THE USE OF MICROWAVE DISINFECTION

Dentures are a foci of infection and reinfection. Monitoring the denture due to its infectious properties is perhaps more important than treating the oral mucosa. It has been suggested that C. albicans on denture biofilms and not the mucosa is the causative factor of denture stomatitis.[53] In disinfecting dentures when using a chemical soak, chemicals can be absorbed through the porosities on the surface of acrylic denture resin. These chemicals are not fully eliminated upon rinsing, and can subsequently be introduced into the mouth after the dentures are removed and inserted.[14, 54] Dentures serve as reservoirs for microorganisms and may become deeply colonized and infected.[14] In a study by Glass et al, they found that when samples were taken from dentures in vivo to identify the amount of microbial contamination, they found that the depths of most dentures were more contaminated than the surface, and that the distribution of bacteria differed anteroposteriorly. Posterior samples were found to be more heavily contaminated than anterior samples. This is consistent with greater hydraulic pressure in the molar regions during mastication. They also found that there was substantial denture contamination after only 24 hours, and in some case after only 8 hours. When contaminated dentures are placed in the mouth this allows for continuous reinfection on a daily basis.[58]

Tissue conditioners are used to help denture bearing tissues recover from the trauma of ill-fitting dentures. These liners in dentures have been associated with candida growth and in a study by Makila and Hopsu-Havu reported that candida can be present in as high as 85 percent of dentures.[16]

In addition to infection of the individual, there is also concern on a communal level of infection when dentures are out of the mouth, namely in the dental office and dental lab. Several articles site that proper disinfection of dentures is important to prevent cross contamination in dental office and dental lab.[10,14,34,53,54,68] Powell et al state that the majority of materials sent from the dental clinic to the laboratory are contaminated with pathogenic bacteria.[64]

A study by Kahn, Lancaster, and Kate demonstrated that microorganisms could be found in pumice slurry from contaminated prosthetics that were polished without prior disinfection, and that these microorganisms could be transferred from one prosthesis to another during finishing and polishing.[68]

Dental personnel may be at risk of contracting infection if prostheses have not been properly disinfected. Due to the infectious nature of dentures and the possibility of cross contamination in the dental laboratory environment, major research efforts have been focused on a simple inexpensive method of disinfection that does not have the deleterious effects associated with chemical soaking, that guards against the development of antimicrobial resistant microorganisms.[15, 47]

5.1 A BRIEF HISTORY OF USING MICROWAVE IRRADIATION IN THE DECONTAMINATION OF DENTURES

Oral problems related to poor denture hygiene need to be addressed with a disinfection protocol that is effective, clinically viable, inexpensive, and is easy to comply. Chemical disinfection, afore mentioned, is commonly used, soaking dentures in alkaline glutaraldehyde, sodium hypochlorite, aqueous formaldehyde, or enzymatic solutions. Recently, microwave irradiation has been considered as a means for denture disinfection. Unlike chemical solutions, it requires no special storage, has no expiration date, and does not induce resistance to C. albicans or other microorganisms.[69] Microwave energy has been shown to be an effective method to disinfect complete dentures and prevent cross contamination, and It has been recommended in the treatment of denture stomatitis.[2,12,34,68]

Initially microwave irradiation was used to disinfect laboratory dishes and eventually medical devices. Hume and Makinson in 1978 were the first to publish about the potential of using microwave irradiation to disinfect against microorganisms. Sanborn, Wan, and Bulard reported that microwave sterilization could be used in the sterilization of plastic tissue culture vessels intended for reuse. They were able to sterilize against Escherichia coli, Psedomonas fluorencens, Klebsiella pneumoniae, Proteus vulgarus, Sarcina lutea, Corynebacterium equi, Bacillus alvei, bacillus globigii, and Streptococcus faecium. Also viruses polio type 1, parainfluenza type 1, and bachteriophage T4.[70]

Wan and Bulard 1982, Young et al 1985 disinfected medical instruments, Rohrer et al 1986 hydrophilic contact lenses, Griffith et al 1993 polyethylene catheters. Microwave irradiation has also been shown to be effective in the decontamination of food.[43]

One of the first to subject dentures to microwave irradiation was Rohrer and Bulard 1985 who used a 3D rotisserie. They demonstrated that when acrylic dentures contaminated with C. albicans were attached to a 3D rotating device, disinfection could be achieved after 8 min of microwave irradiation. Furthermore they demonstrated that dentures that were inoculated with C. albicans could be disinfected after 10 minutes of microwave irradiation.[1]

Webb et al 1998 was able to sterilize acrylic dentures at 2 min at 650 Watts, they also suggested from their study in comparing microwave verses chemical disinfection that microwaving dentures at a medium setting for 6 min may be a more effective method of denture disinfection than soaking for 8 h in a 0.02% sodium hypochlorite solution.[13]

Griffith et al. 1993 used as standard household microwave 650 Watts at 2, 4, 6, and 8 minute settings. They showed that disinfection was achieved after 6 minutes of microwave radiation. Furthermore they introduced the concept of using a rotating platter, and a small beaker of water that acts as a heat sink to avoid overheating.[13]

Neppelenbroek et al 2008 used a domestic microwave irradiating the dentures in 200 ml of sterile water 3 times per week for 30 days at 650 W for 6 min.[2] Rubeiro et al 2009 in there clinical study, where they swabbed the palates of maxillary dentures, found that they could

achieve complete disinfection of dentures subjected to microwave radiation for 3 min at 650 W, and disinfection for 2 min at 650 W.[15]

Altieri et al. 2011 demonstrated that microwave disinfection can be effective in the elimination of methicillin-resistant Staphylococcus aureus (MRSA) biofims. In their experiment Altieri et al contaminated 36 simulated complete dentures with MRSA and divided them into 4 groups, 1 control, and 3 test, of which one was soaked in 1 percent sodium hypochlorite for 10 minutes, another 2 percent chlorhexidine gluconate for 10 minutes, and the last microwave irradiation 650 W for 3 minutes. The results were that soaking the dentures in chlorhexidine gluconate, and microwave irradiation resulted in complete disinfection of all dentures both in short and long term measurments.[41]

5.2 VARIOUS MICROWAVE IRRADATION DISSINFECTION STUDIES: METHODS AND PROTOCALS

Developing a protocol for using microwave irradiation to disinfect dentures has thus far been a process of trial and error. The protocol must call for irradiation that is intense enough for disinfection, but is not so intense that the irradiation treatment is deleterious to the acrylic denture resin. In general higher wattages were used in earlier studies, and lower wattages more recently. There is yet to be fond a protocol that protects the denture against possible harm, and is still effective for disinfection.

Initally, Rohrer and Bulard were able to disinfect dentures inoculated with four bacteria and 1 fungus after 10 min of microwaving at 720W. In their experiment the denture was microwave in a dry state by simply placing the denture in the microwave and subjecting it to irradiation. Subsequent experiments by other researchers incorporated introducing a glass beaker containing water to act as a heat sink to protect the dentures. Eventually researchers placed the denture in a beaker containing water, resulting in increased protection of the denture, more effective heating, and lower wattage and reduced time necessary to disinfect the denture, as was the case in a study by Silva et al. They found that they could disinfect dentures against S. aureus and C. albicans using a reduce protocol of 6 min of microwave irradiation at 650 watts.[34] This study was one of the first to incorporate the use of 650 watts of microwave irradiation.

The use of 650 watts has been used in several studies and is actual protocol for the disinfection of dentures using microwave irradiation. A complete protocol could be defined as to subjecting dentures to microwave irradiation at 650 watts for 3 minutes in a glass beaker containing 200 ml of water in order to achieve disinfection.

Silva et al carried out microbiologic and clinical assessment of dentures subjected to microwave irradiation at 14, 30, 60, and 90 days using a 650 watt, 3 minute protocol. They demonstrated that this protocol was as effective as topical antifungal therapy using nystatin in the treatment of denture stomatits.[11] Dovigo et al were able to disinfect against P. aeruginosa, and S. aureus after 3 min at 650W.[34] Also using the 3 min 650 W protocol, Sanita et al were able to effectively disinfect dentures inoculated with 5 different species of candida.[68]

Webb, Thomas, and Wittle 1998, did an early study using low wattage, 350 watts. Their study was a 2 year clinical study of sixty complete denture wearing subjects. They examined the efficacy of an overnight sodium hypochlorite denture soak (0.02% Milton's Solution) verses microwaving 350 W for 10 minutes with a water heat sink for the denture. Both methods were used nightly for the duration of 1 week. The results were that both methods greatly reduced the amount of candida and aerobic bacteria on the denture and palate, but did not completely eliminate all candida or aerobic bacteria on either the denture or plate.[32]

After the study by Webb, Thomas, and White, the use of low wattages 350 watts was abandoned due the possibility of not being effective enough to clinically disinfect dentures. Though recently, a study by Senna et al. found that when sterile resin disk were inoculated with C. albicans, then subjected to microwave radiation at 450 watts for 300 minutes at, no viable

cells were found when an enzymatic denture cleanser, Polident, or distilled water was used was used.[71] The significance is that it has now been shown that a lower wattage 450 W, has been proven to remove C. albicans. The question now is, if 450 watts is ideal for disinfection against microorganism, and if so is the acrylic resin of the denture affected dimensionally?

