Journal of Chemical Health Risks

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JCHR (2023) 13(3), 1217-1224 | ISSN:2251-6727

Comparative Evaluation of Strength and Color Stability of Heat Cured Acrylic Resin Incorporated with Antifungal Agents- Melaleuca Alternifolia & Fluconazole

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(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS

color stability, compressive strength, fluconazole, flexural strength, Melaleuca alternifolia, PMMA.

ABSTRACT:

Introduction: Partial and completely edentulous arches are being restored with heat cured acrylic resin (PMMA). Even though there are innumerable benefits its surface topography, renders it susceptible for the oral bacteria to flourish. The use of antifungal drugs avoids candidal growth. Along with the antifungal properties, the denture should also set forth acceptable physical and optical properties. Therefore, this invitro study was done to examine and assess PMMA's strength and color stability after adding antifungals.

Method: Fluconazole was incorporated in ratio 1:9 of powder whereas Melaleuca alternifolia was 20% by vol of monomer. Custom dies were fabricated for flexural strength (65x10x4) mm, compressive strength (38x25) mm & color stability (2x20)mm according to ADA specification no. 12, ISO 20795 & ASTM D 695-02a (ISO 604). 22 samples were made for 3 groups: Control, Fluconazole & Melaleuca alternifolia. 198 samples were tested under UTM & spectrophotometer machines.

Results & Conclusion: Statistical tests were performed using SPSS software by inter group & pairwise comparison with one-way ANOVA and Tukey post hoc test respectively. Mean flexural strength was 131.82, 115.20 & 73.41 for Group 1, 2 & 3 respectively which showed slight difference with Group 2 but statistically significant change with Group 3. Mean compressive strength was 71.10, 68.07 & 33.28 for Group 1, 2 & 3 respectively where Group 1 & 2 had comparable values as compared to group 3 where it changed drastically. Mean color stability was 0.750, 4.90 & 10.57 for Group 1, 2 & 3 respectively which was statistically significant for 3 groups.

1. Introduction

Removable prosthesis, have been a crucial asset to the world of prosthodontics since time immemorial. They have excellent appearance, simple in processing, color stable, can be easily polished and relatively easy to maintain.[1] Regardless of their immense service in the field of dentistry, these prostheses can strike a balance in one's oral health if not taken care of. One such drawback which is commonly seen is denture stomatitis. The mucosa beneath a complete denture might become inflamed due to denture stomatitis (DS).[2] Currently, antifungal agents with denture acrylic resin are being explored to overcome this hurdle.[3] Fluconazole which is a commercial antifungal is being incorporated into PMMA (poly methyl methacrylate). The other antifungals such as essential oils, example, Melaleuca alternifolia or tea tree oil (T.T.O) is a well-established topical antiseptic. Currently, its reputation as a natural, safe and effective anticandidal has gained popularity.[4]

It is fundamentally significant that a denture base resin (DBR) material not only proclaim its antimicrobial effect but also does not jeopardize its mechanical & physical

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JCHR (2023) 13(3), 1217-1224 | ISSN:2251-6727

properties.[2] Dentures are put through the flexural stress during the process of mastication. Thus, flexural strength which is high is required to prevail over fracture of denture under flexural loading.[2] PMMA exhibits a property i.e., compressive strength which is a property of all materials that makes it resistant to vertical static loads. The base of a denture, which typically contracts under strong occlusal stresses, needs to have this property.[1] One of the telltale signs of a material's deterioration or aging is color.[5] The importance of color stability of acrylic resins is shown by the striking disparity between the perceptibility and acceptability limits for denture base resins.[6]

Although adding T. T. O. and fluconazole to heat cured acrylic resin offers antifungal benefits, the material's flexural strength, compressive strength, and color stability after such addition are still being thoroughly researched. Therefore, the purpose of this in-vitro study was the comparative evaluation of strength and color stability of heat cured acrylic resin incorporated with antifungal agents- Melaleuca alternifolia & Fluconazole.

