EVALUATION OF LINEAR DIMENSIONAL STABILITY OF VARIOUS DENTURE BASE RESINS SUBJECTED TO STERILIZATION BY MICROWAVE IRRADIATION- AN INVITRO STUDY

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In partial fulfillment for the degree of

MASTER OF DENTAL SURGERY

BRANCH - I

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled **"EVALUATION OF LINEAR DIMENSIONAL STABILITY OF VARIOUS DENTURE BASE RESINS SUBJECTED TO STERILIZATION BY MICROWAVE IRRADIATION- AN INVITRO STUDY"** is the Bonafide work done by **DR.T.SARANYA**, Post Graduate Student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the speciality of **DEPARTMENT OF PROSTHODONTICS AND CROWN & BRIDGE**, Vivekanandha Dental College for Women, Tiruchengode during the period of 2017-2020.

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TITLE OF DISSERTATION	Evaluation of linear dimensional stability of various denture base resins subjected to sterilization by microwave irradiation- An invitro study
PLACE OF STUDY	Vivekanandha Dental College for Women, Elayampalayam, Tiruchengode, Namakkal District.
DURATION OF COURSE	3 Years (2017-2020)
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CERTIFICATE – II

This is to certify that this dissertation work **"EVALUATION OF LINEAR DIMENSIONAL STABILITY OF VARIOUS DENTURE BASE RESINS SUBJECTED TO STERILIZATION BY MICROWAVE IRRADIATION- AN INVITRO STUDY"** of the candidate, **DR.T.SARANYA**, with registration number **241711451** for the award of degree **MASTER OF DENTAL SURGERY** in the branch of **PROSTHODONTICS AND CROWN & BRIDGE**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and the results shows **4%** of plagiarism in the dissertation.

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LIST OF ABBREVIATIONS

W	Watts
PMMA	Polymethyl Methacrylate
SDB	Sabouraud Dextrose Broth
SDA	Sabouraud Dextrose Agar
OD	Optical Dentistry
UV	Ultra Violet
Mm	Millimeters
ANOVA	Analysis of Variance
SD	Standard Deviation
N	Number of samples

INTRODUCTION

INTRODUCTION

Practice of Removable prosthesis has increased the potential of cross contamination between dental office and laboratory. Patient who has come for the corrections of the existing dentures are heavily contaminated with microorganisms from saliva and blood. There is a constant exposure of dentist, dental assistant to these microorganisms and when the dentures are sent to the dental laboratory, there is a cross contamination to the dental technicians. There should be proper disinfection of the dentures before it is handled by dentist, dental assistant and laboratory technicians. Therefore, the use of an improper disinfection procedure in the handling of dental materials not only places the dental personnel at risk, but also results in a high level of cross-contamination.

The different techniques used to disinfect the infected dentures are chemical, mechanical, and physical methods.[3]

The chemical disinfectants are glutaraldehyde, chlorhexidine, sodium perborate and peroxide solutions. Deleterious effects on acrylic resins is seen when the dentures are soaked in the chemical solution. Sodium hypochlorite is fungicidal and is effective in dissolving mucin and other organic substances. It may discolour or whiten the prostheses. Glutaraldehyde has several risks of cytotoxicity. When the dentures are soaked in the chemical solutions for disinfection it takes several hours for complete disinfection of dentures. And in common dental practice it is difficult to disinfect the dentures by soaking in the chemical solution for several hours.[3]

Mechanical techniques are use of ultrasound and brushing technique. In the brushing technique, tooth brush and paste are used to clean the denture. This does not cause the complete removal of the microorganisms.[3]

In physical technique the microwave irradiation is used. Microwave irradiation is being recommended as a practical physical sterilization method that is as effective as autoclaving and less time-consuming.[7]

In the previous study Rohrer and Bulard showed that microwave energy can easily kill microorganisms on denture acrylic resin surfaces within 15 minutes of exposure.[2] And another study has demonstrated that microwave energy irradiation in a conventional domestic oven for 3 minutes at 650 W was capable to sterilize all complete dentures inoculated with Candida species. A study comparing chemical and microwave disinfection showed that the hardness, flexural strength and dimensional changes of the acrylic resin were not significantly altered by these disinfection procedures. More recent studies verified that a single microwave disinfection cycle decreased the hardness and had no effect on the impact, and flexural strength of different commercial acrylic resins.[3]

However, it has also been shown that microwaving could cause dimensional changes or distortion of the acrylic resin denture base, which could compromise the denture stability, retention, and durability. A different time and power settings of microwave irradiation has been proposed for denture disinfection, but their side effects on material properties still are unclear. High wattage and long irradiation time seem to yield significant denture distortion, and alteration of surface roughness, but resin hardness is not affected. Also, a previous study found that repeated microwave disinfection at 650 W for 6min promoted significant increase of baseplate distortion in a denture resin polymerized by microwave energy but not when the same resin was polymerized by conventional heat cure method. Dovigo et al in his study said that microwave irradiation for 3 minutes at 650 W produced sterilization of complete dentures contaminated with Staphylococcus aureus and Pseudomonas aeruginosa.

Dentures contaminated with Bacillus subtilis were disinfected by microwave irradiation after 3 and 5 minutes at 650 W.[4]

The method of processing the acrylic dentures are heat cure processing method, injection moulding processing method, microwave processing method, light cure processing method, and flexible acrylic method. In this study the conventional heat cure processing method, injection moulding method and flexible acrylic processing methods are used. The acrylic samples are prepared using these processing methods and both the sterilization effect and the change in the linear dimensional stability of the acrylic samples are evaluated. The most commonly Methacrylate polymers are preferred material for the fabrication of removable dental prostheses. The previous studies had used the heat cure acrylic resin and soft liners for evaluating the change in the physical properties and the sterilization effect. In this study the heat cure acrylic resin, injection moulding acrylic resin and flexible acrylic materials are used. These are the materials which are commonly used in preparing dentures in common dental practice.

The most infected subjects, from 40% to 70%, suffer from stomatitis as a direct result of Candida albicans infection.[5] In this study to evaluate the effect of disinfection of the acrylic samples the candida albicans species are coated over it and heated in the microwave oven. There is no study had said the correct wattage and timing for the complete sterilization using microwave oven. So in this study the two different wattages are used. One is at 650W and second is at 810W is used to evaluate sterilization effect and change in the linear dimensional stability at 3 minutes. From the result it can be concluded that which wattage and timing is used for the complete sterilization and whether there is a change in the linear dimensional stability of three different acrylic resins used in this study.

AIM AND OBJECTIVES

AIM AND OBJECTIVES

AIM:

To evaluate the dimensional stability of previously polymerized different denture base resins subjected to sterilization by microwave irradiation.

OBJECTIVE:

1. To determine whether there is a change in dimensional stability of different denture base resins after it has been subjected to sterilization by microwave irradiation.

2. To determine the sterilization status of the denture base resins tested.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In **1980 Wakefield** reported that among the purposes of microwave energy in cross-infection prevention, one of the most important is the disinfection of removable dentures. In addition to its contamination by the oral microorganisms, it has been reported that dentures are contaminated at various stages during their fabrication.[8]

In **1985 Rohrer & Bulard** who found that dentures subjected to microwaves for up to 16 minutes showed no dimensional changes when evaluated by analyzing the fit of the denture into impressions that had been made into die stone. No data were provided to support their finding. Then Rohrer and Bulard first reported on microwave sterilization of various dental instruments and the effect of microwave sterilization on acrylic resin dentures. They found that contaminated dentures can be sterilized in a short time. However, little information is available concerning the effect of disinfection methods upon the physicomechanical properties of denture base resins.[2]

In **1999 Border & Rice-Spearman** said application of microwave irradiation in preventing cross-infection is the disinfection of disposable materials. Although there is little scientific support for this purpose, some reports documented the disinfection of contaminated gauze and swabs with the use of microwave irradiation.[9]

In **1999 Kansu et al** showed that 15 min at 500 W irradiation achieved complete sterilisation of acrylic resin-base materials contaminated by Staphylococcus aureus, Escherichia coli, C. albicans and Streptococcus mutans. Others have shown that 6 min of microwave irradiation at 650 W was sufficient to achieve sterilisation of

the acrylic base-resin colonised by the same microorganisms. Although 3 min at 650 W may be sufficient to sterilise dentures contaminated by the Pseudomonas and Candida species[10]

In **2001 Glass et al** used several protocols; positive results were obtained by in vitro and in vivo studies, proving that microwave energy can be an effective method in the disinfection of dentures.[11]

In **2001 Banting & Hill** conducted the first study that evaluated the effectiveness of microwave energy for denture disinfection as a co-adjuvant in the treatment of denture stomatitis. The authors observed that the method was effective in the reduction of the clinical signs of infection. These findings are in agreement with those found by Webb et al in 2005 a few years later.[6]

In **2005 Campanha et al** studied the Effect of microwave sterilization and water storage on the Vickers hardness of acrylic resin denture teeth and concluded that for specimens immersed in water for 90 days, 2 cycles of microwave sterilization had no effect on the hardness of most of the acrylic resin denture teeth.[12]

In **2005 Pavan et al** in his study evaluated the Effect of Microwave Treatments on Dimensional Accuracy of Maxillary Acrylic Resin Denture Base and concluded that Treatment in microwave oven at 604 W for 10 min produced the greatest discrepancies in the adaptation of maxillary acrylic resin denture bases to the stone casts.[13]

In **2007 Sanita et al** and others verified that microwave irradiation for 3 min at 650 W for three consecutive exposures resulted in sterilisation of the dentures

contaminated with six species of Candida. Although structural and dimensional changes might be more prominent when the denture is left dry while being micro waved, there is no consideration of the daily, long-term effect of microwave use for everyday denture hygiene.[10]

In **2008 Vergani et al** studied the Flexural strength of autopolymerizing denture reline resins with microwave postpolymerization treatment and concluded that microwave postpolymerization irradiation can be an effective method for increasing the flexural strength of Duraliner II (at 650 W) and Kooliner (at 550 W and 650 W for 5 minutes).[14]

In **2008 Consani et al** studied the Effect of the Simulated Disinfection by Microwave Energy on the Impact Strength of the Tooth/Acrylic Resin and results indicate that the simulated microwave disinfection decreased the impact strength in all treatments.[15]

In **2008 Neppelenbroek et al** evaluated the effectiveness of complete denture disinfection by microwave energy in the treatment of patients with denture stomatitis. In agreement to Banting & Hill (2001) and Webb et al. (2005), it was observed that disinfection of the dentures by microwaves was effective for the treatment of denture stomatitis.[16]

In **2008 Consani et al** studied the Effect of Repeated Simulated Disinfections by Microwave Energy on the Complete Denture Base Adaptation and concluded that repeated simulated disinfections by microwave energy did not cause deleterious effect on the base adaptation, when the traditional cramp and RS system flask closure methods were compared.[17]

In **2009 Ribeiro et al** studied the in vivo effectiveness of microwave irradiation on the disinfection of upper complete dentures and showed that a 3-min microwaving cycle at 650W was capable to sterilize all dentures. This result was confirmed by the absence of microbiological growth in both selective and non-selective culture media after 48 hours. [7]