Sesma et al tested the temperature of dentures exposed to different microwave protocols, at 700 W for a duration of 3 and 6 minutes respectively, and under two conditions, being microwaved in a beaker containing water, or microwaved without water. They found that when dentures were microwaved in water that they had significantly higher temperatures then dentures that were microwaved under dry conditions. They also found that there was no difference in temperature between heating dentures for 3 or 6 minutes in the microwave.[12] Their study points to more efficient heating when the denture is in water. It also shows that at 700 W the maximum time need to reach the maximum temperature of the acrylic is 3 minutes. They also go on to point out, that various studies have shown significant changes in internal adaptation, surface roughness, hardness and dimensional stability when dentures are subjected to microwave radiation. They also point out that there are other studies have not found this to be the case. To credit why such wide results have been found in various studies, it is noted that two factors have been key, the ratio of time to irradiation power, and whether the acrylic resin is irradiated under water or not.[10,12]

Various other studies include Banting and Hill, who used an 850 Watt for 1 min. They found in there study that by using this protocol that the irradication of C albicans was more effective then soaking in a 0.2% solution of chlorhexidine.[54] Banting and Hill 2001, in addition to Webb

et al 1998, Mima et al 2008, and Ribeiro et all 2008, Ribeiro et al demonstrated that flexural strength and hardness of different acrylic resin specimens were not detrimentally affected by microwave irradiation at 1,2,3,4 or 5 min intervals under a wet conditions.[15,54] Mima et al found that when specimens wire irradiated for durations of 3, 4, and 5 minutes, complete disinfection was achieved, whereas after 2 min, specimens inoculated with C. albicans were not disinfected. They concluded that 3 minutes of microwave irradiation at 650 Watts is sufficient for disinfection.[54]

5.3 THE MECHANISM BEHIND MICROWAVE IRRADIATION AND STERILIZATION OF DENTURES

Although the mechanism behind the lethality of microwave irradiation has not been fully elucidated, microwave irradiation has been shown to be effective in vitro against the cells of C. albicans, and the cells contained in young (24 hour) biofilms. Effectiveness is best achieved when irrigation of the microorganisms has been performed through introducing the contaminated denture into water by full immersion.29 Water effects the coagulation and denaturation of proteins, while dry heat promotes oxidation of organic components of cells, as such, moist heat is more efficient at killing microorganisms than dry heat.[54]

It has been demonstrated in studies by Dixon, Fredrich, and Baysan, that candida can survive under dry microwave conditions, but when dentures are immersed in water, the use of microwave irradiation appears more effective[2] Fitzpatric et al speculated that disinfection by microwaves is only possible when a specimen is sufficiently moistened as water acts on the coagulation of essential proteins in microorganisms during sterilization. Studies by Dixon, Breeding, and Faler 1999, Neppelenbroek et al 2003, and Silva et al 2006, have shown that when specimens are immersed in water they are more likely to experience disinfection.

The lethal action of microwave radiation is not fully understood, it has been shown that water osmotic pressure can disrupt cells. Since distilled water is used in most of the literature, there is a hypotonic relation to the solute content of the cells which contributes to and influx of

water into the cells. In addition heat has been attributed to the lethality of the microorganisms, producing alterations in the structural integrity of the cell membrane and cell metabolism.[30] Rosaspina et al suggest that there are also non-thermal effects also from the electromagnetic field created by the microwaves and the interaction with the cells, in which there are molecular and mechanical interactions. The molecules of water act in several ways, absorbing the energy from microwave radiation, and the friction between molecules creates heat increasing temperature.[2,15,54] Non-thermal effects of microwave irradiation may be resultant from the selective spectrum of absorption of microwaves by different molecules such as nucleic acids, proteins, and lipopolysaccharides. In addition, the volume and composition of the surrounding liquid can modify the effect of the microwave radiation, and the presence of charged molecules in a high frequency field can lead to lethal effects.[54]

Microwave disinfection may work from the heat generated during microwaving, or possibly the killing of microorganisms resulting from the interaction of the electromagnetic field with the molecules of the cells and surrounding liquid.[4] The destruction of microorganisms below the thermal point have been observed. It is possible, depending on composition, that individual cells may be selectively targeted. Based on the relative composition of cells, including ionizable compounds, cells may absorb thermal radiation from microwaving at a greater rate than the surrounding liquid, which may account for this phenomenon. Furthermore, microwave irradiation works on the principle of dielectric heating. When cells are heated the oscillations created in the electromagnetic field are rapid and can cause enough displacement to exceed the elastic limitations of the cell membrane.[2,34] The rotational energy from intermolecular

collisions is converted into thermal energy, resulting in an increase in temperature which can cause denaturation of proteins, DNA, and destruction of the extracellular matrix.[4,30,71]

Bindo et al state that heating by microwave irradiation is independent on thermal conductivity, the dielectrict effect, and the direct intermolecular friction that takes place between molecules. The materials experience this friction and it is responsible for their warming.[72] In a study by Campanha et al, they site that the Injury of S. aureus exposed to microwave radiation at even at sublethal temperatures has shown to be more effective than conventional heating.[30] Rosaspina et al 1993 demonstrated that progressive changes in cells was proportional to the microwave exposure time.[13]

Olseson et al. believe that non thermal effects of microwaves, such as formation of hydrogen peroxide molecules and other molecular changes are key to inactivation of microorganisms. Goldblith, Wang, and Lechowich et al found that in liquid systems disinfection is directly related to heat. Wayland et al found that thermal and electromagnetic function of microwaves are interdependent of each other. Jeng et al. examined the effects of heat on sterilization. They compared a dry-heat oven and microwave oven to sterilize against dry spores of Bacillus subtilis subsp. Niger due to their resistance to extreme heat., and found that there was no significant difference between convection and microwave heat in the number of spores inactivated, leading them to believe that heat and not other factors is the reason for inactivation.[73] Experiments by Lechowich et al demonstrated that heat was the only factor effective in killing Streptococcus faecalis and Saccharomyces cervisial.[74]

5.4 HOW MICROWAVE DISINFECTION HAS BEEN SHOWN TO EFFECT AND NOT EFFECT DIMENSIONAL STABILITY OF DENTURE BASES

Studies on the effect of microwave disinfection on dimensional stability of acrylic resin have produced varied results.[25] Rohrer and Bulard 1985 showed that there were no dimensional changes in dentures exposed to microwaves for up to 16 min at 720 W. Polyzois, Zissis and Yannikakis 1995 using a protocol of microwave irradiation of 3 and 15 min at 500 W also did not observe significant dimensional changes. Thomas and Webb 1995 though, found that microwaving acrylic dentures at 604 W for 10 min caused significant dimensional changes.[13]

Another study by Satori et al found when comparing microwave versus chloride disinfection that there was a significant dimensional change in internal adaptation of resin denture bases that were subjected to microwave irradiation. The acrylic denture bases used in the study were 2mm thick. The bases were placed in 500ml deionized water and subjected microwaving at 690 W for 6 minutes. To measure dimensional changes the denture bases were replaced on a metal master cast and silicone rubber injected underneath the gap between the denture base and cast, and then weighed. In their study Satori et al found that the dimensional changes and internal adaptation of the denture bases were so significant after a second round of exposure to microwave irradiation that the denture bases could not be reseated on the metal master cast and measured. Satori et al feel this is suggestive of structural changes within the acrylic resin denture base.[75, 76]

Nirale, Thombre, and Kubasad in their study used maxillary denture bases with a standard being 3 mm thick, and relined bases, 1.5 mm acrylic, 1.5 mm reline material. They found that chemical disinfection with sodium hypoclorite safe as a disinfection method in preserving the dimensional stability of the denture bases over microwave disinfection (650 W for 6 minutes). Denture bases treated with microwave irradiation had significant distortion. They also found that dimension changes of denture bases was less when bases were relined with a soft reline material rather than being hard acrylic.[77]

Dimensional changes from microwave irradiation should not be produced in acrylic denture bases. According to Consani et al, who microwaved denture bases at 650 watts for 3 minutes, tissue can compensate 0.25 mm and still allow for a maxillary denture base to have almost complete seating on the oral tissues. They correlate better adaptation to linear shrinkage of the resin from residual polymerization. Also they found in their study that the posterior palatal zone most affected.[78]

In another study by Pavan et al, they examined 3mm thick denture bases, submitted to either 500 W for 3 min, or 604 W for 10 minutes, found the greatest discrepancy with bases that had undergone 604 W for 10 minutes when the gap was measured after the denture base was replaced on their respective cast.[79] They found that lower wattage results in less dimensional change over the same amount of time. They speculated that asymmetric distortion could be caused from the complex design of denture bases.[77]

Dimensional change of dentures during processing may affect the stability, support, and retention of the denture. Dimensional change or the resin base is effected by size, shape, thickness of the denture, thermal expansion and contraction of the acrylic resin and stone, the polymer/monomer ration, presence of artificial teeth and the processing methods used.[25]

The shape of the acrylic sample is important. Previous studies on microwave disinfection have assessed linear measurements in selected points of dentures or rectangular specimens, but so far there is a lack of data on tridimensional distortion of denture bases.[75] Burns et al used cylindrical specimens for conventional denture base acrylic and only had changes of 0.02-0.03%, after disinfection in the microwave for 15 minutes. Polyzois et al used rectangular specimens and only found changes of -0.005 to 0.0095 mm but used linear samples, not complex geometries as denture bases.[76] Rohrer and Bulards in their used complete maxillary dentures. They found that there were no significant changes after disinfecting the denture for 16 minutes in the microwave. Thomas and Webb also used complete maxillary dentures and had dimensional changes of -0.17 to -0.80% after ten minutes of microwaving at 604 watts.[13]

Recently published results from Senna et al. suggests that a microwaving protocol of 450 and 630 W is safe for PMMA polymers.[79] Again, only linear dimensional change was measured in their study. Further studies must be done using standardized acrylic denture bases of even thickness, if an accurate understanding of what happens dimensionally to acrylic denture resin when subjected to microwave irradiation.