2. METHODOLOGY

i. Master Die Fabrication:

A. Flexural Strength: According to ADA specification No.12 and ISO 20795, a custom rectangular die in stainless steel with internal measurements of 65mm by 10mm by 4mm (length, breadth & height) was created. (Fig 1)

B. Compressive Strength: A cylindrical stainless steel custom die having internal dimensions of 38mm x 25mm (height x diameter) was made according to ASTM D 695-02a (ISO 604). (Fig 2)

C. Color Stability: A ring-shaped stainless-steel die was prepared with internal dimensions of 2mm x 20mm (height x diameter) was made according to ADA specification No. 12. (Fig 3)

Fig 1: Custom-made die for flexural strength

Fig 2: Custom-made die for compressive strength

Fig 3: Custom-made die for color stability

ii. Wax Samples Preparation:

To get samples of heat-cured acrylic resin later, modelling wax was heated and poured into the master die. The samples were then flasked, dewaxed, packed, and successively cured. After cooling, the wax samples were checked for any discrepancy. Twenty-two samples were made for each group that equals to 66 samples for each property which will account for total of 198 samples. Flexural strength, compressive strength, and color stability were the three attributes tested; the control, fluconazole, and tea tree oil (T.T.O.) groups were further split.

iii. Mold Preparation:

For the preparation of molds, the wax samples were flasked. Firstly, Type 2 dental plaster after mixing was put within the metal flask's lower half. Wax samples were placed inside the plaster, which was then given time to harden before separating media were applied, Plaster was poured into the flask's upper half, which was then clamped shut. Using a boiling-out machine the wax was boiled out. Any residual wax was then manually removed. Moulds that were needed to fabricate the acrylic samples were then obtained.

iv. Fabrication of heat cured acrylic resin samples: (Fig 4-6)

59 g of heat-cured acrylic resin powder and 30 g of methyl methacrylate (MMA) monomer were combined to create the control group (group 1) (Fig 4). Before

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packing, the mixture was allowed to reach the dough stage. 100 N pressure was used on a hydraulic press machine. For Group 2 Fluconazole (Fluka 150) tablets were crushed and finely powdered with the help of a mortar and pestle. The tablets were weighed. Powder mass extracted from each tablet was 340 mg. For each flask 59.4g powder and 6.6 g fluconazole powder was taken in the ratio 9:1 i.e., 9 parts of heat cure powder with 1 parts of fluconazole and mixed with 30g of monomer. For Group 3 i.e., Melaleuca alternifolia (a.k.a T.T.O) was measured by 20% by volume of monomer. For groups 2 & 3 (Fig 5 & 6) the process was repeated. All the flasks were kept for bench curing overnight. The samples underwent a short curing cycle that lasted 740°C for two hours and then 1000°C for an hour. After curing was complete, the flasks were left to cool down. All the samples were retrieved and trimmed with conventional DFS acrylic burs and sandpaper & buffed to avoid any irregularities. The samples were then set aside in water bath till they were tested.

v. Testing of Samples:

The finished samples were tested by the use of universal testing machine for flexural and compressive strengths and spectrophotometer was used to measure color stability.

A. FLEXURAL STRENGTH: (Fig 7)

In this study, Universal testing machine (UTM) (Asian Test Equipments) was the choice of machine for conducting the experimental tests. At the site of the previously used benchmarks, the samples were clamped into it, gripped on both sides by pneumatic clamps. The jig was set before to the test so that the samples were neither in compression nor tension. At a crosshead speed of 1 mm/min and a cross-sectional area of 20 mm^2, the test was performed. After noting the peak load at fracture (F), the flexural strength was computed using the formula: $\sigma_{f=3FL} \frac{3FL}{2WD^2}$; where, σ_f - flexural strength in MPa, W- sample width in mm F - fracture load in Newton, D – sample thickness in mm, L - span length in mm

B. COMPRESSIVE STRENGTH: (Fig 8)

Under UTM and a cross head speed of (5 mm/min), compressive force was applied to the samples. for calculating of the compressive stress by dividing of maximum force on the crosses sectional areas and the deformation at various loads were recorded.

C. COLOR STABILITY: (Fig 9)

The Commission Internationale de l'Eclairage L*a*b* values were obtained for all the samples which was done with the help of a spectrophotometer (14600A0010). The measurements were performed by blocking of light by a black background. The formula to evaluate the difference in color was calculated using the: $\Delta E = ([\Delta L]^2 + [\Delta a^2 + [\Delta b]^2)^{½}$.