In **2009 Consani et al** studied the Effect of Repeated Disinfections by Microwave Energy on the Physical and Mechanical Properties of Denture Base Acrylic Resins and concluded that repeated simulated microwave disinfections decreased the Knoop hardness of Clássico and Onda-Cryl resins and had no effect on the impact strength of QC-20. The flexural strength was similar for all tested resins.[18]

In **2009 Machado et al** studied the Hardness and surface roughness of reline and denture base acrylic resins after repeated disinfection procedures and concluded that Disinfection by immersion in sodium perborate or microwave irradiation did not adversely affect the hardness of all materials evaluated. The effect of both disinfection methods on the roughness varied among materials.[19]

In **2010 Ibrahim M. Hamouda** studied the Effect of microwave disinfection on mechanical properties of denture base acrylic resin concluded that the microwave oven is not acceptable for sterilization of dentures because of its weakening effects on the dentures that prone for fracture during clinical use. This method of sterilization increased the brittleness of acrylic resin specimens.[20]

In **2010 Sanita et al** performed tests in order to evaluate the effectiveness of denture irradiation in the treatment of diabetic denture wearer patients with denture stomatitis, showing promising outcomes.[21]

In **2011 Nelson-Filho et al** studied the efficacy of microwave irradiation for disinfection of toothbrushes and tongue cleaners, suggesting that this may be a practical and low-cost alternative method of disinfection that can be easily used in the oral hygiene care practices.[22]

In **2013 Newton Sesma** studied the Effectiveness of Denture Cleanser Associated with Microwave Disinfection and Brushing of Complete Dentures and said that microwave irradiation in combination with soaking in denture cleanser and brushing effectively disinfected the dentures and removed denture biofilm.[23]

In **2013 Deepthi Kalahasti et al** studied the Evaluation of Efficacy of Microwave Irradiation in Disinfecting Dental Gypsum Casts: An Ex Vivo Study and concluded that Microwave irradiation was found to be effective in disinfecting gypsum casts when compared to chemical disinfectant in disinfecting dental impressions.[24]

1n **2014 Mojarrad et al** showed that the use of both 2% glutaraldehyde for 10 min and microwave irradiation at an output power of 650 W for 3 min completely removed Candida colonies from denture surfaces; however, brushing and denture-leansing tablets did not fully remove the Candida colonies.[7]

In **2015 Neha Ahuja** studied the effect of microwave disinfection on the hardness of heat cure and self cure acrylic resin: an in-vitro study and concluded that

microwave disinfection can be used safely to disinfect prosthesis made of heat cure and self-cure acrylic resin in clinical prosthodontic procedures. This study evaluated the effect of microwave disinfection on the hardness of heat cure and self-cure acrylic resin. 15 Samples each of self-cure and heat cure acrylic resin without microwave disinfection were used as a control group and the same samples were microwave disinfected and were used as a experimental group. 6 minute exposure at 650 W for 15 days to microwave was employed as disinfection procedure. The samples were stored in distilled water at 37 degree Celsius for 24 hours prior to disinfection. There were no statistically significant differences in the hardness of heat cure and self-cure acrylic resin after disinfection with microwave.[25]

MATERIALS & METHODS

MATERIALS AND METHODS

The present invitro study was conducted for the evaluation of the linear dimensional stability of the various denture base resins namely heat cure acrylic resin, injection moulding acrylic resin, flexible acrylic resin which is subjected to sterilization by microwave irradiation at 650W and 810W for 3 minutes.

Materials used:

1. Stainless steel rectangular die (64x10x3.3mm^3- ISO 1567:1999)
2. Heat cure Acrylic resin (DPI, Bombay Burmah Trading Corp Ltd, Uttarkhand, India)
3. Injection moulding Acrylic resin (SR- IVOCAP high Impact, Ivoclar Vivadent, Liechtenstein)
4. Sunflex semiflexible Acrylic resin (Sun Dental Laboratories LLC , St. Petersburg)
5. Digital vernier calliper (Bombay Tools Center Pvt Ltd, Mazgaon, Mumbai)
6. Candida albicans (ATCC 24433) obtained from Department of Microbiology, Periyar University, Tamilnadu.
7. Microwave oven (Model - Panasonic NN- G335WF)

Methods:

I. Preparation of Heat cure PMMA acrylic resin

II. Preparation of Injection moulding acrylic resin

III. Preparation of Flexible acrylic resin

IV. Grouping of prepared samples

V. Testing of samples for Dimensional Stability

VI. Testing of samples for Sterilization

Step 1: Preparation of Heat cure PMMA acrylic resin:

A stainless steel die measuring 64x10x3.3mm3 (ISO 1567: 1999) [Fig- 1] was fabricated.

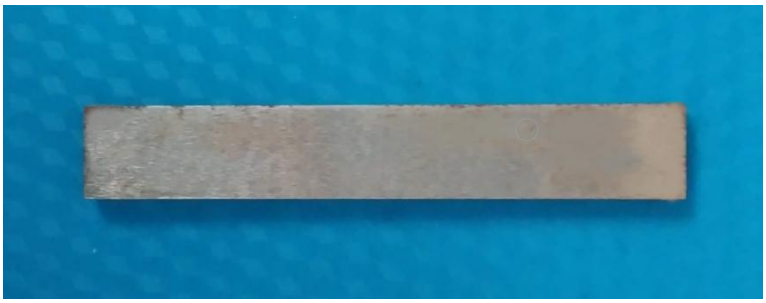


Fig- 1: Stainless Steel Die

These dies were invested in a flask using type III dental stone. The flasks were separated and the dies were retrieved from molds without distorting the mold space. The sodium alginate separating media [DPI heat-cure cold mould seal] was applied to the mold space and allowed to dry for few minutes. For fabricating the specimens of Group-A, using Heat cure acrylic resin ([DPI, Bombay Burmah Trading Corp Ltd, Uttarkhand, India)[Fig- 2] polymer and monomer were mixed in a ratio of 3:1 and in the dough stage the resin was packed into mold space. Trial closure was done and compressed with hydraulic press for 1 hr at 1200 psi. The flasks were bench cured for 20 min and heat cured at 74°C for 2 hrs and 100°C for 1 hr. After curing, the flasks were bench cooled to room temperature[26]. The 40 bar shaped specimens were retrieved. The specimens of each sub-group A1, A2, A3, A4 contains 10 samples each were finished using sandpaper and the specimens are not polished [Fig-3]. All the specimens are immersed in distilled water at 37°C for 7 days.

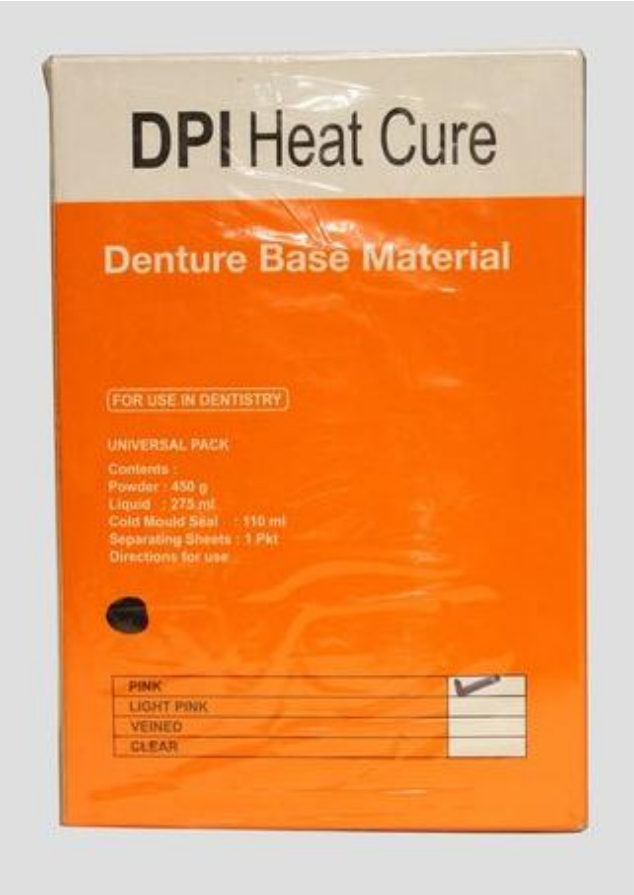


Fig- 2: DPI Heat Cure Resin

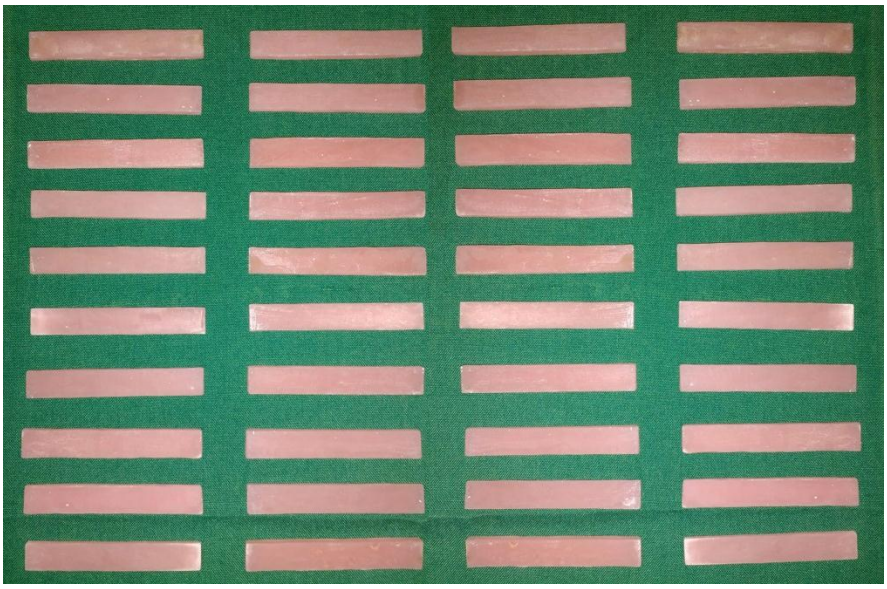


Fig- 3: Heat Cure Acrylic Resin Samples

Step 2: Preparation of Injection moulding acrylic resin:

For fabricating the specimens of Group B using injection moulding acrylic resin (SR-Ivocap High Impact, Ivoclar Vivadent, Liechtenstein) [Fig- 4] the 40 specimens were flasked according to the manu-facturer's instructions using the Ivocap flask. Before injecting into the flask premeasured SR-Ivocap capsules of resin and monomer (20 g powder, 30 mL monomer) were mixed in Cap vibrator (Ivoclar AG) for 5 minutes. For the curing process of the SR-Ivocap system, hydraulic pressure of 6 atm at 100°C was maintained for 35 minutes. using running water with a pressure of 6 atm, a 10-minute cooling process was used before deflasking the denture. Then, the specimens were deflasked and the surfaces were finished using sandpapers[27]. All the rectangular specimen of subgroup B1, B2, B3, B4 contains 10 samples each were stored in distilled water at room temperature until measured [Fig- 5].