6.0 MATERIALS AND METHODS

20 standardized acrylic resin denture bases were fabricated from Lucitone 199, an acrylic denture resin (Lucitone 199, Dentsply International Inc, York, PA). The acrylic resin denture bases were fabricated on standardized stone models (Microstone, Whipmix Coperation, Louisville, KY) that were poured from a single duplication of a metal cast of an edentulous maxillary arch. (Figure 1)

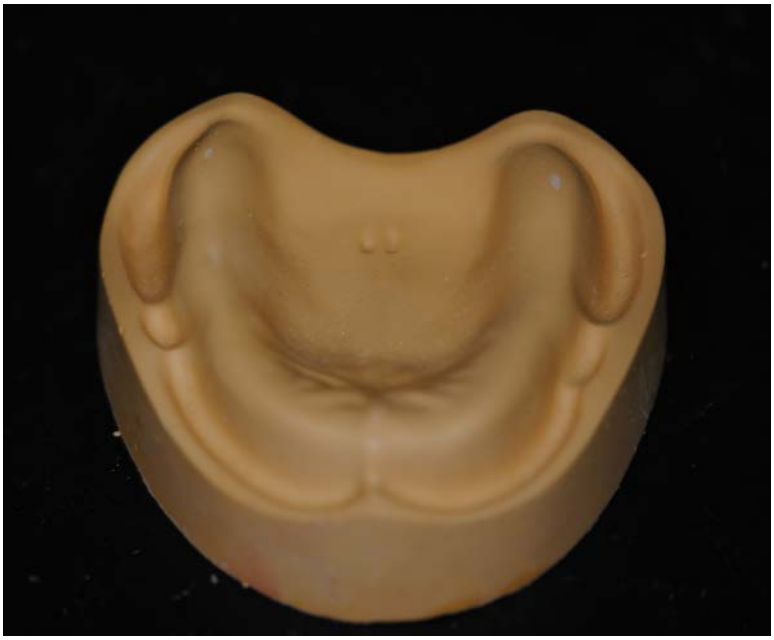


Figure 1: Maxillary Edentulous Cast

A standardized 4mm spacer was placed on top of the cast to allow for an even thickness of acrylic resin throughout the denture base. The spacer was sealed down using a thin bead of denture adhesive (Poligrip, GlaxoSmithKline, Moon Township, PA) (Figure 2)

Figure 2: Acrylic Spacer on Cast

The casts were then invested in standard two piece brass flasks. (Figure 3)

Figure 3: Maxillary Cast Ready for Investment

All stone used for investing was measured and mixed to manufactures instructions (Microstone, and Mounting Plaster, Dentsply International Inc, York, PA) When the stone was set, the flasks were opened and the spacers removed. The space left after removal of the spacer was replaced with Lucitione 199, which also was measured and mixed to manufactures directions.

The Lucitone 199 was then packed once, and put under 3500 psi pressure using a pneumatic denture press (Coe-bilt, Coe Laboratories Inc, Chicago, IL) for the duration of 10 minutes. (Figure 4)

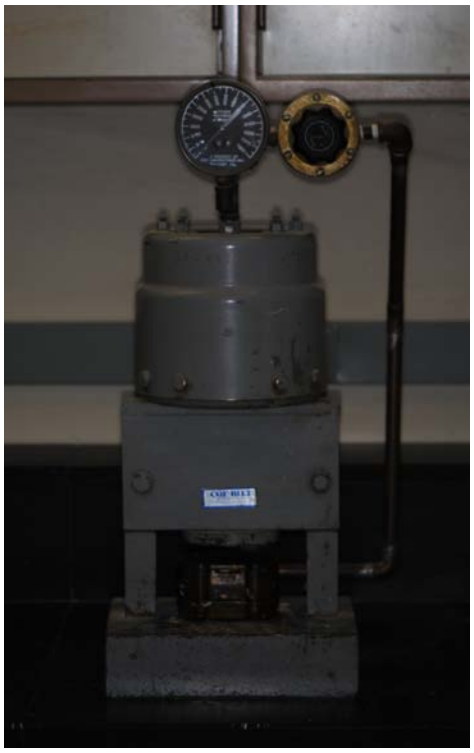


Figure 4: Pneumatic Denture Press

Each was then placed in a denture bath (Hanau Curing Unit, Haunau Engineering, Inc, Buffalo, NY) for the recommended long curing time of 9 hours at 163 F. (Figure 5)

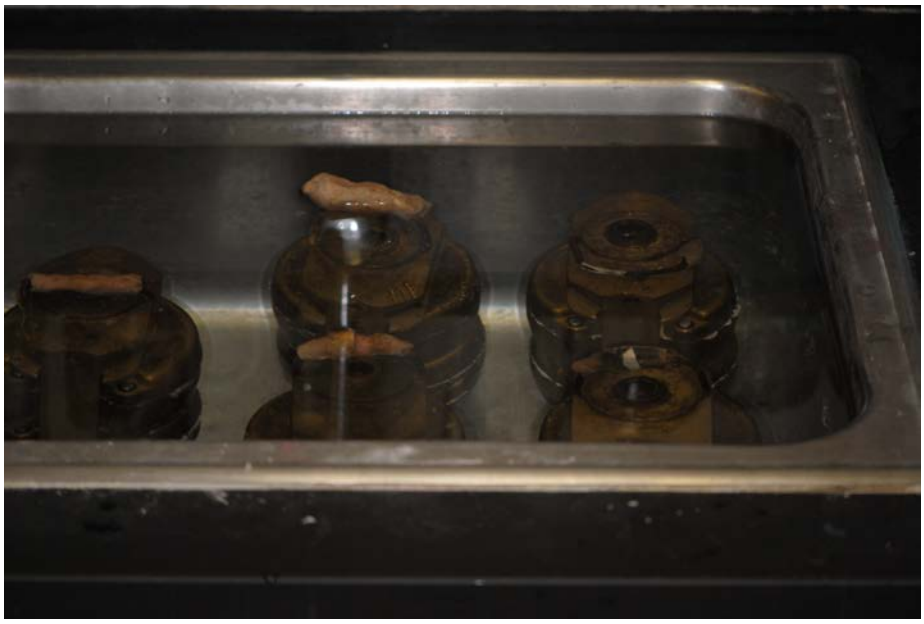


Figure 5: Denture Bases Curing in Water Bath

The denture bases were then cooled for one hour until reaching room temperature and then deflasked following manufactures instructions. The bases were removed from the cast and the acrylic flash was removed using an acrylic bur. They were then placed and stored at room temperature 21C in deionized water for 48 hours prior to measuring. (Figure 6)

Figure 6: Finished Denture Bases Soaking in Deionized Water

The denture bases were fabricated in two separate experimental groups of 10. Bases were then randomly assigned 1 through 10, and a series of 9 points were placed on the intaglio surface of the denture bases using a standardized template with a fine felt tip marker. (Figure 7)

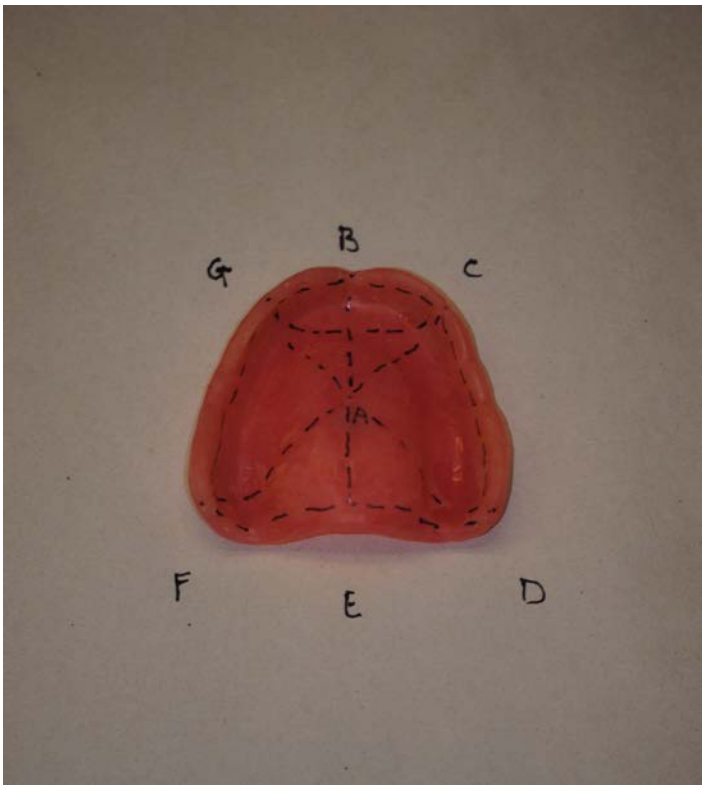


Figure 7: Denture Base with Points of Measure Marked

These points would be used to as references to measure changes in dimension of the denture base.

The distances between the points on the denture bases were then measured and recorded using a digital microscope (Keyence digital optical microscope (VHX-600), Keyance, Elmwood Park, NJ) under 5 times magnification, measuring to 10^{-2} mm. (Figure 8)

Figure 8: Measuring Denture Bases using Digital Microscope

The denture bases were measured in two halves due to the size of the specimens and the inability for the entire denture base to be displayed on the computer monitor. Each measurement was recorded three times and then averaged. Denture bases were mounted on the digital microscope stage using modeling clay, and leveled with a surveyor's level. (Figure 9)

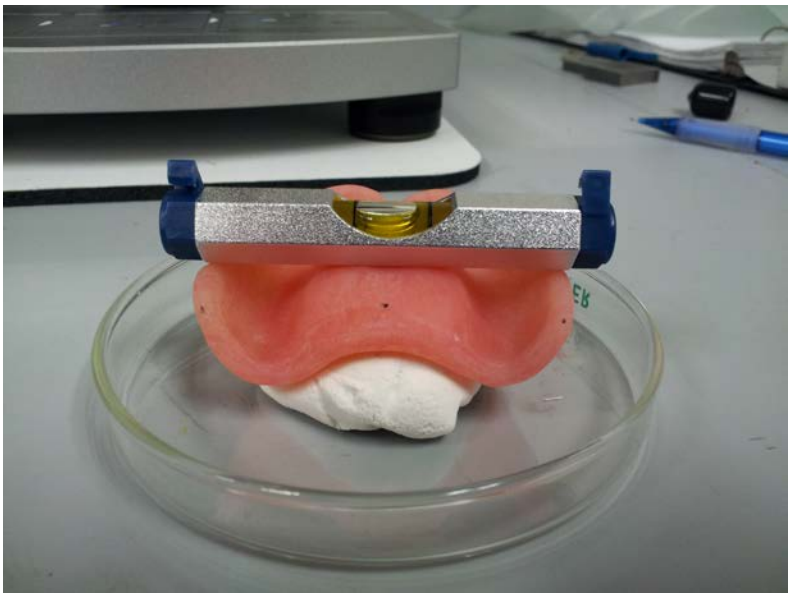


Figure 9: Leveling the Denture Base Prior to Measurement

This was done to account for repeatability of measurement and reduce variation in measurement as the material deformed. After this initial measurement was preformed, the denture bases were then subjected to microwave irradiation either at 700 watts or 420 watts, depending on the experimental group, using a 700 watt 0.7 cu ft microwave with rotating turn table (Magic Chef Microwave Oven, MC Appliance Corporation, Wood Dale, IL). Each of the denture bases were individually placed into a standard 500 ml glass beaker, filled with 200 ml of deionized water at 21C. (Figure 10)