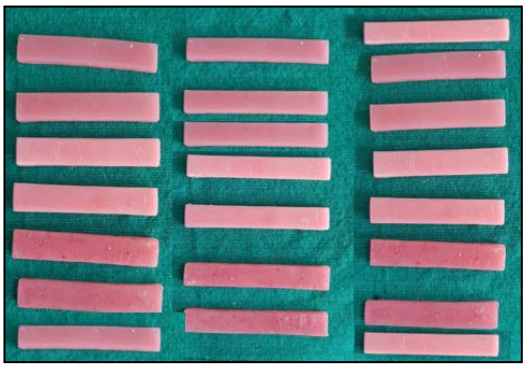

Fig 4: Group of samples for flexural strength

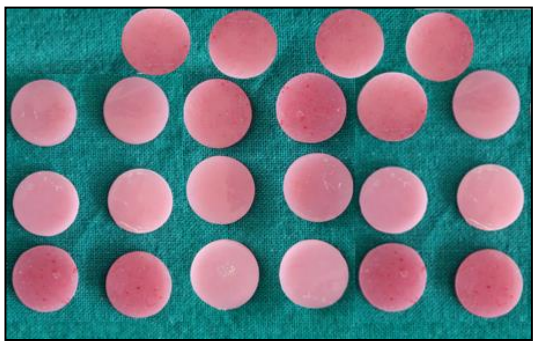

Fig 5: Group of samples for compressive strength

Fig 6: Group of samples for color stability

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JCHR (2023) 13(3), 1217-1224 | ISSN:2251-6727

3. Results

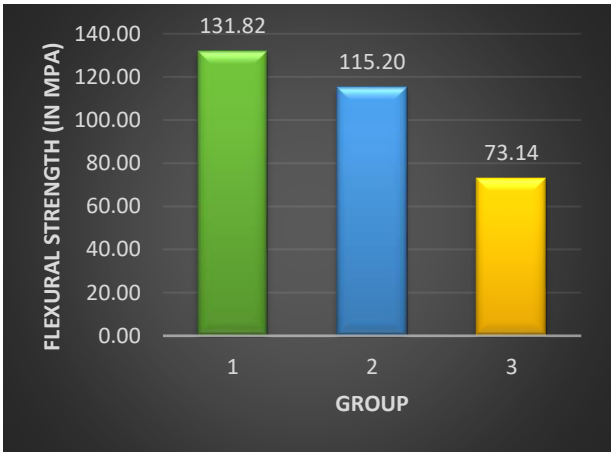
The data obtained for the three physical properties of heat-cured acrylic resin were recorded and put through statistical analysis. The data was recorded in excel sheet. The mean and SD of the parameters was calculated for each group. The SPSS software, Version 23, was used to run statistical tests. The data distribution was demonstrated to be normal by the results of the Kolmogrov-Smirnov test used to determine the normality of the data. As a result, parametric tests were employed. One-way ANOVA was used to compare the means between the groups, and Tukey's post hoc test was run when it was determined that the difference was significant. P values that were less than or equal to 0.05 were deemed significant.

Fig 7: Testing of sample using Universal Testing Machine for flexural strength

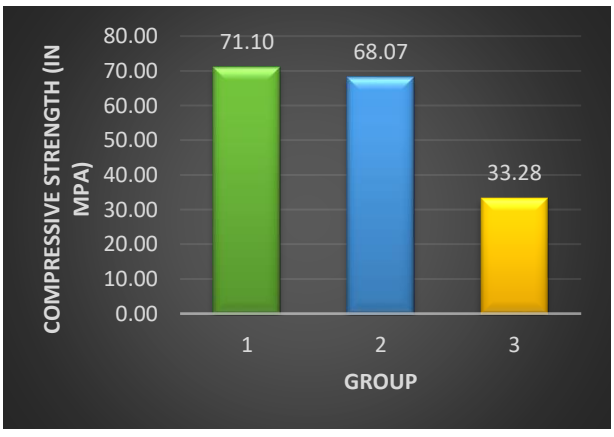
Fig 8: Testing of sample using Universal Testing Machine for compressive strength

Fig 9: Testing of sample using spectrophotometer for color stability

The results of the study pertaining flexural strength, compressive strength and color stability in all three groups i.e., Control, Fluconazole and Tea Tree Oil (T.T.O) are plotted in graphs 1-3 after descriptive statistical analysis for the above parameters.