Fig- 4: SR- Ivocap Injection molding Resin

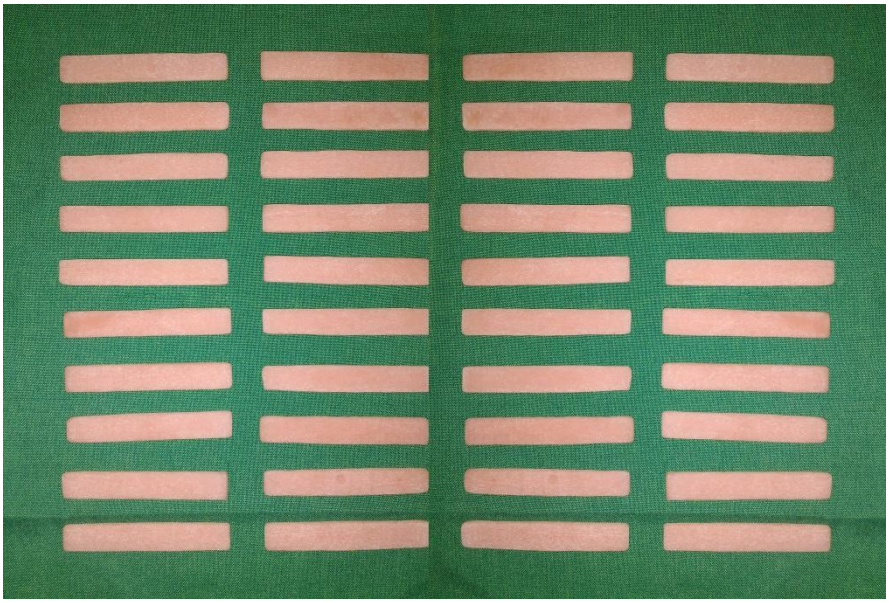


Fig-5: Injection Molding Acrylic Resin Samples

Step 3: Preparation of Flexible acrylic resin:

For fabricating the specimens of Group C the Sunflex semiflexible acrylic resin are used. The 40 specimens were prepared in a mold made by investing a stainless steel die as master models. For preparing samples the injection type oven is used. Stainless steel die specimens with specific dimension were inserted into the investing gypsum die stone and after final setting of material; these samples were connected with each other with hole of flask by three sprues wax. The separating medium is coated over the gypsum surface. The master models with sprues have been removed when the gypsum sets. After applying separating medium the flasks are locked by screws tightly on the surface of gypsum. Sunflex capsule of flexible material were used. At 288 degree C, using a special holder the capsule was grasped, and placed inside the oven for 16 minutes. Using a manual press the material was injected through a hole inside the flask. The flask was left inside press for 5 min, then

removed, and left for bench cooling for about 1 h, then deflasked and the samples were trimmed and sandpapering is done[28]. All the rectangular specimens of subgroup C1, C2, C3, C4 contain 10 samples each were stored in distilled water at room temperature until measured [Fig-6].

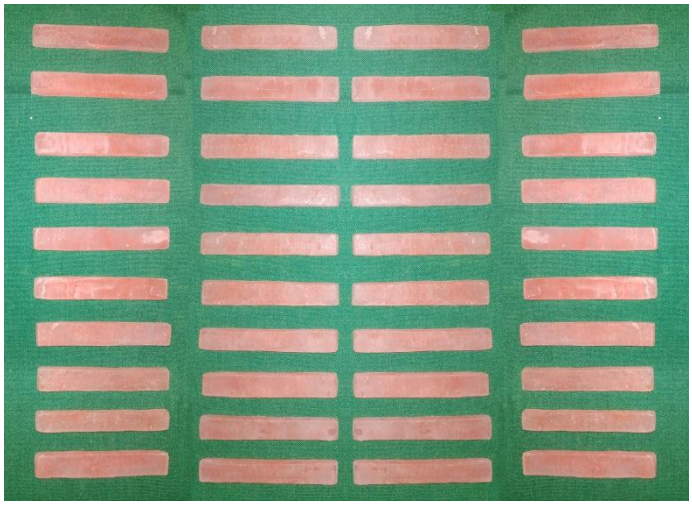
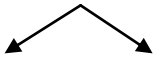


Fig-6: Flexible Acrylic Resin Samples

Step 4: Grouping of prepared samples

120 samples



60 samples (Linear dimensional stability)

60 samples (Sterilization)

Methods	Dimensional stability		Sterilization	
	Microwave irradiation for 650W for 3 mins	Microwave irradiation for 810W for 3 mins	Microwave irradiation for 650Wfor 3 mins	Microwave irradiation for 810W for 3 mins
Group – A Heat Cure	A1- 10	A2 – 10	A3 -10	A4 – 10
Group – B Injection molding	B1 – 10	B2 – 10	B3 -10	B4 – 10
Group – C Flexible acrylic	C1-10	C2 – 10	C3 – 10	C4 – 10

Group A (n=40) heat cure acrylic samples were prepared. Group A (n=40) samples were further divided into subgroup A1, A2, A3, A4.

Subgroup A1 (n=10) and Subgroup A2 (n=10) were subjected to microwave irradiation at 650W and 810W for 3 minutes respectively for testing the dimensional stability of samples.

Subgroup A3 (n=10) and subgroup A4 (n=10) were subjected to microwave irradiation at 650W and 810W for 3 minutes respectively for sterilization of heat cure acrylic samples.

Group B (n=40) injection moulding acrylic samples were prepared. Group B (n=40) samples were further divided into subgroup B1, B2, B3, B4.

Subgroup B1 (n=10) and subgroup B2 (n=10) were subjected to microwave irradiation at 650W and 810W for 3 minutes respectively for testing the dimensional stability of samples.

Subgroup B3 (n=10) and subgroup B4 (n=10) were subjected to microwave irradiation at 650W and 810W for 3 minutes respectively for sterilization of injection moulding acrylic samples.

Group C (n=40) flexible acrylic resin samples were prepared. Group C (n=40) samples were further divided into subgroup C1, C2, C3, C4.

Subgroup C1 (n=10) and subgroup C2 (n=10) were subjected to microwave irradiation at 650W and 810W for 3 minutes respectively for testing the dimensional stability of samples.

Subgroup C3 (n=10) and Subgroup C4 (n=10) were subjected to microwave irradiation at 650W and 810W for 3 minutes respectively for sterilization of flexible acrylic resin samples.

Step 5: Testing of samples for Dimensional Stability

In Group A heat cure acrylic samples Subgroup A1 (n=10) and Subgroup A2 (n=10) were measured for linear dimensional stability using the digital vernier calliper (Bombay Tools Center Pvt Ltd, Mazgaon, Mumbai) [Fig-7]. The linear measurements were performed over the length of the samples and values were note.

Fig-7: Digital vernier caliper

Fig- 8: Microwave oven

Subgroup A1 (n=10) samples were kept in microwave oven [Fig- 8] at 650W for 3 minutes and Subgroup A2 (n=10) were kept in microwave oven at 810W for 3 minutes. The samples were taken from the microwave oven. Again the samples were checked for the linear dimensional stability using the digital vernier calliper. Then the values were noted.

Similar procedures were followed for the injection moulding acrylic resin samples Subgroup B1 (n=10) & Subgroup B2 (n=10) and flexible acrylic resin

samples Subgroup C1 (n=10) & Subgroup C2 (n=10). The values were noted respectively.

Step 6: Testing of samples for sterilization status

a. Fungal Culture and Medium

The strain Candida albicans are being used because the main cause of denture stomatistis in denture wearing patients is candidiasis. For the enrichment, basic culture media namely, Sabouraud Dextrose Broth (SDB) containing (g/l) 20g of dextrose, 10g of peptone and pH 5.6 was used. The medium was prepared and sterilized at 121^{o}C. To it, a loopful of Candida albicans was inoculated and incubated at 37^{o}C under shaking condition. Further, the enriched strain from the broth was purified by repeated streaking on Sabouraud Dextrose Agar (SDA); individual viable colonied were picked and stored in SDA slants for further studies.

Candida albicans

b. Adherence of Candida albicans in acrylic samples

The Candida albicans strain was cultured in Sabouraud Dextrose Broth for 18 hrs at 37°C. The culture broth was centrifuged at 5000 rpm for 20 min. The culture suspensions were adjusted to 1.0 Optical Density (OD) at 600 nm using a UV-Vis spectrophotometer (Model: Cyber Lab UV 100, USA) by diluting with sterile distilled water to get an approximate cell density of 10^{7} CFU/ml. About 1% of the above suspension was used as inoculum for the adherence study.

For the adherence of Candida albicans in acrylic samples, Sabouraud Dextrose Broth containing (g/l) 20g of dextrose, 10g of peptone and pH 5.6 was prepared. There three sets of medium (100 ml v/v) were prepared in

separate conical flasks since we used three types of acrylic samples namely heat cure acrylic resin, injection moulding acrylic resin, flexible acrylic resin. The medium was sterilized by autoclaving at 121^{o}C. After medium cooled, the prepared inoculum suspension of Candida albicans were inoculated under aseptic conditions and incubated under Orbital shaker (120 rpm) at 37^{o}C for 24 h. The samples were autoclaved at 121 degree C for complete sterilization of the samples. Then the acrylic samples (n=10 in each type) were dipped in to the each flasks containing enrich medium. Samples were allowed for one hour to adhere with Candida albicans [Fig-9].

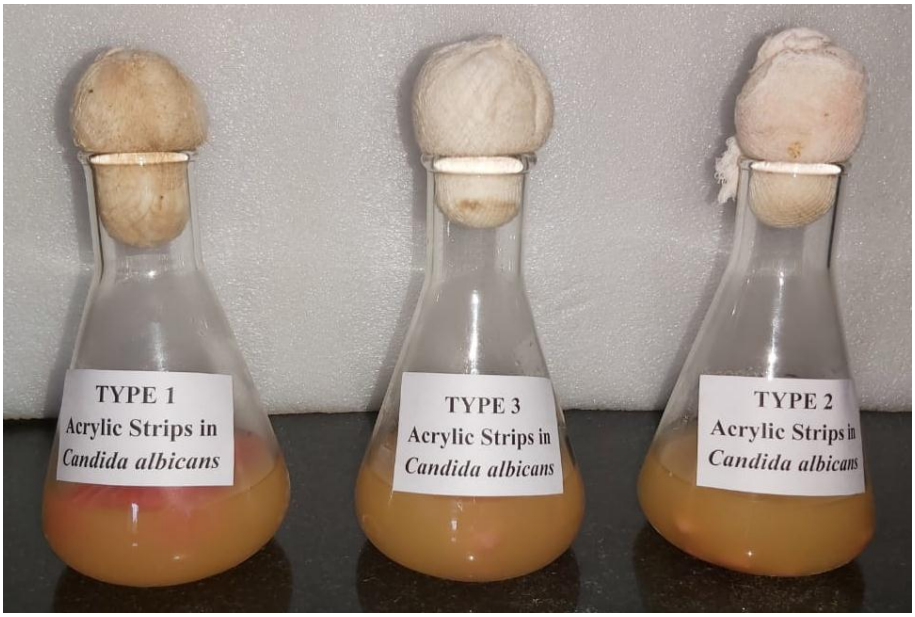


Fig-9: Type 1- Heat cure Acrylic resin
Type 2- Injection moulding Acrylic resin
Type 3- Flexible Acrylic resin

c. Assessment of Candida albicans adherence

The samples allowed with adherence of Candida albicans were tested for their survivability in SDA plates. For the heat cure acrylic samples, samples were taken from the flask containing Candida albicans enrichment and

Subgroup A3 (n=10) were sterilized by keeping them in a microwave oven at 650W for 3 minutes and Subgroup A4 (n=10) were sterilized by keeping them in a microwave oven at 810W for 3 minutes. Similar procedures were followed for the Subgroup B3 (n=10) and Subgroup B4 (n=10) injection moulding acrylic resin samples and Subgroup C3 (n=10) & Subgroup C4 (n=10) flexible acrylic resin samples.