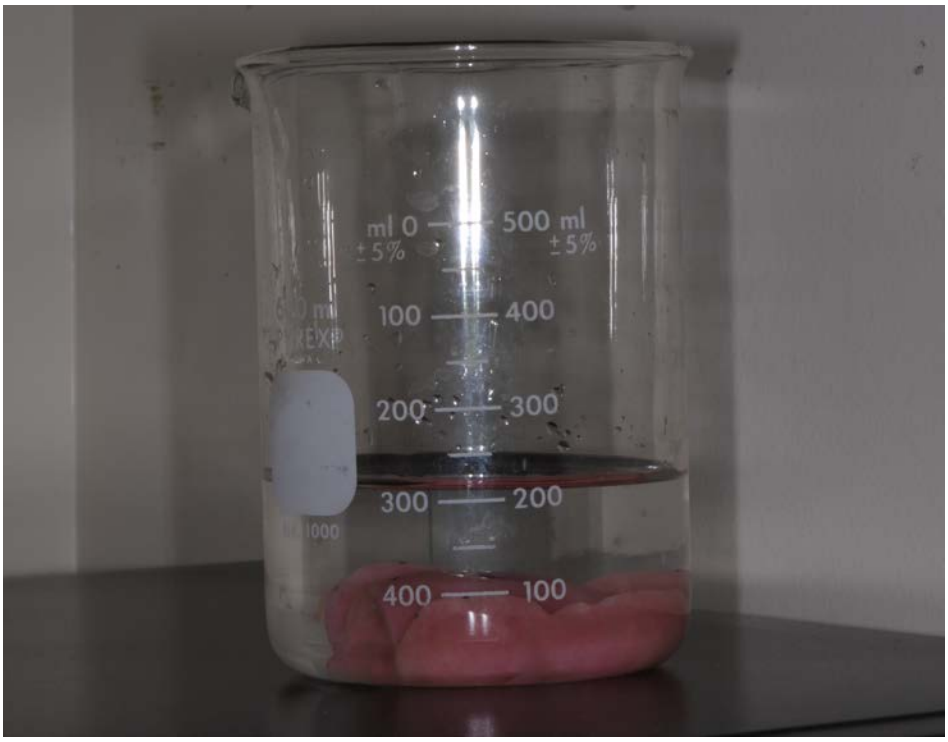


Figure 10: Denture Base Placed in Flask Prior to Microwave Radiation Exposure

The bases were then exposed to microwave radiation one at a time for a duration of 3 minutes for each sample. This brought the water in the flask up to boiling at approximately 1.5 minutes. (Figure 11)

Figure 11: Denture Base in Microwave

After the 3 minutes had elapsed the flask with the denture base sample was removed and allowed to cool on the bench top until reaching room temperature again. This procedure was repeated for all 20 denture bases.

Measurements of the distances between points on the denture bases were made 24 hours after each round of exposure to microwave radiation. (Figure 12)

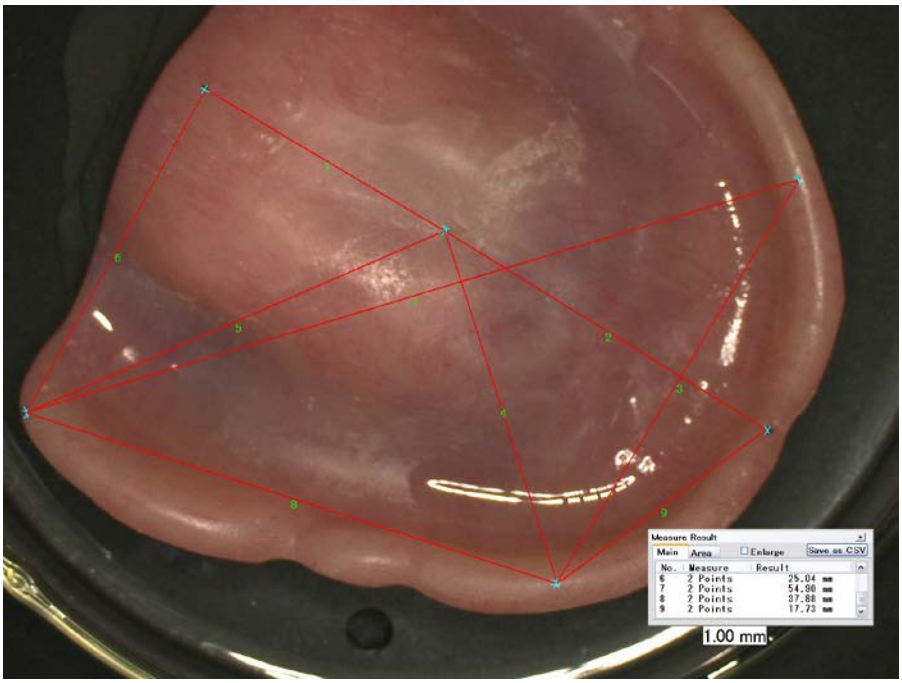


Figure 12 Image of Measurement Recorded from Denture Base

The denture bases overall were subjected to two rounds of microwave radiation, and the measurements were as follows: First measurement as a base or pre-microwave exposure state, once after one round of microwaving, and second after a second round of microwaving.

All data was recorded and subjected to student t-tests using spreadsheets (Excel, Microsoft Corporation, Redmond, WA) to test for the significance of the data. The student t-test was used to find differences in data between all the trial groups, baseline, microwaved once, and microwaved twice, for 700 watts and 420 watts respectively.

7.0 RESULTS

All acrylic resin denture bases exhibited approximately 1.0-2.0 mm of deformation in all recorded measurements after each period of microwaving. Results were very significant with all t-tests having the values of $p<0.05$ for each distance between the measured points.

The denture bases that were subjected to 700 watts of microwave irradiation showed a reduction in the distance measured between reference points after the first period of microwave irradiation, and subsequent expansion in distance between reference points after the second period of microwave irradiation. The denture bases that were subjected to 420 watts of microwave irradiation showed an opposite effect, expansion between reference points after the first period of microwave irradiation, than subsequently contraction between reference points after the second period of microwave irradiation.

Deformation in all groups appeared very uniform, even between each of the individual bases of each experimental group. After exposure to microwave irradiation, none of the denture bases returned to their original measured dimensional configuration. The greatest deformation was found when measurements were documented across the complete denture base, length, width, and diagonally.

8.0 DISCUSSION

The purpose of this study was to test whether acrylic resin denture bases, when subjected to multiple cycles of microwave radiation will exhibit dimensional instability. Two testing protocols, 700 watts of microwave irradiation for 3 minutes, and 420 watts of irradiation for 3 minutes were selected based on current published research data. Several studies have found that 650 watts of microwave irradiation for 3 minutes is effective in reducing and even disinfecting acrylic resin dentures.[8, 34, 54] What has not been determined is whether 650 watts of microwave irradiation for 3 minutes is safe or has negative effects on the acrylic denture resin. As previously stated, studies have not been conclusive as to their agreement with regards to the dimensional stability of irradiated acrylic denture resin. Studies can vary greatly in the type acrylic resin tested, the configuration of the test material, whether they are acrylic resin disks, rectangular sticks, standardized denture bases, or full form complete dentures.

A conventional 700 watt microwave with turntable was selected for this study due to having a close comparison to the 650 watts used in many of the prior studies, and due to its common availability. Many studies are arcane in that the protocols used to disinfect dentures with microwave irradiation are beyond what an average person would use. In the United States, 650 watt microwaves are uncommon, and finding a microwave with higher wattage that can be reduced to exactly 650 watts are not available. The premise behind microwave disinfection is

that it should be simple and easy to do for the average person. What advantage is there to this technique if it is inaccessible? The second 420 wattage was selected due to a recent article by Sienna et al, which demonstrated that a lower wattage of 420 watts was also effective in disinfecting acrylic resin, with possible reduced but unknown physical effects on the denture resin.

This study offered strict controls to eliminate variability or experimental error that can be introduced through the processing and even measurement of the denture bases. All materials were weighed, and processed specifically to manufactures instructions. Measurements were made on mounted specific denture bases that were leveled before measuring to reduce the influence of a three dimensional deformation. This provided an increase in the reliability and repeatability of measurement. Asymmetric distortions may not be detected by only looking at linear changes.[10]

The experimental results showed great consistency between all measurements and experimental groups. The results demonstrate that the deformation that the acrylic denture bases underwent was uniform when exposed to heat. This fact can most likely be accounted for due to uniform heating with the denture base being placed in a beaker of water. This could explain the findings of Basso et al., that disinfection is improved when specimens are irradiated while immersed in water.[10]

Craig reports heat distortion in acrylic denture resin can occur from 71 to 90 C. Boiling raises to 100 C and goes well beyond the T(g) of the resin material.[80] This accounts in fact why there

were deformational changes observed after each period of exposure to microwave irradiation. What remains unexplained is what is actually happening on a molecular level within the acrylic. The deformation that occurred, is it related to the internal rearrangement of molecular particles and the release of internal stresses that are captured in the material during processing, or is it something else? Also, what is unexplained, why the acrylic would expand or contract uniformly. Would these processes of expansion and contraction continue as the material is subjected to additional rounds of microwave irradiation? Does this pattern follow a wave pattern, such as a sin wave as suggested by the data? Lastly, why did the acrylic resin appear to expand and then contract for the denture bases tested at 700 watts, and have the opposite effect, contraction then expansion, in the denture bases tested at 420 watts?