Graph 1: Descriptive statistics for flexural strength in group 1, 2 & 3



Graph 2: Descriptive statistics for compressive strength in group 1, 2 & 3

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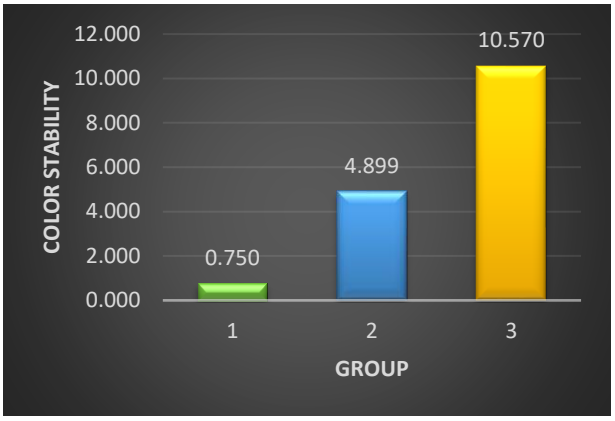
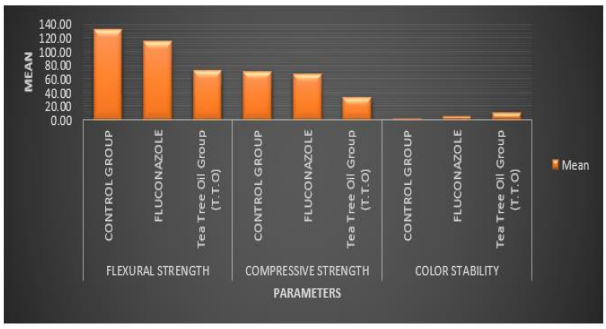


Graph 3: Descriptive statistics for color stability in group 1, 2 & 3



Graph 4: Comparison of parameters between group 1, 2 & 3

The graph depicts the comparison between the three groups for flexural strength, compressive strength and color stability.

The mean flexural strength for groups 1, 2 and 3 were 131.82 ± 5.24, 115.20 ± 7.68 and 73.14 ± 1.50 MPa respectively. When compared, it was discovered that there was a statistically significant difference in the mean amongst the groups with F value 680.558 and p value 0.000.

The mean compressive strength for groups 1, 2 and 3 were 71.10 ± 0.80, 68.07 ± 1.42 and 33.28 ± 1.83 MPa respectively. Comparing the two groups' mean differences revealed that they differed statistically significantly with F value 4877.812 and p value 0.000

The mean color stability for groups 1, 2 and 3 were 0.75 ± 0.364, 4.899 ± 0.642 and 10.570 ± 0.884 MPa respectively. Comparative analysis revealed that the mean difference between the groups was statistically significant with F value 1209.875 and p value 0.000.

4. Discussion

The production of removable partial and complete denture prosthesis was aided by the introduction of the PMMA resin material in 1936.[7] Though the benefits are abundant, if not cleaned and taken care of, these appliances can debilitate the oral health. The production of biofilms on bioprosthetic surfaces is caused by the far more prevalent type of fungal infection of oral cavity called candidiasis. It is generally agreed upon that the main factor contributing to the onset of denture stomatitis is *Candida albicans* colonization on denture materials.[8]

In order to upkeep the oral and systemic health of a denture wearer, the only thing that is of paramount importance is the adequate denture cleansing for which numerous mechanical and chemical methods are practiced. Presently, the entire emphasis is on developing DBR that have natural antibacterial characteristics to prevent germ adherence & eventually oral lesions that follow. Organic & inorganic drugs are being incorporated to prevent candidal infection.[9] In order to maintain the highest therapeutic medication levels at the infection site for the sustained release of the drug after integration into PMMA, local drug carriers increase the effectiveness of oral treatment.[10] Such medications are Fluconazole and Melaleuca alternifolia (a.k.a Tea Tree Oil).

Due to its good toleration and minimal side effects, peroral fluconazole is commonly used as a therapy. However, the major clinical concern is the microbiological and clinical resistance. Additionally, it can be challenging to maintain therapeutic medication levels in patients with decreased salivary output.[11]

Natural chemicals, such as concentrated, hydrophobic liquids called essential oils that are collected from plants, have therefore come into play in recent years to address the aforementioned problem.[12] Therefore, a substitute to the synthetic chemical substances can be the natural ones, as a result, interest in medicinal plants as a source of antibacterial compounds is increasing.[13] A modern multipurpose herb that is a natural alternative is tea tree oil (Melaleuca alternifolia) indicating its use in dental hygiene as an extremely effective antimicrobial agent.[14]

When heat cured DBR is exposed to water sorption, the main cause of the degradation of its mechanical & optical qualities occurs. To reduce the chance of breakage,

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JCHR (2023) 13(3), 1217-1224 | ISSN:2251-6727

different preparation methods as well as the physical and chemical characteristics of DBR should be taken into account. In this study the following physical properties were evaluated i.e., flexural strength, compressive strength and color stability.