After sterilization of Candida albicans in microwave oven, all the samples were horizontally placed under aseptic conditions on the medium in SDA plates and incubated at 37^{o}C for 24 h [Fig-10].

Fig-10: After Sterilization samples placed in SDA plates

Comparatively, a loopful culture of Candida albicans were picked from each acrylic samples and they were in inoculated on SDA plates by simple streaking. Control was maintained by streaking live Candida albicans on agar plate and incubated at 37^{o}C for 24hrs [Fig-11, 12, 13]. Finally the agar plates were examined for any growth of candida albicans.

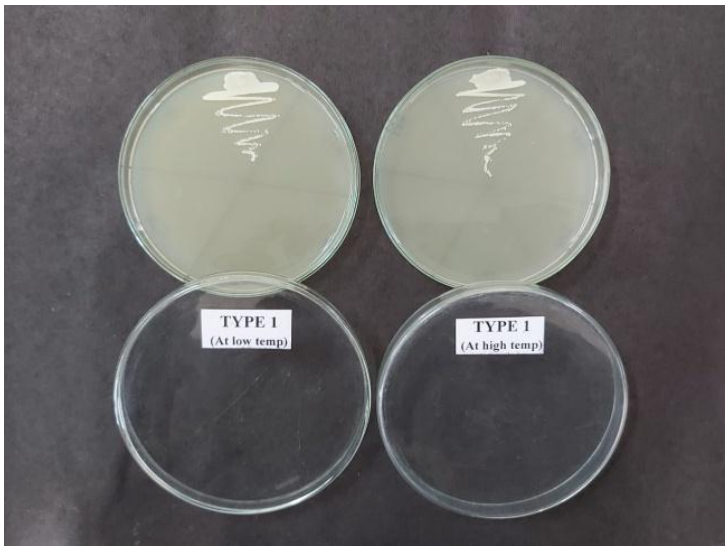


Fig – 11: Assessment of Candida albicans growth in heat cure acrylic resin samples

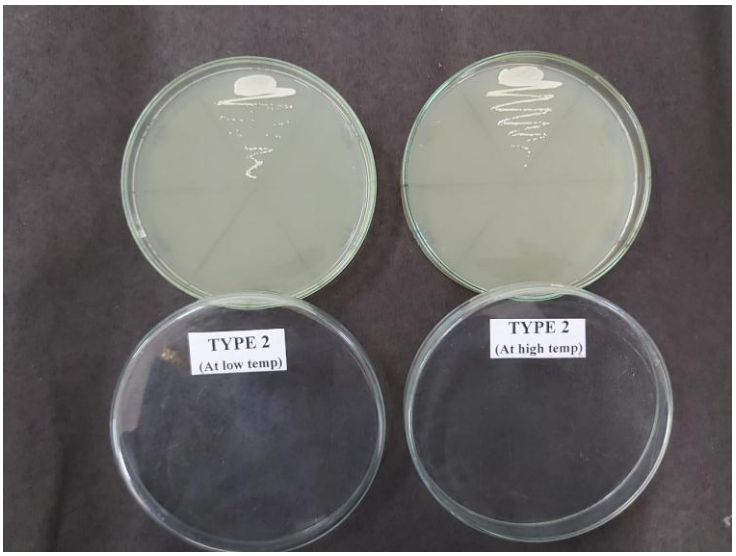


Fig – 12: Assessment of Candida albicans growth in Injection molding acrylic resin samples

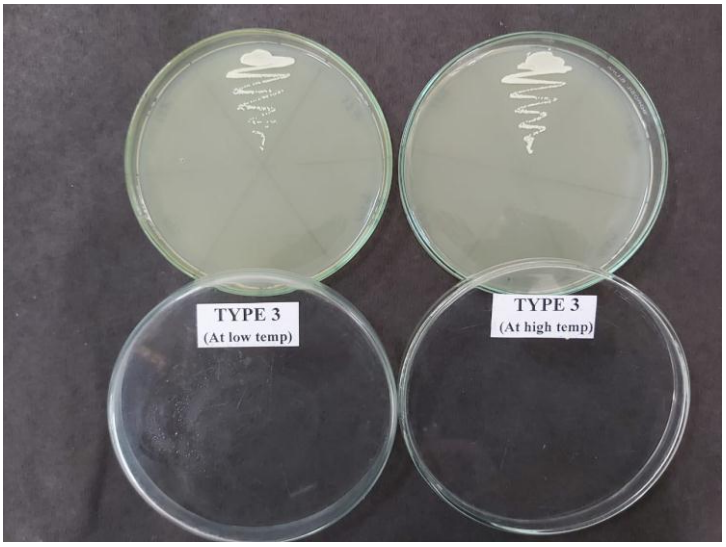


Fig- 13: Assessment of Candida albicans growth in Flexible acrylic resin samples

TESTING VALUES

TESTING VALUES

1: Dimensional stability of Heat cure acrylic resin subjected to Microwave Irradiation at 650W and 810W for 3 minutes

Sl.no	Before microwave irradiation at 650W for 3mins (mm)	After microwave irradiation at 650W for 3mins (mm)
1.	64	64.1
2.	64	64.1
3.	64	64
4.	64	64.2
5.	64	64.3
6.	64	64.1
7.	64	64.1
8.	64	64
9.	64	64
10.	64	64.1

Sl.no	Before microwave irradiation at 810W for 3mins (mm)	After microwave irradiation at 810W for 3mins (mm)
1.	64	64.1
2.	64	64
3.	64	64.1
4.	64	64
5.	64	64
6.	64	64
7.	64	64.1
8.	64	64.1
9.	64	64
10.	64	64

2: Dimensional stability of Injection moulding acrylic resin subjected to Microwave Irradiation at 650W and 810W for 3 minutes

Sl.no	Before microwave irradiation at 650W for 3mins (mm)	After microwave irradiation at 650W for 3mins (mm)
1.	64	63.9
2.	64	63.9
3.	64	63.9
4.	64	64
5.	64	64
6.	64	64
7.	64	63.9
8.	64	63.9
9.	64	64
10.	64	64

Sl.no	Before microwave irradiation at 810W for 3mins (mm)	After microwave irradiation at 810W for 3mins (mm)
1.	64	63.8
2.	64	63.9
3.	64	64
4.	64	64
5.	64	64
6.	64	63.9
7.	64	63.9
8.	64	63.9
9.	64	64
10.	64	64

3: Dimensional stability of Flexible acrylic resin subjected to Microwave Irradiation at 650W and 810W for 3 minutes

Sl.no	Before microwave irradiation at 650W for 3mins (mm)	After microwave irradiation at 650W for 3mins (mm)
1.	64	64
2.	64	64
3.	64	63.9
4.	64	63.9
5.	64	64
6.	64	64
7.	64	63.9
8.	64	64
9.	64	64
10.	64	63.9

Sl.no	Before microwave irradiation at 810W for 3mins (mm)	After microwave irradiation at 810W for 3mins (mm)
1.	64	63.9
2.	64	64
3.	64	64
4.	64	64
5.	64	63.9
6.	64	64
7.	64	64
8.	64	63.9
9.	64	63.9
10.	64	64

Sterilization status of the Acrylic samples subjected to Microwave Irradiation at 650W and 810W for 3 minutes:

The presence of Candida albicans in acrylic samples was investigated using Sabouraud Dextrose Agar. The results revealed that there is no growth of Candida albicans when the medium directly streaked with Candida albicans. The growth was shown only in control (Table 4).

4.Assessment of Candida albicans presence in acrylic samples by streaking on SDA medium

Name of the Sample	Type 1- Heat cure acrylic resin samples		Type 2 – Injection moulding acrylic resin samples		Type 3 – flexible acrylic resin samples	
	Microwave irradiation at 650W for 3 Min	Microwave irradiation at 810W for 3 mins	Microwave irradiation at 650W for 3 Min	Microwave irradiation at 810W for 3 mins	Microwave irradiation at 650W for 3 Min	Microwave irradiation at 810W for 3 mins
Control	+	+	+	+	+	+
Sample 1	-	-	-	-	-	-
Sample 2	-	-	-	-	-	-
Sample 3	-	-	-	-	-	-
Sample 4	-	-	-	-	-	-
Sample 5	-	-	-	-	-	-
Sample 6	-	-	-	-	-	-
Sample 7	-	-	-	-	-	-
Sample 8	-	-	-	-	-	-
Sample 9	-	-	-	-	-	-
Sample10	-	-	-	-	-	-

RESULTS

Table 1: Comparison of the mean dimensional stability of Heat cure acrylic resin, Injection moulding acrylic resin, Flexible acrylic resin before and after microwave irradiation at 650W and 810W for 3minutes

Paired Samples Test									
Group		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Heat cure acrylic resin	before and after microwave irradiation at 650W	-0.10000	0.09428	0.02981	-0.16744	-0.03256	-3.354	9	0.008*
	before and after microwave irradiation at 810W	-0.04000	0.05164	0.01633	-0.07694	-0.00306	-2.449	9	0.037*
Injection moulding acrylic resin	before and after microwave irradiation at 650W	0.05000	0.05270	0.01667	0.01230	0.08770	3.000	9	0.15
	before and after microwave irradiation at 810W	0.06000	0.06992	0.02211	0.00998	0.11002	2.714	9	0.24
Flexible acrylic resin	before and after microwave irradiation at 650W	0.04000	0.05164	0.01633	0.00306	0.07694	2.449	9	0.370
	before and after microwave irradiation at 810W	0.04000	0.05164	0.01633	0.00306	0.07694	2.449	9	0.370

*Significant at $p<0.05$, Paired t-test

Table 2: Comparison of the mean dimensional stability among Heat cure acrylic resin, Injection moulding acrylic resin and Flexible acrylic resin after microwave irradiation at 650W for 3minutes

Group	N	Mean	Std. Deviation	F value	p-value
Heat cure acrylic resin	10	64.1000	0.09428	14.721	<0.001*
Injection moulding acrylic resin	10	63.9500	0.05270		
Flexible acrylic resin	10	63.9600	0.05164		
Total	30	64.0033	0.09643		

*Significant at p<0.05, One-way ANOVA

Post-hoc test: TUKEY HSD

Group (1)	Group (2)	Mean Difference (1-2)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Heat cure acrylic resin	**Injection moulding acrylic resin**	0.15000*	0.03091	0.000	0.0734	0.2266
	Flexible acrylic resin	0.14000*	0.03091	0.000	0.0634	0.2166
Injection moulding acrylic resin	**Heat cure acrylic resin**	-0.15000*	0.03091	0.000	-0.2266	-0.0734
	Flexible acrylic resin	-0.01000	0.03091	0.944	-0.0866	0.0666
Flexible acrylic resin	**Heat cure acrylic resin**	-0.14000*	0.03091	0.000	-0.2166	-0.0634
	Injection moulding acrylic resin	0.01000	0.03091	0.944	-0.0666	0.0866
* The mean difference is significant at the 0.05 level.						