It is suggested that further investigation be accomplished to study the behavior of the acrylic denture resin, and to determine a protocol that does not cause significant deformation of acrylic dentures.

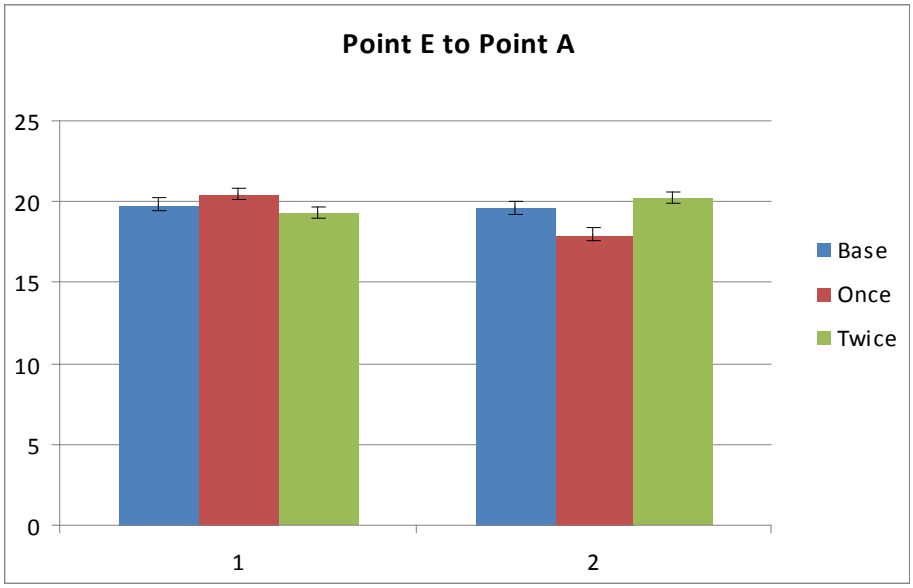
9.0 CONCLUSIONS

It may be concluded that these tested denture bases deformed a significant amount under experimental conditions at either 700 watts for 3 minutes in 200 ml of water or 420 watts for 3 minutes in 200 ml of water.

It may also be concluded that this specific acrylic denture resin utilized in this study, experiences uniform expansion and contraction of when subjected to microwave irradiation.

TABLES

Table 1: Denture Resin Bases

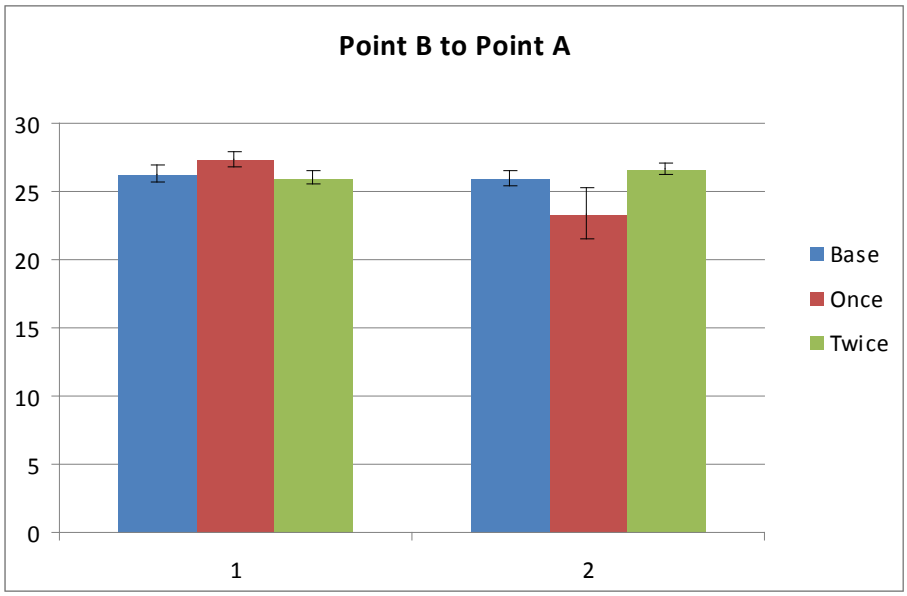


700 W **420 W**

	Values per Line Segment				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700W	19.82	20.49	19.36	700W	0.42	0.36	0.33
420W	19.63	17.97	20.26	420W	0.43	0.41	0.35

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
8.64E-07	1.52E-04	1.33E-09
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
5.69E-09	4.88E-05	6.23E-13

Table 2: Denture Resin Bases

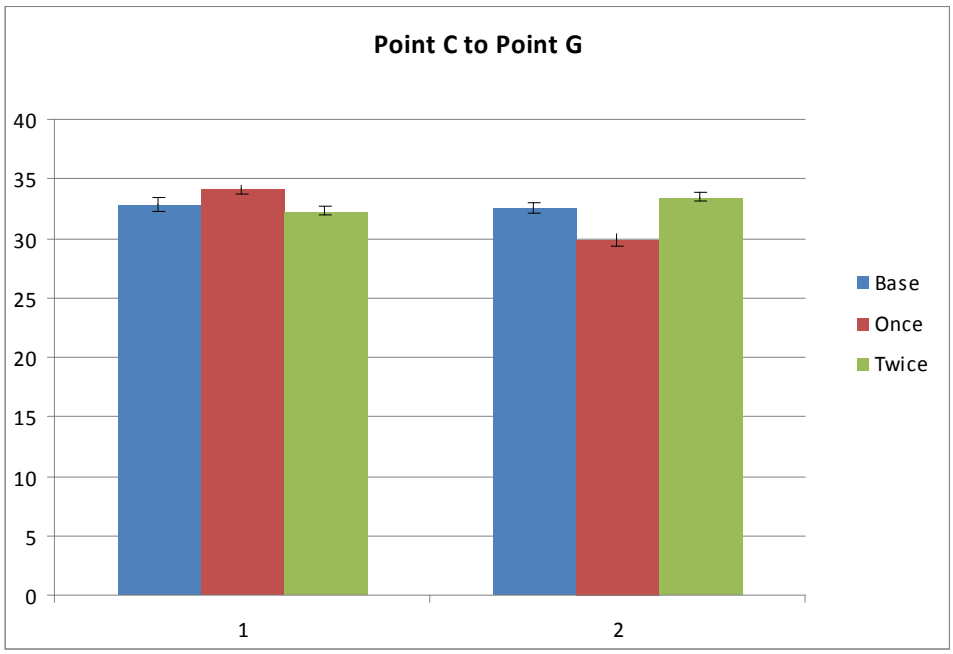


700 W **420 W**

	Values per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700W	26.30	27.40	26.00	700W	0.58	0.57	0.48
420W	25.96	23.36	26.71	420W	0.55	1.9	0.43

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
4.03E-07	4.70E-04	1.87E-09
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
2.28E-03	1.56E-05	2.39E-04

Table 3: Denture Resin Bases

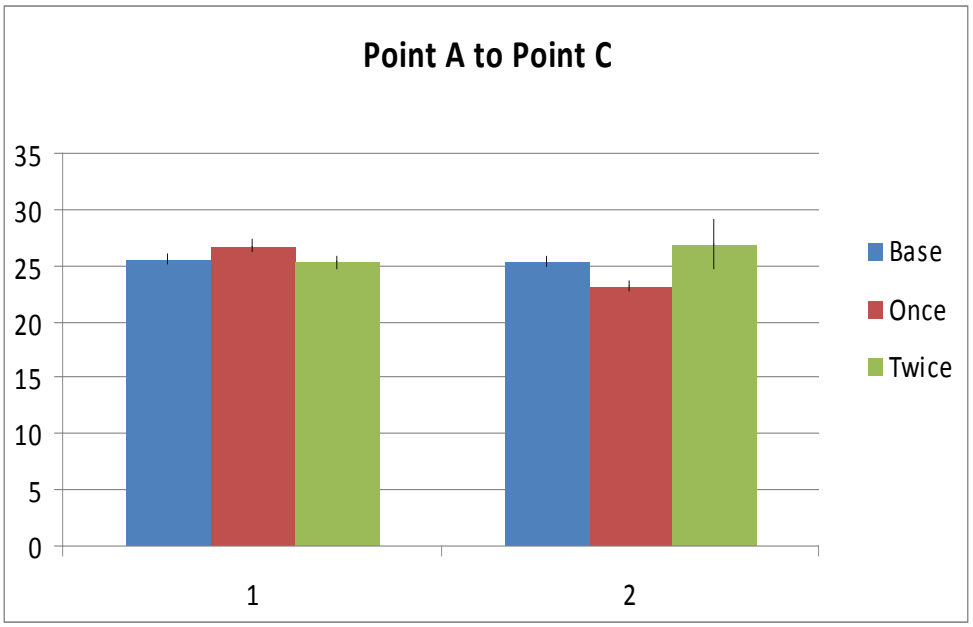


700W **420 W**

	Values per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700W	32.86	34.12	32.30	700W	0.52	0.41	0.40
420W	32.51	29.88	33.46	420W	0.42	0.50	0.35

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
1.01E-06	1.4E-04	7.44E-11
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
3.60E-11	1.32E-06	8.21E-12