Flexural strength is obtained by three-point bending test.[15] The compressive strength of a base of the denture can be used to predict how materials will behave under crushing stresses. A cylindrical sample that is supported vertically and compassed in the same direction is measured for its compressive strength to determine its strength and stiffness.[15] A working knowledge of differential colorimetry and color space is necessary to identify and measure variations in the color of dental materials.[16] Therefore, this in vitro study was aimed to compare & evaluate strength and color stability of heat cured acrylic resin incorporated with antifungal agents-Melaleuca alternifolia & Fluconazole.

The current study's null hypothesis is that combining antifungal agents i.e., fluconazole and T.T.O with PMMA heat cure DBR that doesn't possess adverse effect on the flexural strength, compressive strength and colour stability of PMMA heat cure denture base resin.

Statistics were applied to all the values for the three physical parameters of heat-cured acrylic resin that were acquired from various equipment. Each group's mean and standard deviation were determined following which statistical analyses were performed. The Kolmogrov-Smirnov test results, which demonstrated that the data distribution was normal, were used to evaluate the data's normality. Hence, parametric tests were employed. One-way ANOVA was used to compare the means between the groups, and Tukey's post hoc test was run when it was determined that the difference was significant. P values that were ≤ 0.05 were deemed significant.

Graph 1 depicts that the mean flexural strength for groups 1, 2 and 3 were 131.82 ± 5.24, 115.20 ± 7.68 and 73.14 ± 1.50 MPa respectively. This demonstrated that the flexural strength of heat-cured acrylic resin combined with group 2 (fluconazole) differed very slightly, whereas group 3 (Melaleuca alternifolia) caused a significant shift in flexural strength. The study by Mirizadeh[3], which demonstrated a decrease in flexural strength when an antibacterial agent was added to PMMA, was comparable to this one in that it also exhibited a similar result. Studies by Rajeev[17] and Neven[18] revealed that flexural properties of a DBR gets changed when an antifungal agent is incorporated into the polymer.

Graph 2 depicts that the mean compressive strength for groups 1, 2 and 3 were 71.10 ± 0.80, 68.07 ± 1.42 and 33.28 ± 1.83 MPa respectively. According to the results of this investigation, group 2 (fluconazole) and heat-cured acrylic resin had compressive strengths that were almost equivalent as compared to group 3 (Melaleuca alternifolia) where the compressive strength changed drastically.

Graph 3 depicts the mean color stability for groups 1, 2 and 3 were 0.75 ± 0.364, 4.899 ± 0.642 and 10.570 ± 0.884 MPa respectively. The results of this investigation showed that all three groups' differences in the color stability of heat-cured acrylic resin after adding both antifungal agents were statistically significant which agreed with Aziz's study, in which Ti-O nanofillers were used. These fillers displayed increased opacity because they absorbed more light than the polymer matrix, which was in agreement with that study.

5. Conclusion

The forgoing study drew conclusions that the flexural strength of PMMA incorporated with group 2 (Fluconazole) was found to have a slight difference whereas with group 3 (Melaleuca alternifolia), the flexural strength changed drastically. Compressive strength of heat cured acrylic resin incorporated with group 2 (Fluconazole) was found to have comparable values as compared to group 3 (Melaleuca alternifolia) where the compressive strength changed drastically. When both antifungal drugs were added to heat-cured acrylic resin, color stability was discovered to be statistically significant across all three groups.

We can conclude, within the constraints of this investigation, that while the addition of antifungal medications has a beneficial impact on candidal infections, it may change the physical properties at specific concentrations. Additional research is needed to examine these findings as well as the impact of fluconazole and melaleuca alternifolia on the other physical and mechanical properties of heat-cured acrylic resin.

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JCHR (2023) 13(3), 1217-1224 | ISSN:2251-6727

Acknowledgements:

I would like to thank my family and friends for their constant encouragement and understanding during the research process. I am deeply grateful to my research guide, [Dr Jyotsna Seth], and co- guides (Dr Himanshu Aeran & Dr Varun Kumar) for their guidance, mentorship, and unwavering support. Lastly, I extend my thanks to my colleagues Dr. Goutami Deshpande & Dr Neha Sehrawat for engaging in insightful discussions and providing constructive feedback during the data analysis phase.

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List of Abbreviations:

1. PMMA: Poly methyl methacrylate
2. MMA: Methyl methacrylate
3. UTM: Universal Testing Machine
4. T.T.O: Tea Tree Oil
5. DS: Denture Stomatitis
6. DBR: Denture Base Resins
7. ADA: American Dental Association
8. ISO: International Organization for Standardization
9. ASTM: American Society for Testing and Materials
10. SD: Standard Deviation