Table 3: Comparison of the mean dimensional stability among Heat cure acrylic resin, Injection moulding acrylic resin and Flexible acrylic resinafter microwave irradiation at 810W for 3minutes

Group	N	Mean	Std. Deviation	F value	p-value
Heat cure acrylic resin	10	64.0400	0.05164	8.217	0.002*
Injection moulding acrylic resin	10	63.9400	0.06992		
Flexible acrylic resin	10	63.9600	0.05164		
Total	30	63.9800	0.07144		

*Significant at p<0.05, One-way ANOVA

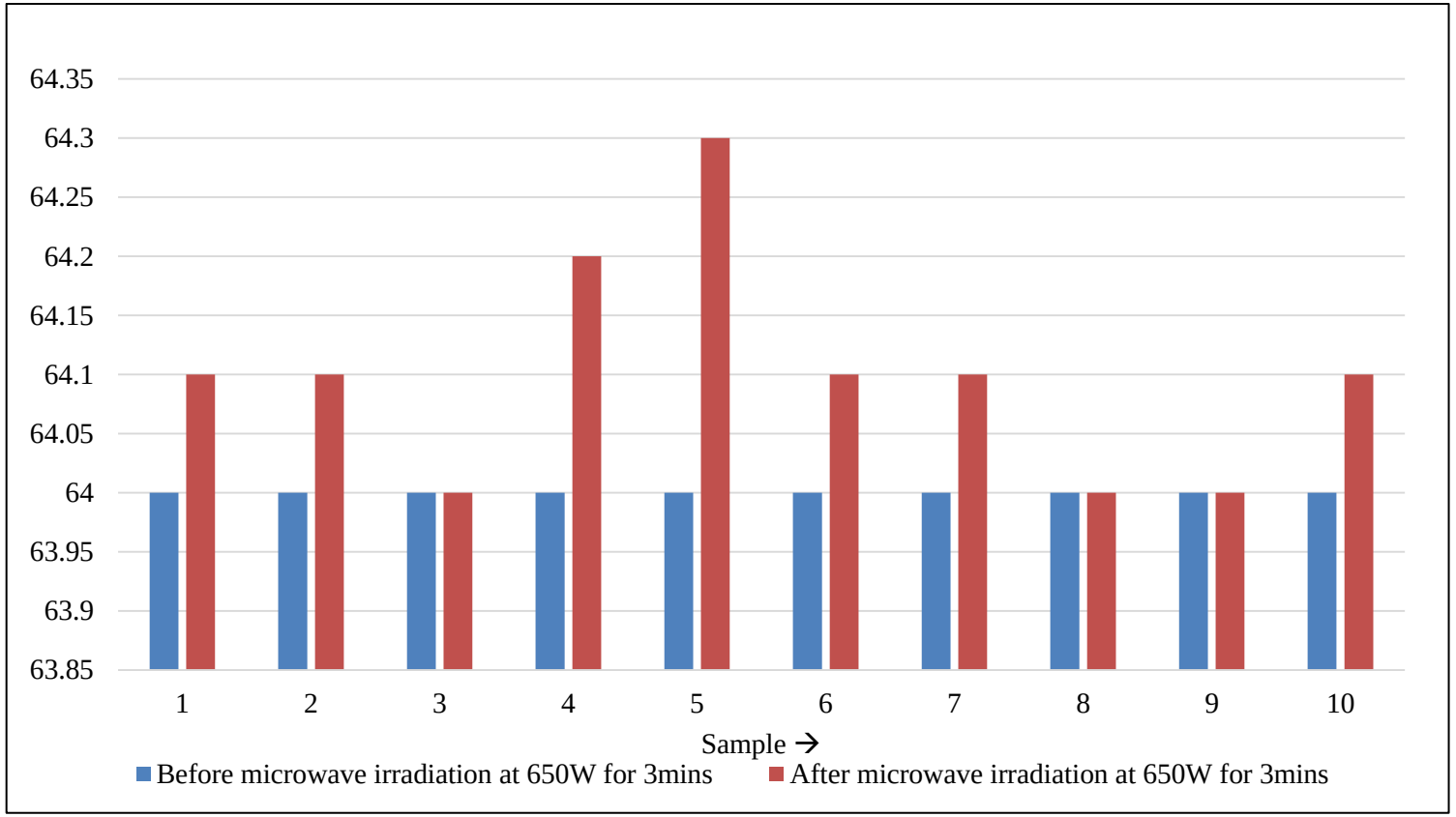
Post-hoc test: TUKEY HSD

Group (1)	Group (2)	Mean Difference (1-2)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Heat cure acrylic resin	**Injection moulding acrylic resin**	0.10000*	0.02611	0.002	0.0353	0.1647
	Flexible acrylic resin	0.08000*	0.02611	0.013	0.0153	0.1447
Injection moulding acrylic resin	**Heat cure acrylic resin**	-0.10000*	0.02611	0.002	-0.1647	-0.0353
	Flexible acrylic resin	-0.02000	0.02611	0.727	-0.0847	0.0447
Flexible acrylic resin	**Heat cure acrylic resin**	-0.08000*	0.02611	0.013	-0.1447	-0.0153
	Injection moulding acrylic resin	0.02000	0.02611	0.727	-0.0447	0.0847
* The mean difference is significant at the 0.05 level.						

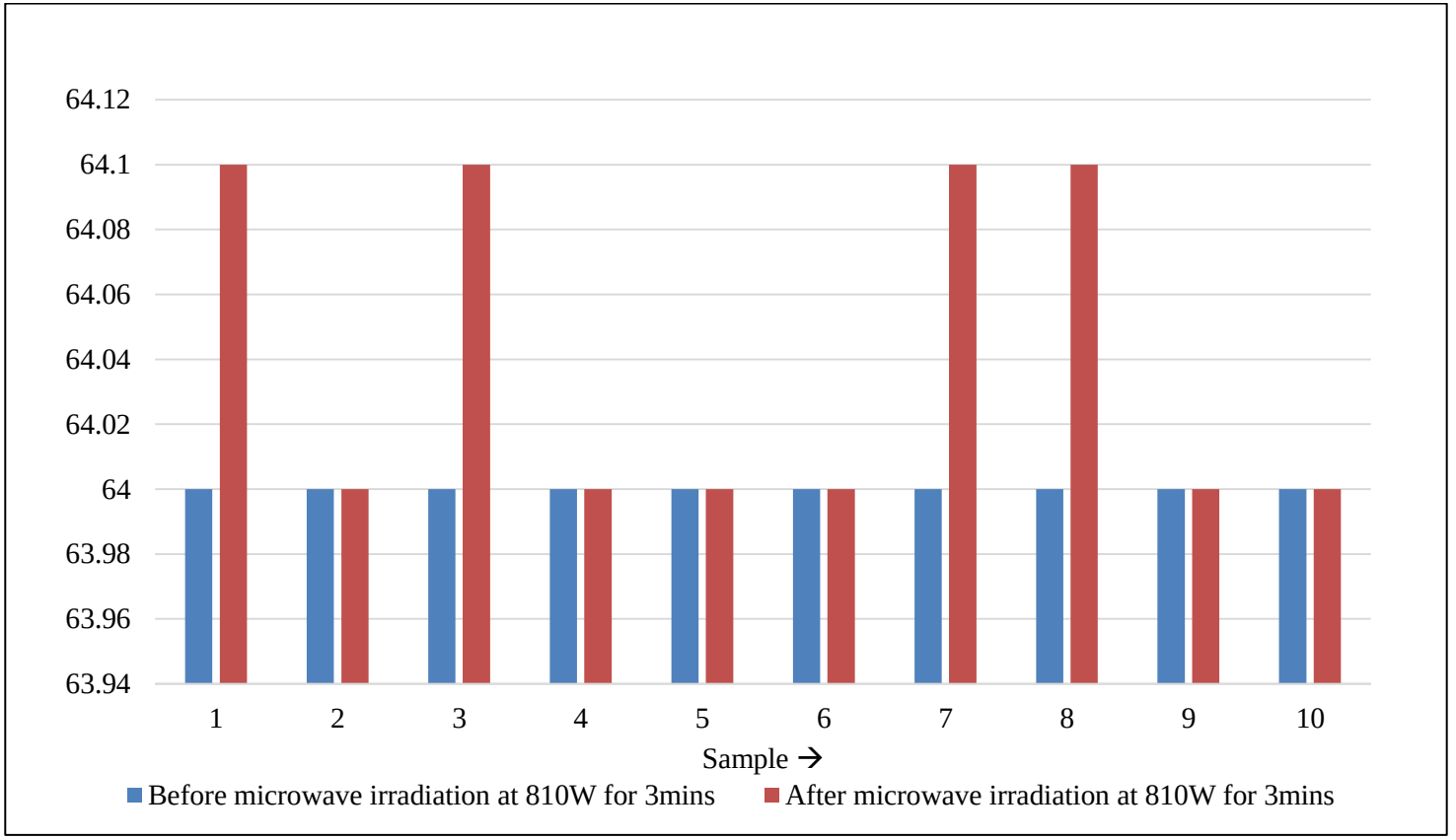
Table 4: Assessment of Candida albicans presence in acrylic samples by streaking on SDA medium

Name of the Sample	Type 1- Heat cure acrylic resin samples		Type 2 – Injection moulding acrylic resin samples		Type 3 – flexible acrylic resin samples	
	Microwave irradiation at 650W for 3 Min	Microwave irradiation at 810W for 3 mins	Microwave irradiation at 650W for 3 Min	Microwave irradiation at 810W for 3 mins	Microwave irradiation at 650W for 3 Min	Microwave irradiation at 810W for 3 mins
Control	+	+	+	+	+	+
Sample 1	-	-	-	-	-	-
Sample 2	-	-	-	-	-	-
Sample 3	-	-	-	-	-	-
Sample 4	-	-	-	-	-	-
Sample 5	-	-	-	-	-	-
Sample6	-	-	-	-	-	-
Sample7	-	-	-	-	-	-
Sample 8	-	-	-	-	-	-
Sample9	-	-	-	-	-	-
Sample10	-	-	-	-	-	-