Table 4: Denture Resin Bases

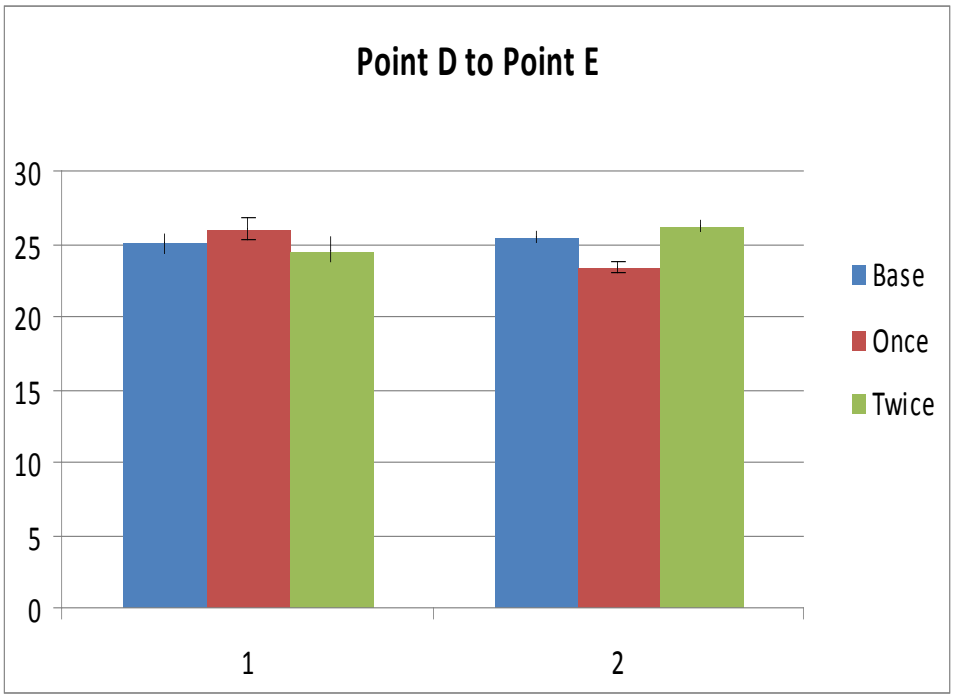


700 W **420 W**

	Values per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	25.66	26.79	25.28	700 W	0.46	0.47	0.46
420 W	25.32	23.27	26.90	420 W	0.42	0.39	2.21

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
4.92E-08	3.66E-04	1.28E-12
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
2.64E-08	4.85E-06	7.10E-04

Table 5: Denture Resin Bases

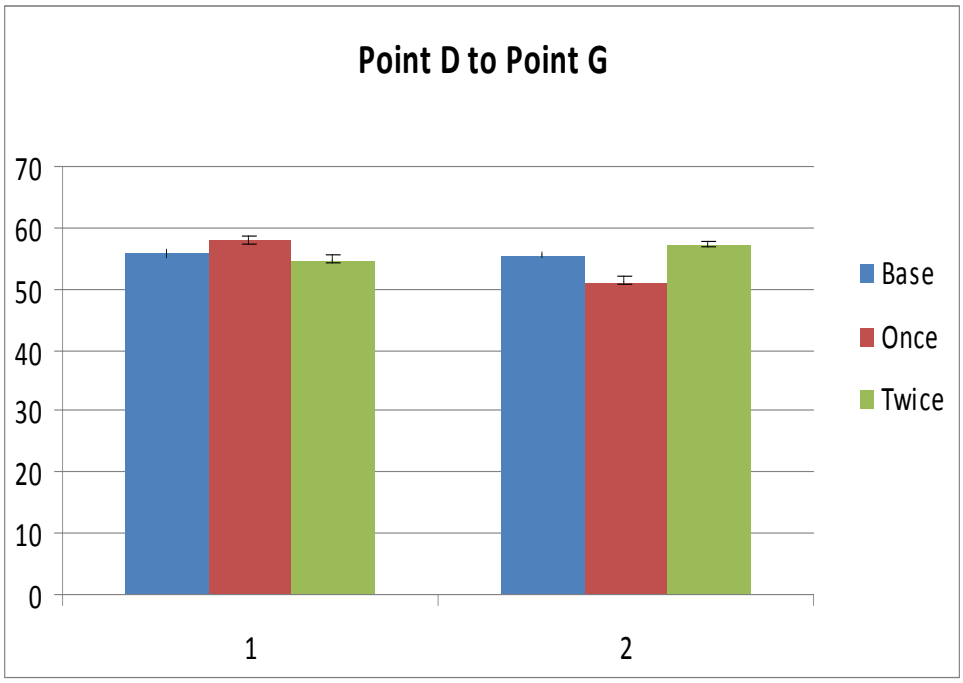


700 W **420 W**

	Values per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	25.06	26.03	24.59	700 W	0.67	0.72	0.79
420 W	25.48	23.41	26.25	420 W	0.41	0.42	0.41

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
6.78E-07	4.15E-04	2.43E-09
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
1.89E-09	8.025E-07	1.65E-10

Table 6: Denture Resin Bases

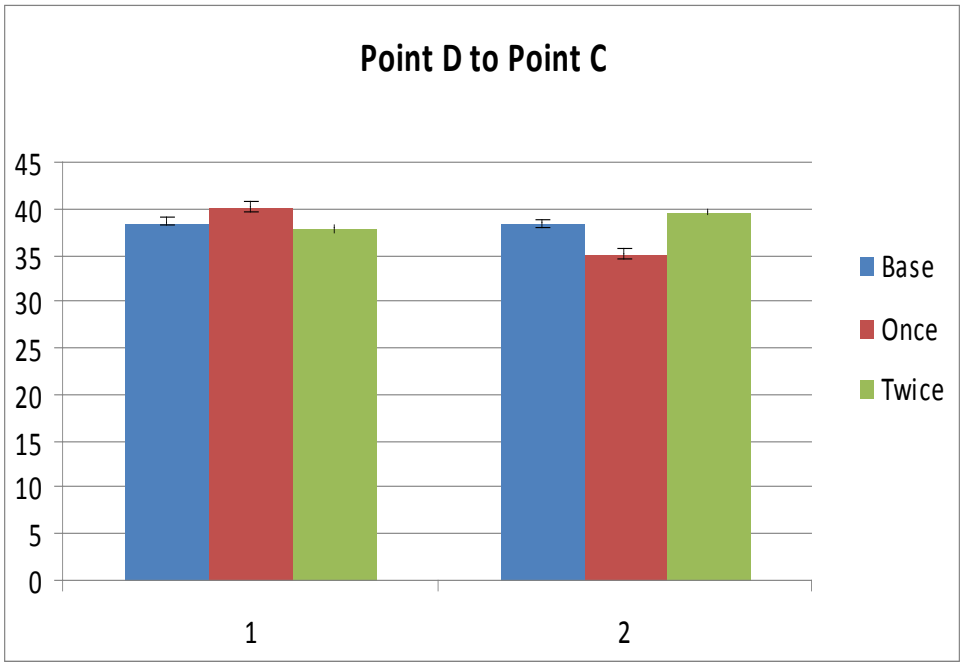


700 W **420 W**

	Values per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	55.83	58.01	54.85	700 W	0.64	0.51	0.59
420 W	55.69	51.25	57.43	420 W	0.42	0.60	0.35

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
4.42E-08	1.19E-04	2.21E-11
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
1.16E-09	7.55E-08	2.16E-10

Table 7: Denture Resin Bases

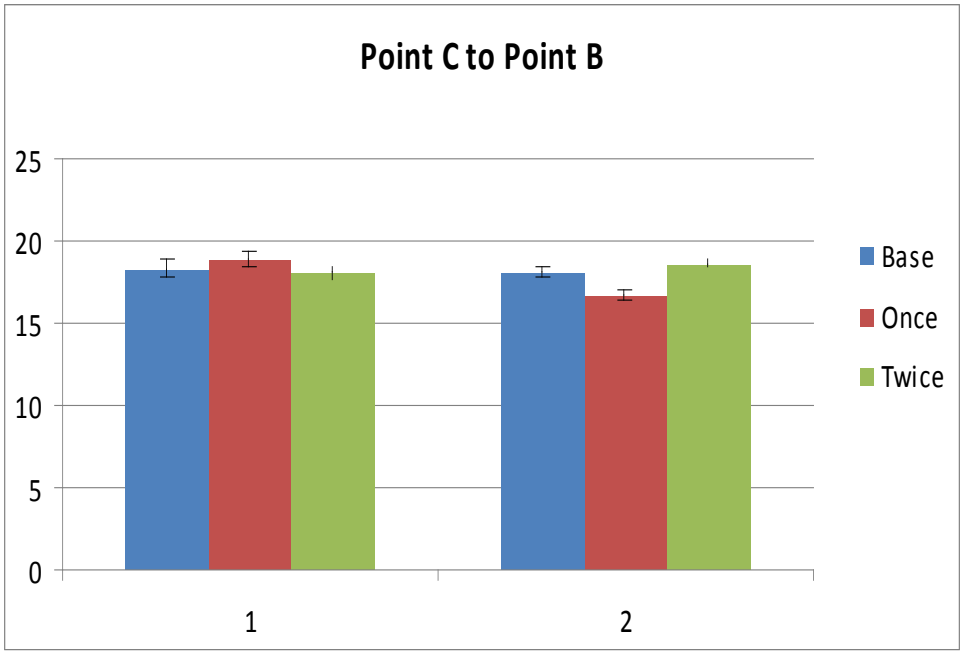


700 W **420 W**

	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	38.61	40.17	37.85	700 W	0.47	0.48	0.49
420 W	38.41	35.23	39.61	420 W	0.33	0.55	0.23

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
2.92E-07	2.74E-04	1.45E-09
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
3.25E-09	8.18E-08	3.20E-10