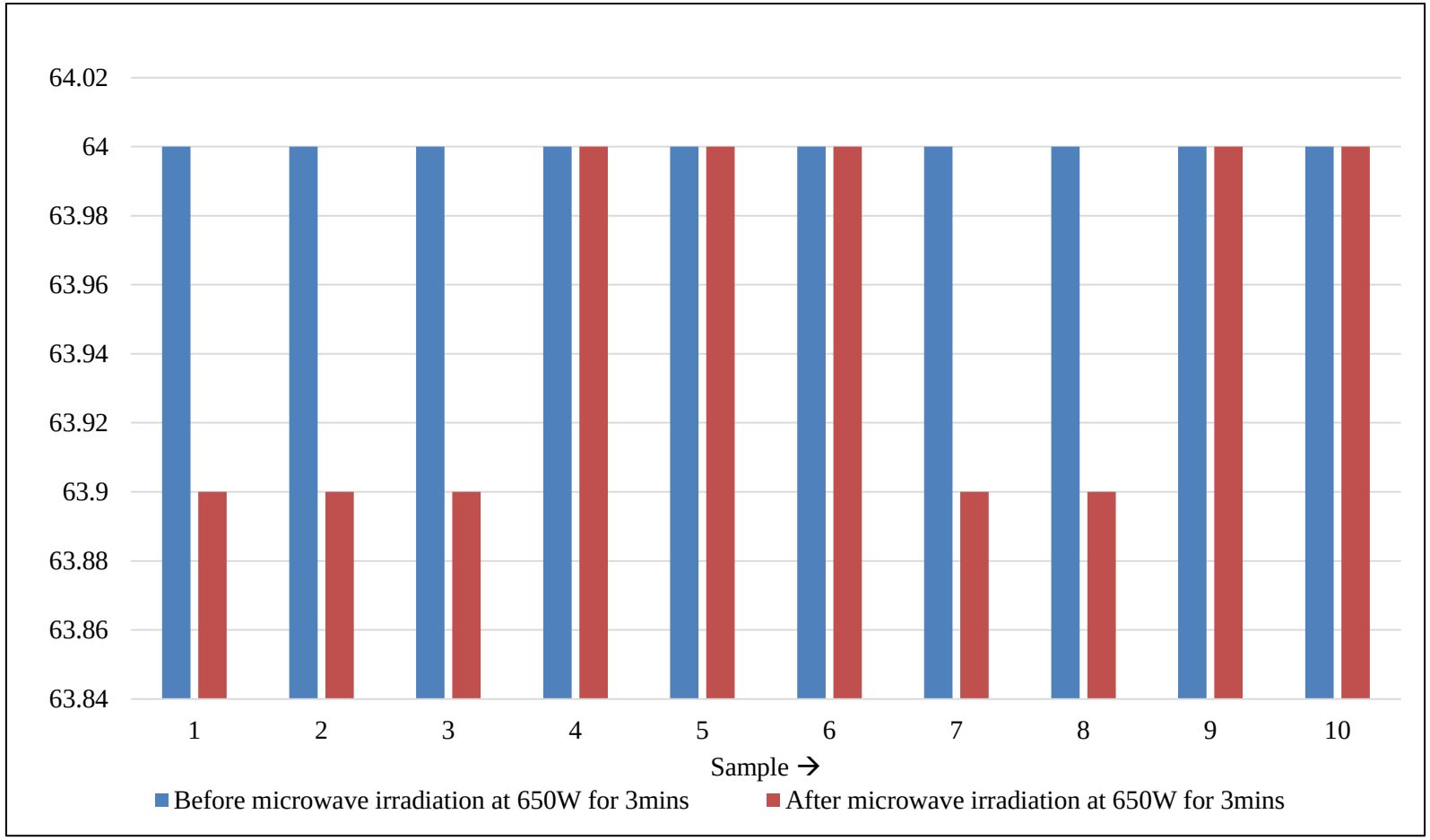
Graph 1: Distribution of the dimensional stability of Heat cure acrylic resin samples (N=10) before and after microwave irradiation at 650W for 3minutes



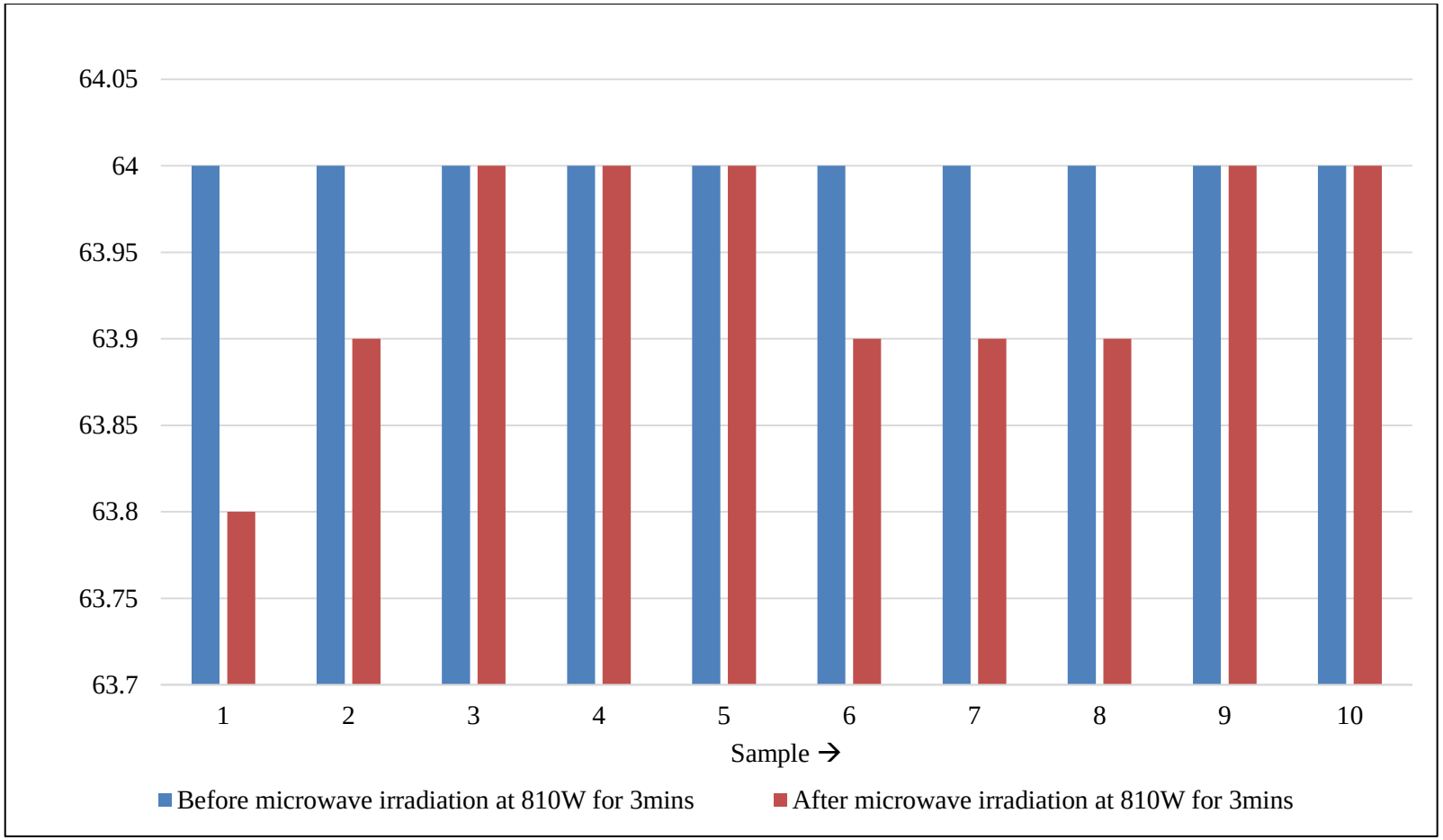
Graph 2: Distribution of the dimensional stability of Heat cure acrylic resin samples (N=10) before and after microwave irradiation at 810W for 3minutes



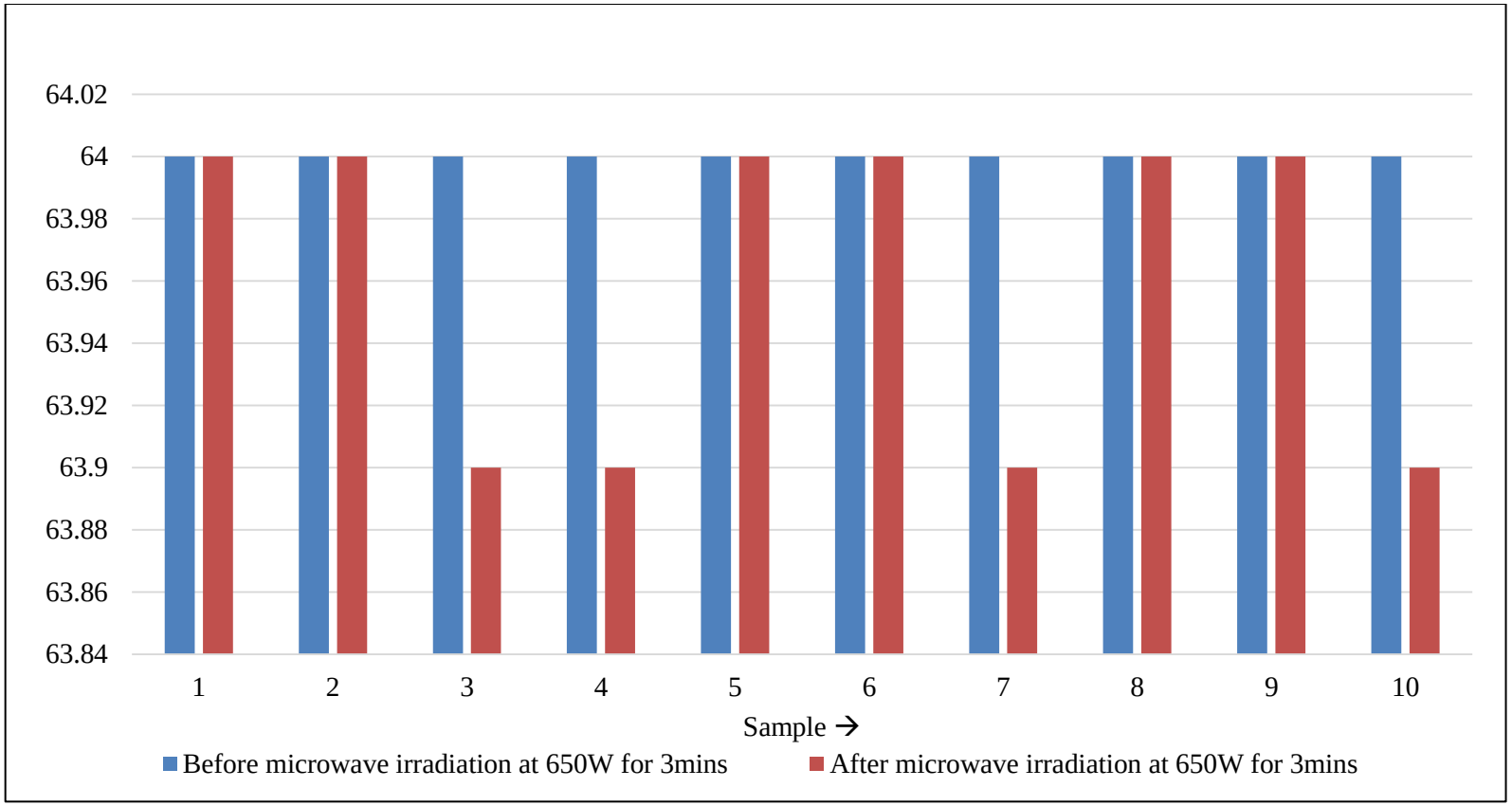
Graph 3: Distribution of the dimensional stability of Injection moulding acrylic resin samples (N=10) before and after microwave irradiation at 650W for 3minutes



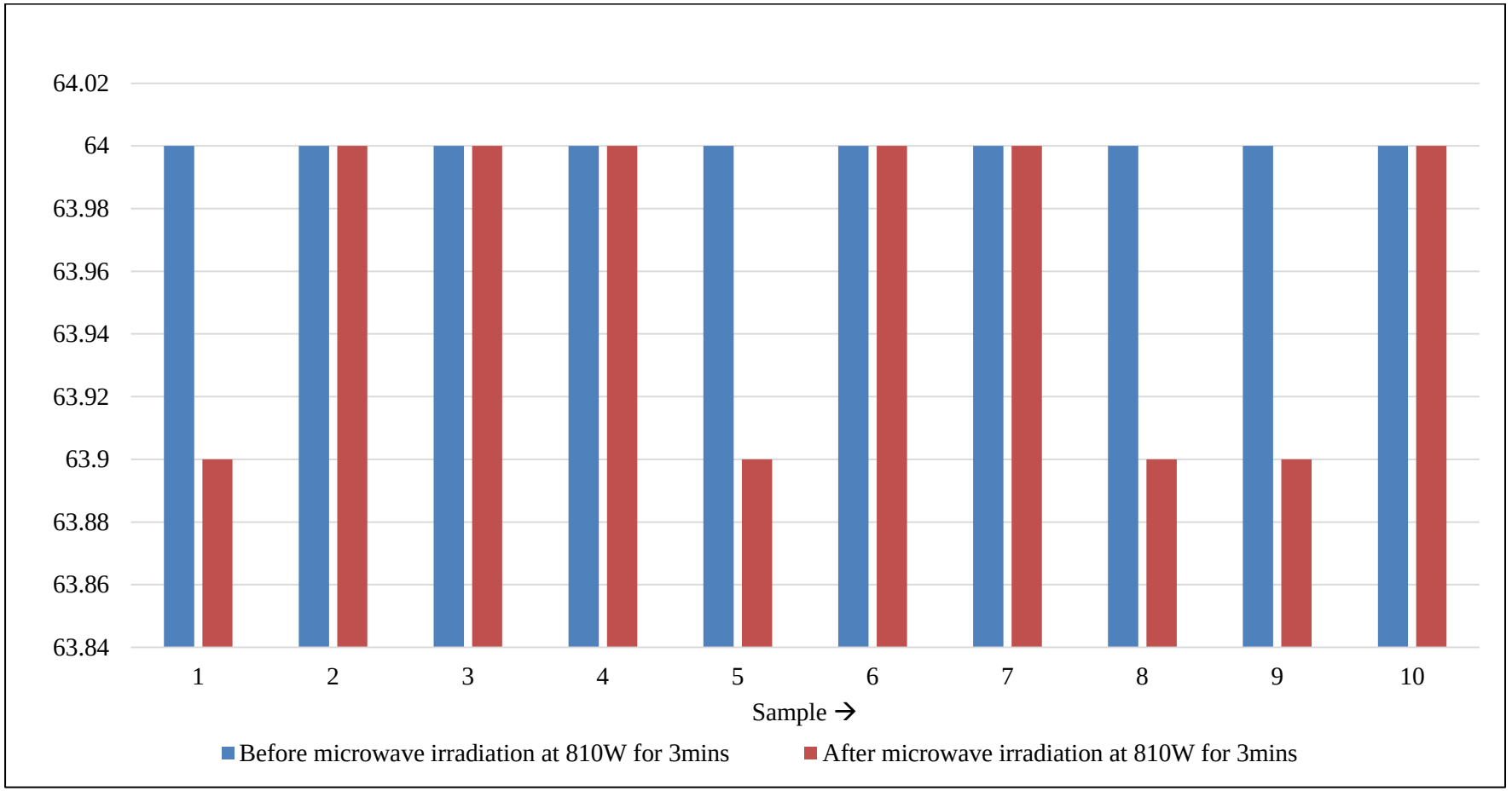
Graph 4: Distribution of the dimensional stability of Injection moulding acrylic resin samples (N=10) before and after microwave irradiation at 810W for 3minutes



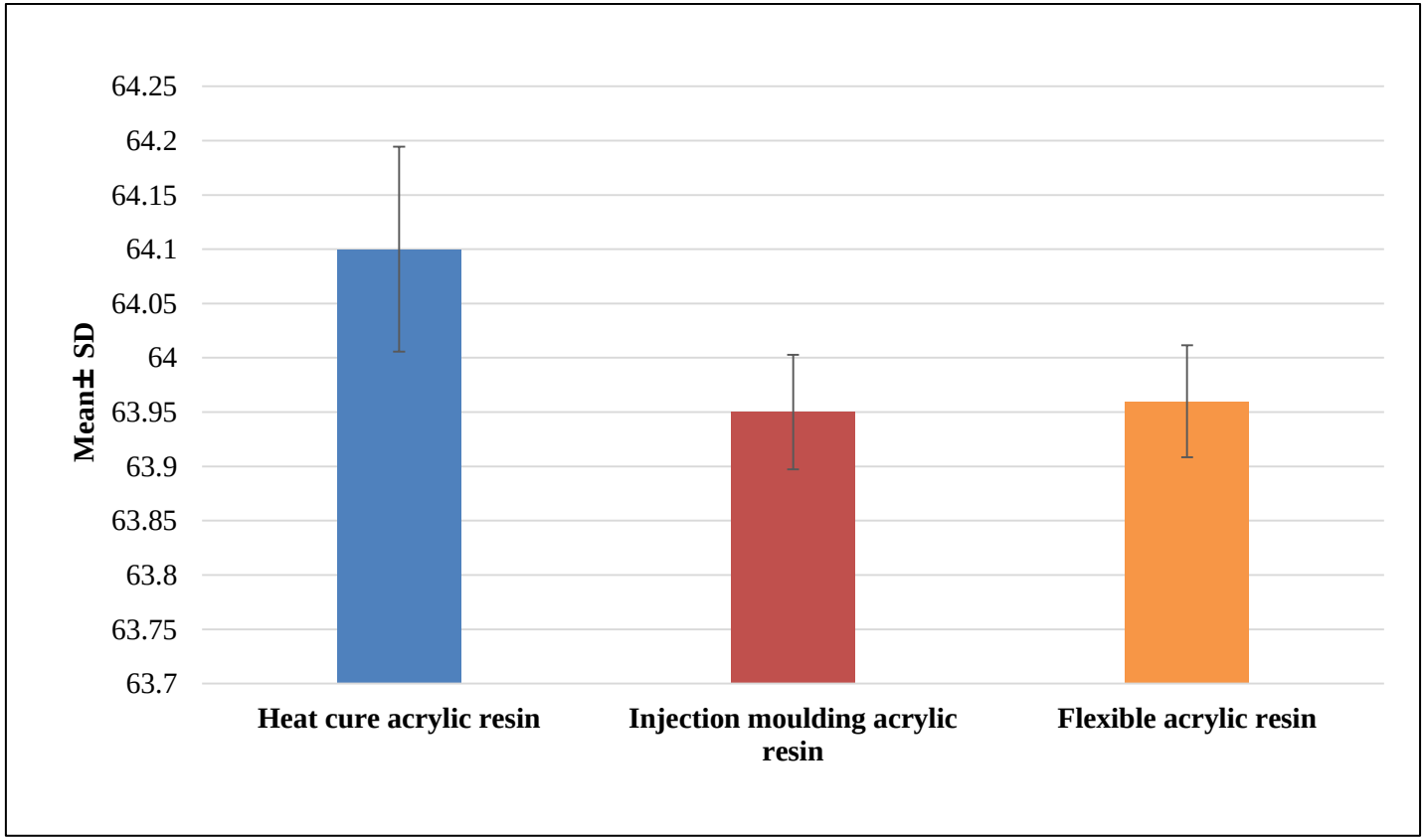
Graph 5: Distribution of the dimensional stability of Flexible acrylic resinsamples (N=10) before and after microwave irradiation at 650W for 3minutes



Graph 6: Distribution of the dimensional stability of Flexible acrylic resin samples (N=10) before and after microwave irradiation at 810W for 3minutes



Graph 7: Distribution of the mean dimensional stability among Heat cure acrylic resin, Injection moulding acrylic resin and Flexible acrylic resin after microwave irradiation at 650W for 3minutes



Graph 8: Distribution of the mean dimensional stability among Heat cure acrylic resin, Injection moulding acrylic resin and Flexible acrylic resin after microwave irradiation at 810W for 3minutes

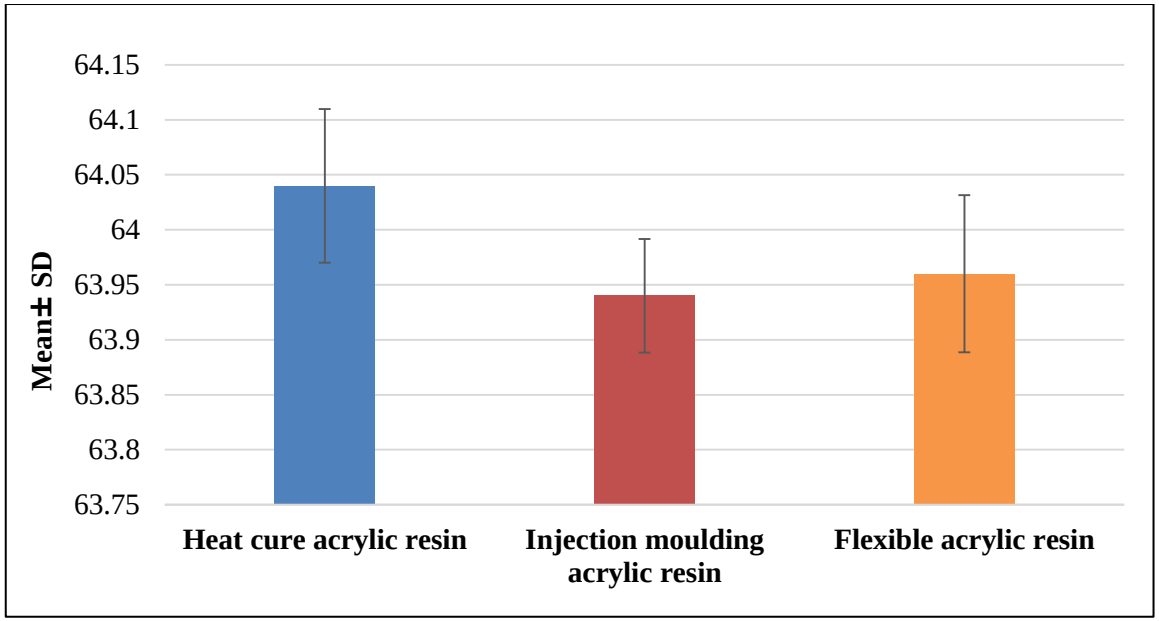


Table 5: Percentage change in dimension

Temp	Group	Mean	SD	Std. Error	95% Confidence Interval for Mean		F	P value
					Lower Bound	Upper Bound		
650W	Heat Cure	.00156220	.001473021	.000465810	.00050846	.00261594	14.72	0.001
	Inj Moulding	-.00078000	.000822192	.000260000	-.00136816	-.00019184		
	Flexible Resin	-.00062400	.000805581	.000254747	-.00120028	-.00004772		
	Total	.00005273	.001505757	.000274912	-.00050953	.00061499		
810W	Heat Cure	.00062480	.000806613	.000255074	.00004778	.00120182	8.21	0.002
	Inj Moulding	-.00093700	.001092988	.000345633	-.00171888	-.00015512		
	Flexible Resin	-.00062400	.000805581	.000254747	-.00120028	-.00004772		
	Total	-.00031207	.001115773	.000203711	-.00072870	.00010457		

Test: One-way ANOVA

Inference: the mean percentage change in dimension is significantly different between the 3 groups at both 650 and at 910 W

DISCUSSION

DISCUSSION

Acrylic resins have been used for fabrication of dentures for about 60 years. For the patients who visits the dentists for corrections of the existing dentures. The denture has to be disinfected. So there is need to establish a disinfection protocol that is effective, inexpensive and less time consuming.