Table 8: Denture Resin Bases

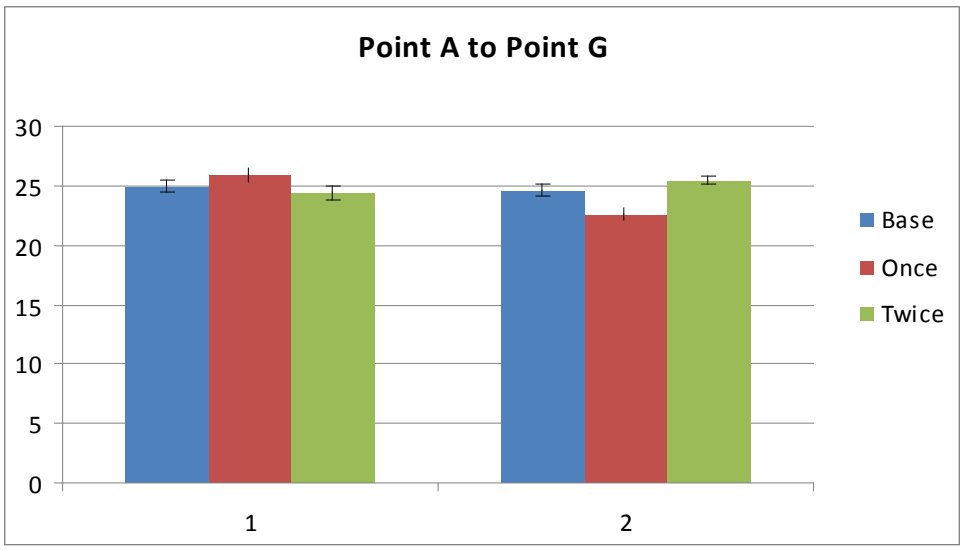


700 W **420 W**

	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	18.34	18.98	18.05	700 W	0.50	0.47	0.45
420 W	18.10	16.74	18.66	420 W	0.29	0.29	0.17

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
1.71E-06	4.33E-05	2.49E-09
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
5.79E-10	1.10E-05	1.81E-10

Table 9: Denture Resin Bases

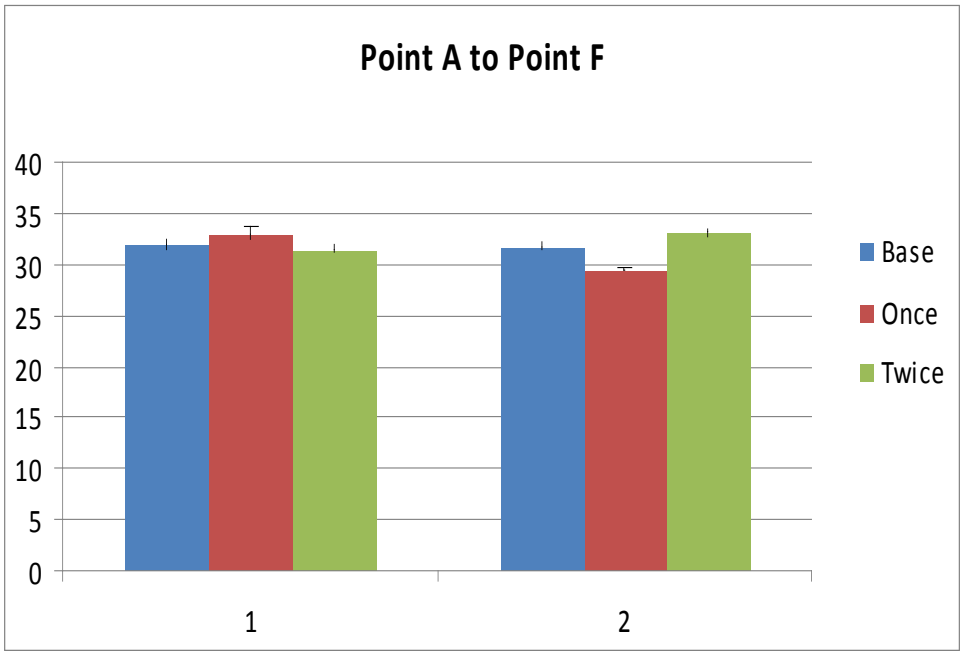


700 W **420 W**

	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	24.96	25.92	24.42	700 W	0.54	0.60	0.59
420 W	24.57	22.61	25.41	420 W	0.50	0.43	0.34

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
7.04E-06	4.11E-06	3.71E-08
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
6.78E-09	2.97E-06	1.62E-12

Table 10: Denture Resin Bases

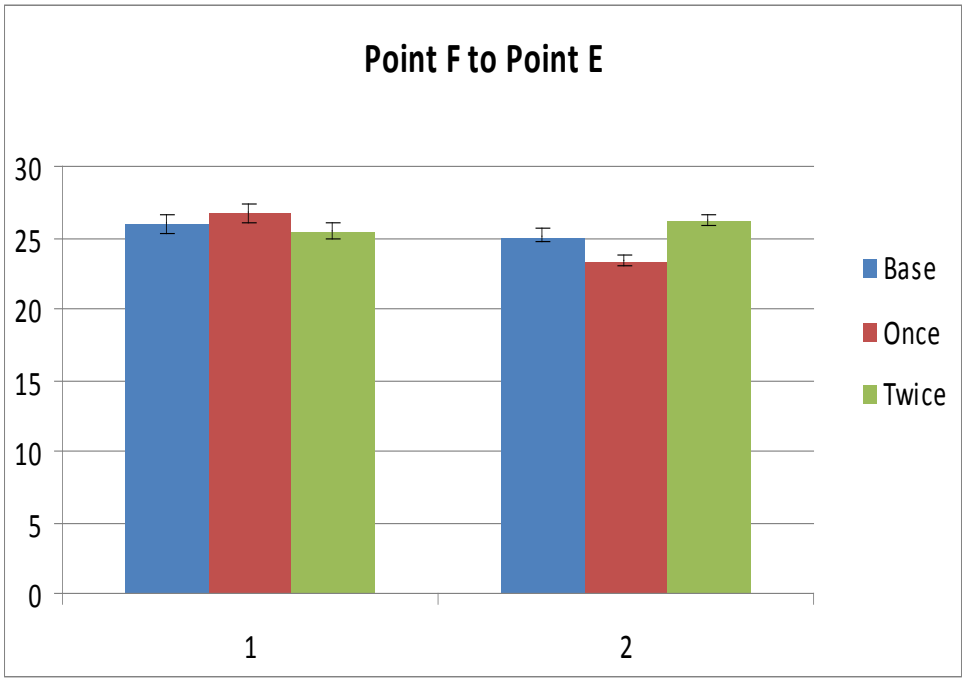


700 W **420 W**

	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	31.98	33.01	31.55	700 W	0.47	0.65	0.29
420 W	31.82	29.50	33.11	420 W	0.39	0.19	0.36

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
2.61E-04	8.96E-04	2.71E-06
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
1.10E-07	1.88E-04	5.53E-11

Table 11: Denture Resin Bases

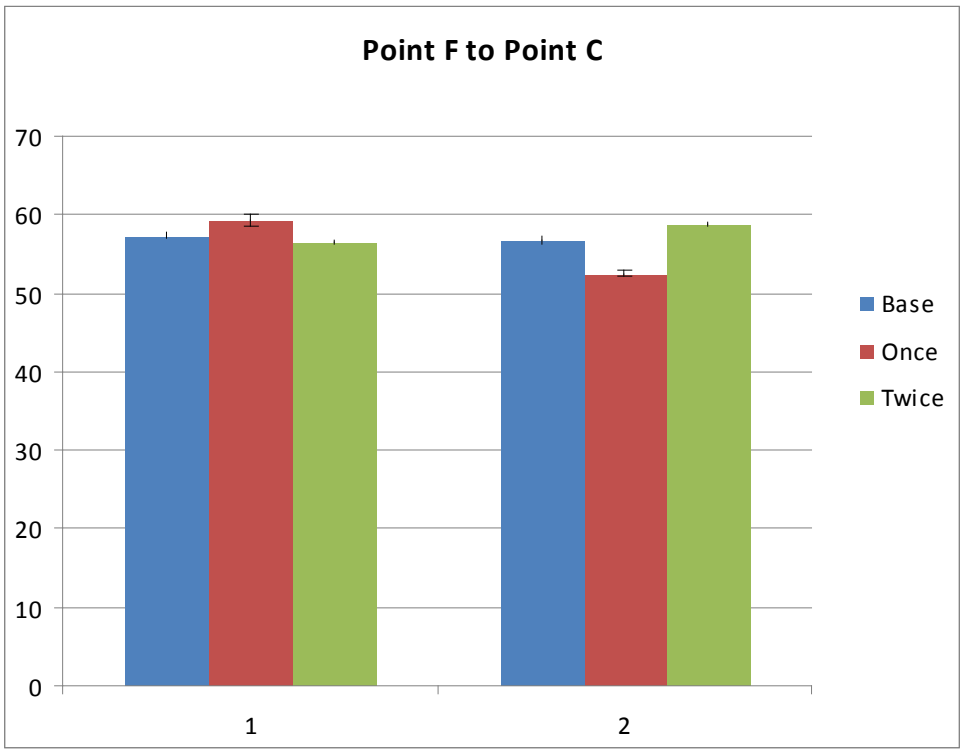


700 W **420 W**

	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	25.95	26.75	25.54	700 W	0.70	0.69	0.57
420 W	25.18	23.39	26.22	420 W	0.39	0.39	0.45

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
3.77E-4	1.07E-4	2.30E-06
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
2.31E-09	5.49E-06	2.00E-11