Chemical disinfection of dentures is commonly achieved by soaking in alkaline glutaraldehyde, sodium hypochlorite, aqueous formaldehyde, or enzymatic solutions. These are still controversial because they may alter some material properties and may induce resistance to candida albicans. Some alcohol-based disinfectants reduce the flexural strength of non-crosslinked denture base acrylic resins. Surface alteration and roughness may occur by continuous use of some disinfection methods, for example, staining (by soaking in chlorhexidine) or bleaching (by soaking in sodium hypochlorite). As a simple alternative to overcome the disadvantages of chemical disinfection on prosthesis, the use of microwave energy has been suggested. It is considered an easily accessible, simple to perform, and effective low-cost method, yet its effect on resin properties after repeated procedures is not clear.[1]

Webb et al. in 1998 did a study to test the in vitro efficacy of two methods of denture sterilization (microwave irradiation and sodium hypochlorite soak). The results of his study indicated that microwaving may be a more effective method of denture sterilization than denture soaking in sodium hypochlorite. There have been a number of studies carried out by other investigators in relation to the sterilization of microorganisms by microwave irradiation (Rohrer et al., 1985). But (Rohrer and

Bulard, 1985) was concerned with the sterilization of microorganisms on denture surfaces and its effects on the dentures.[1]

There has been no studies examined the effect of microwave irradiation on the different acrylic resins (conventional heat cure acrylic resin, injection moulding acrylic resin, flexible acrylic resin) which is commonly used in daily practice. In this study conventional heat cure acrylic resin, injection moulding acrylic resin, flexible acrylic resin were checked for linear dimensional stability before and after subjected to the microwave irradiation. It was observed in conventional heat cure resin, injection moulding resin and flexible acrylic resin there was change in the linear dimensional stability after subjected to microwave irradiation at 650W and 810W for 3 minutes and there was statistically significant change in linear dimensional stability for conventional heat cure resin, injection moulding resin and flexible acrylic resin.

Polyzois et al in his study established that storage of acrylic resin in water induces linear changes; the linear expansion of up to 0.03% partially compensates the overall polymerization shrinkage. The finding that microwave disinfection sterilization procedures produced negligible dimensional changes (-0.005% to 0.009%) is in agreement with a previous report by Burns et al, who found dimensional changes, shrinkage up to 0.03%, of cylindrical specimens (36 mm long, 6 mm diameter) after 15 minutes of microwave exposure.[29]

Rohrer and Bulard, who found that dentures subjected to microwaves for up to 16 minutes, showed no dimensional changes when evaluated by analyzing the fit of the denture into impressions that had been made into die stone.[2]

PMMA resin irradiated in the dry state may distort up to 0.03%. However, when immersed in water, Basso et al reported distortion values of up to 0.5%, with a limit of 1% considered clinically acceptable.[30] In the present study, the 650W and

810W for 3 minutes power levels caused less distortion of 0.003 – 0.01% which is below the clinical limit of 1 %. So it is considered safer to use the acrylic resins for microwave irradiation.

Various studies have shown the efficacy of microwave irradiation. However, there is no standard way of using microwaves and there is much controversy regarding the duration and power of its irradiation as a physical technique in disinfecting dentures.[3]

More than 200 different species of the fungi Candida exist and some of them are commonly found in the oral cavity, without causing any disturbance. However, this family of fungi is the predominant oral yeast infection associated with denture stomatitis. Denture stomatitis is a mucosal inflammation of multifactorial nature associated with several local and systemic factors. Allergic reaction to denture material, uninterrupted denture wearing, ill-fitting dentures, poor oral hygiene, acidic saliva, high carbohydrate intake, long-term antibiotic therapy, diabetes mellitus and arterial hypertension are just some of the local and systemic factors that can favour the growth of fungi in the oral cavity. Candida albicans species in particular seems to play a major role in the initiation and progress of denture stomatitis which remains a chronic condition in the elderly. In some cases, patients with stomatitis can have the entire digestive tract colonised by Candida, making them more prone to sudden and severe systemic infections including pneumonia.

Since Candida is the main cause of denture stomatitis, most studies have focused on the effect of microwave irradiation on this microorganism. However, the role of S. aureus and P. aeruginosa has also been documented. In this study the candida albicans strain is used for detecting the sterilization status of the three different acrylic samples.

Microwave oven irradiation for disinfection of non autoclavable dental instruments and dentures was first introduced by Rohrer and Bulard. Rohrer and Bulard reported that complete dentures contaminated with individual suspensions of aerobic bacteria and fungi were sterilized after 10 min of microwave irradiation (720 W). It was also demonstrated that microwave irradiation at 350W for 6min was a more effective method of inactivating microorganisms on dentures than soaking in sodium hypochlorite.[2]

Webb et al recommended microwave irradiation for 2 minutes at 650W has a more effective sterilization method for dentures inoculated with Streptococcus gordonii than soaking them in sodium hypochlorite.[31]

Barnabe et al studied the effect of three different disinfection techniques (i.e., mechanical and chemical techniques and a combination of them) on Escherichia coli, S. aureus, Enterococcus faecalis, Candida albicans, Streptococcus mutans, and P. aeruginosa. It was concluded that these techniques yield different results depending on the type of the microbial biofilms on acrylic resin samples.

Nepelenbroek et al demonstrated that acrylic resin specimens contaminated with individual suspensions of three bacteria (P. aeruginosa, S. aureus, B. subtilis) were sterilized by microwave irradiation (6 minutes/650 W). More recently, the same microwave regimen (6 minutes/650 W) was effective for sterilizing complete dentures contaminated with S. aureus. These findings support that microwave irradiation may present a suitable alternative for disinfecting complete dentures and will possibly help overcome the limitations of current soak treatments.[4]

Mima et al observed that specimens of a hard chairside reline resin showed consistent sterilization of C. albicans, P. aeruginosa, S. aureus, and B. subtilis after exposure times shorter than 6 minutes at 650 W. Microwaving for 3 minutes

promoted inactivation of all species evaluated. In addition, a recent study showed that microwave disinfection for 3 minutes at 650 W significantly improved denture base adaptation when the traditional flask closure method was used.[4]

One possible explanation for complete inactivation of B. subtilis obtained by Mima et al is that the microwave exposure was performed on small-dimension specimens (10-mm long, 1-mm thick), which had been processed against an acetate sheet and glass slab. This procedure resulted in the specimens having a mirror-like finish, which is less likely to facilitate microbial retention than a surface with a higher roughness, such as the tissue surface of a denture base and any unpolished areas, where acrylic resin irregularities are more pronounced.[4]

An earlier study evaluated the effectiveness of microwave disinfection (6 minutes/650 W) of three hard chairside reline resins and showed consistent sterilization of four pathogenic microorganisms. Further evidence of the use of microwave irradiation was provided by Silva et al, who demonstrated that 6 minutes of microwave exposure at 650 W resulted in sterilization of complete dentures contaminated with S. aureus and C. albicans; however, despite its effectiveness, the effect of this disinfection protocol on the physical and mechanical properties of the denture materials must be carefully considered. It has been observed that this procedure promoted a significant increase in the mean linear dimensional change (shrinkage) of denture base and relining materials.

Additionally, Seo et al verified that thermal and mechanical stress exerted deleterious effects on the strength of intact and/or relined denture bases. A decrease in the surface hardness of five brands of acrylic resin denture teeth has also been observed. Thus, reduced microwave exposure should be evaluated to prevent any detrimental effect on acrylic resins. Recently, sterilization of acrylic resin specimens

inoculated with individual suspensions of four microorganisms (C. albicans, P. aeruginosa, S. aureus, B. subtilis) was achieved at 3-, 4-, and 5-minute exposure times at 650 W. Furthermore, the mechanical properties of the materials evaluated were not detrimentally affected by these exposure times to microwave irradiation.

A recent study has demonstrated that microwave energy irradiation in a conventional domestic oven for 3 min at 650 W was capable to sterilize all complete dentures inoculated with 5 Candida species. Considering the probability of the denture base been contaminated internally and externally, microwave energy appears as an effective method for complete denture disinfection and prevention of cross-contamination

In this study the presence of Candida albicans in acrylic samples was investigated using Sabouraud Dextrose Agar. The results revealed that there is no growth of Candida albicans when the medium directly streaked with Candida albicans. The growth was shown only in control. This shows that the Candida albicans is sterilized both at 650W and 810W subjected to the microwave irradiation.

SUMMARY

SUMMARY

In dental clinic, during repair work, relining or adjustment procedures dentists, dental assistants and dental office environment commonly comes in contact with the dentures. These dentures may be infected with loads of bacteria, fungi and viruses. The microorganisms adhered to the denture surface create a potential source of contamination from patients to dental and laboratory personnel. Thus, to reduce the chances of cross-contamination in the dental environment, dentures should be completely disinfected before being sent to the laboratory. Patients with poor denture hygiene exhibit denture stomatitis.

The aim of this study is to evaluate the dimensional stability of previously polymerized different denture base resins subjected to sterilization by microwave irradiation. And to determine whether there is a change in dimensional stability of different denture base resins after it has been subjected to sterilization by microwave irradiation. To determine the sterilization status of the denture base resins tested.

In these study three different acrylic resins is used. Heat cure acrylic resin, injection moulding acrylic resin and flexible acrylic resin. To determine the linear dimensional stability the samples are tested in the digital vernier calliper and subjected to microwave irradiation at 650W and 810W for 3 minutes. Then again the linear dimensional stability is checked with the digital vernier calliper.

Samples were taken for sterilization. Candida albicans strain is taken and coated over the samples of three denture base resins. Each denture base material is subjected to microwave irradiation at 650W for 3mins and 810W for 3mins. After irradiation the specimens are placed in sterile container and culturing is done for detection of any growth of organisms.

It was concluded from the result the there is significant changes in the linear dimensional stability but within the percentage change of 1%. Then there is complete sterilization of the candida coated samples both at 650W and 810W for 3 mins

The acrylic samples can be heated for minimum wattage of 650W for 3 minutes for the complete sterilization of the three different acrylic resins.

CONCLUSION

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The findings of this investigation suggest that microwave irradiation may be a potential treatment to prevent cross contamination between the dental office and dental laboratory. Microwaving for 3 min was an effective method for clinical sterilization of acrylic dentures. There is change in the physical properties of the acrylic resin materials, but within the acceptable changes. The complete sterilization of the acrylic resin is at 650W and 810W for 3 minutes. The power used for sterilization of the acrylic samples can be set at 650W for 3minutes.

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