Table 12: Denture Resin Bases

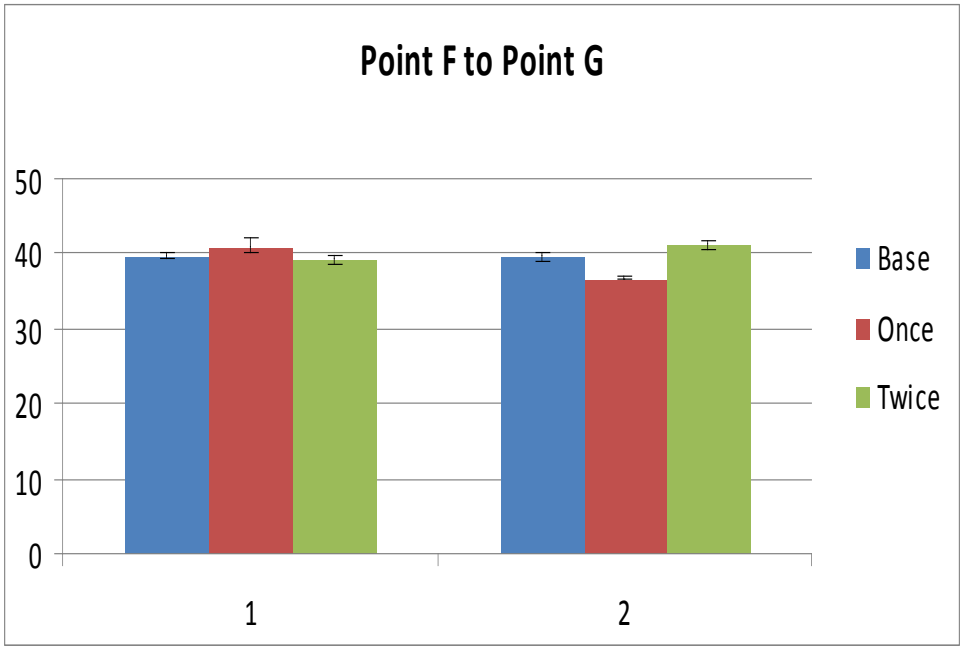


700 W **420 W**

	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	57.35	59.26	56.46	700 W	0.33	0.83	0.28
420 W	56.85	52.47	58.81	420 W	0.54	0.38	0.29

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
2.95E-05	7.86E-06	3.14E-07
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
3.16E-11	3.80E-07	1.25E-12

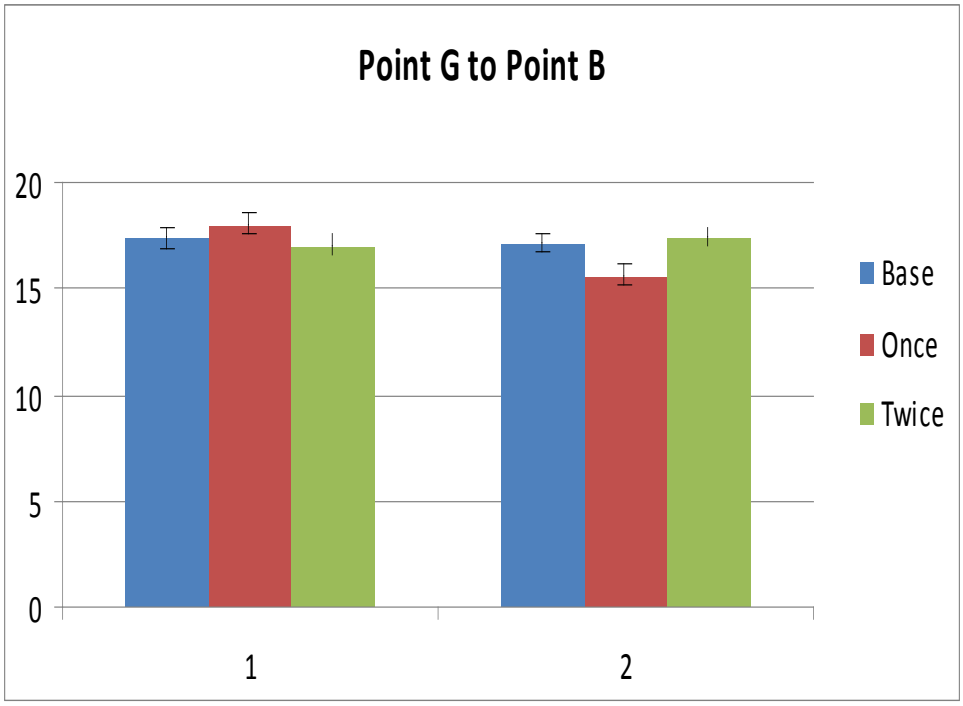
Table 13: Denture Resin Bases



	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	39.76	41.11	39.20	700 W	0.40	0.85	0.49
420 W	39.66	36.72	41.20	420 W	0.50	0.30	0.46

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
5.25E-05	6.17E-06	3.23E-07
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
1.86E-09	3.99E-06	1.85E-11

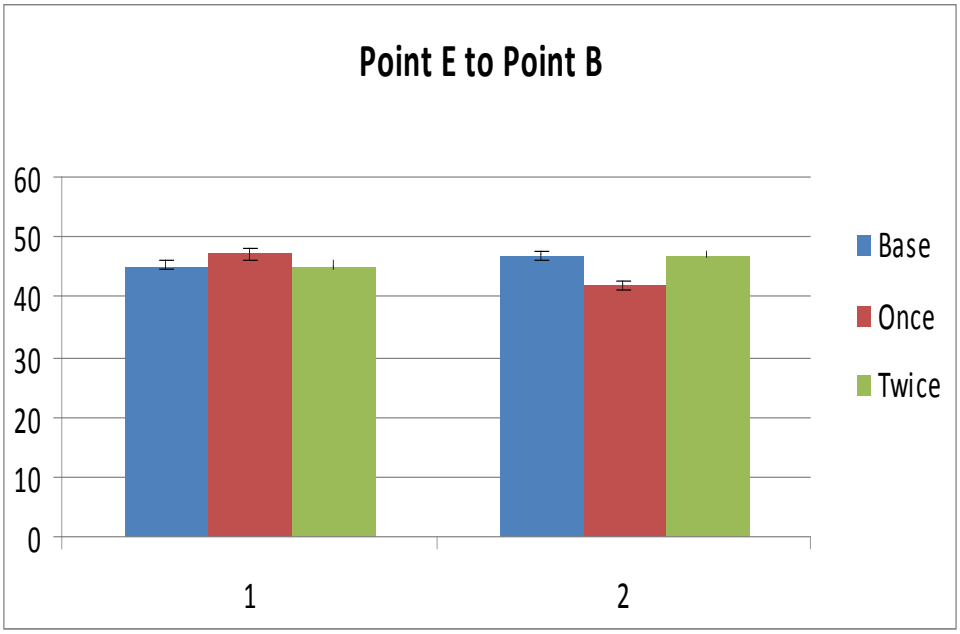
Table 14: Denture Resin Bases



	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	17.40	18.07	17.03	700 W	0.49	0.55	0.49
420 W	17.16	15.67	17.49	420 W	0.39	0.49	0.41

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
2.73E-04	6.77E-04	1.61E-07
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
8.80E-10	1.49E-04	1.30E-08

Table 15: Denture Resin Bases

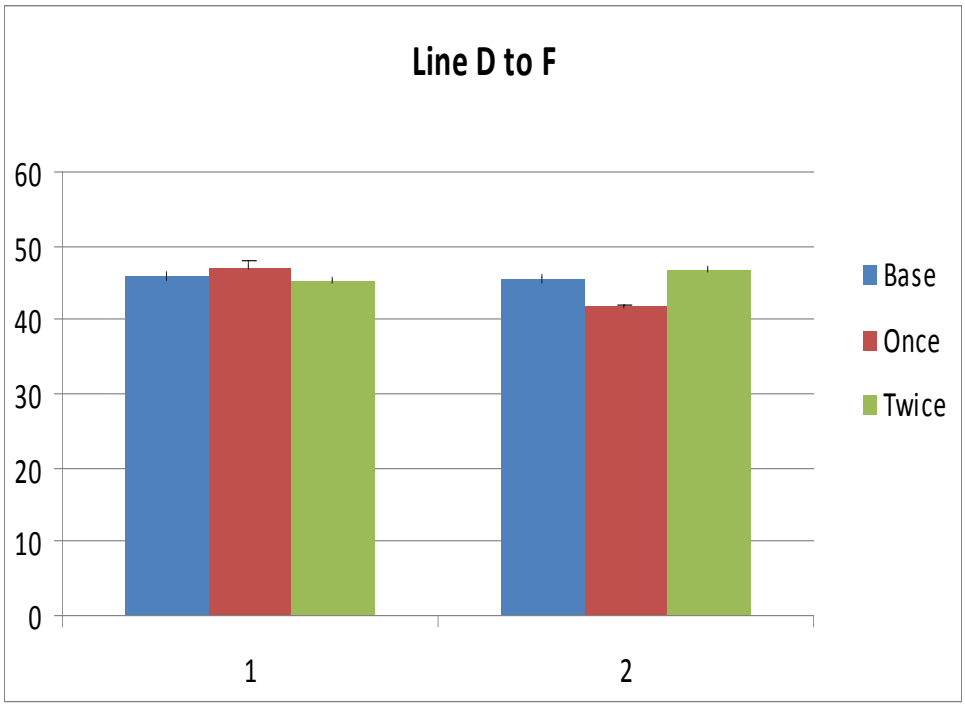


700 W **420 W**

	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	46.10	47.42	45.36	700 W	0.63	1.49	0.51
420 W	45.58	41.94	46.97	420 W	0.67	0.76	0.50

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
8.83E-04	3.36E-06	5.20E-04
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
1.01E-11	7.32E-08	2.04E-12

Table 16: Denture Resin Bases



	Value per Line Segment in Millimeters				Standard Deviation		
	Base	Once	Twice		Base	Once	Twice
700 W	51.11	52.73	50.13	700 W	0.56	0.66	0.50
420 W	50.67	46.80	52.47	420 W	0.42	0.28	0.23

t-test 700 base Vs. 700 once	t-test 700 base Vs. 700 twice	t-test 700 once Vs. 700 twice
7.22E-06	3.11E-04	1.59E-08
t-test 420 base Vs. 420 once	t-test 420 base Vs. 420 twice	t-test 420 once Vs. 420 twice
1.42E-10	4.17E-07	9.71E-13

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