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ORIGINAL RESEARCH

COMPARATIVE EVALUATION OF EFFECT OF AZADIRACHTA INDICA ON SURFACE ROUGHNESS, HARDNESS AND COLOR STABILITY OF POLYMETHYL METHACRYLATE DENTURE BASE MATERIAL - IN VITRO STUDY.

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ABSTRACT

Background:The study consists of two groups. Group-A, prepared with unmodified Polymethylmathacrylate(PMMA) resin and Group-B prepared with PMMA incorporated with neem oil. Colour stability assessment was done using the UV-Vis Spectrophotometer. The initial colour of both groups and after exposure color changes and Delta E (ΔE) value was calculated. Surface roughness assessed using Profilometer. Hardness was assessed by Vickers hardness number. Data subjected to statistical analysis. As the data was normally distributed ($p > 0.05$) parametric tests of significance were used. Independent Sample t-test was used. P value of < 0.05 was considered to be statistically significant.
Materilas and Methods: The results obtained from the study indicate that there is a significant difference in the surface roughness of the experimental group of mean 0.0718 was found to be statistically significant ($p < 0.05$) than the control group at baseline with a mean of 0.5652. The post immersion mean of Group B for surface roughness was also lesser than that of Group A
Results: The results obtained from the study indicate that there is a significant difference in the surface roughness of the experimental group of mean 0.0718 was found to be statistically significant ($p < 0.05$) than the control group at baseline with a mean of 0.5652.The post immersion mean of Group B for surface roughness was also lesser than that of Group A.
Conclusion: This in vitro study shows how PMMA can be modified with fillers such as neem oil to improvise its physical and mechanical properties. The properties of PMMA incorporated with neem oil was comparatively better than conventional resin.Significantly better properties indicate their ability to overcome the shortfalls in PMMA resin.

***Keywords*:** Pediatric dentistry, Early childhood caries, Chlorhexidine varnish, Primary molars, Caries prevention, Fluoride varnish.

1.INTRODUCTION

Poly methyl methacrylate is one of the various polymers used in prosthodontics. Several other materials such as vulcanized rubber, polyamides, gold, porcelain, aluminum, polystyrene etc., have been used as base for complete dentures but they are not proven to be as good as Polymethylmethacrylate (PMMA) resin resulting in PMMA being one of the most commonly used denture base materials[1,2]. Although, it has few shortcomings, PMMA has a variety of applications in prosthodontics which is attributable to its advantages of being aesthetic, with good properties and cost effectiveness[3]. This polymer has undergone numerous modifications through manipulations methods, composition manipulation etc., over time to further enhance its properties and performance through various alterations being done to improve its physical and mechanical properties, as these properties govern the functionality, fit and comfort of the denture[4,5]. Improvising / enhancing the existing properties of the PMMA resin by addition of fillers refers that, addition of few materials has shown to improvise the existing physical/mechanical properties of PMMA resin. For instance, addition of neem has shown to improvise the antibacterial efficacy of PMMA resin. However, the usage of dentures intra-orally changes the bio-environment and causes deposition of biofilms. Biofilm attachment is one of the disadvantages Surface characteristics such as surface roughness plays a significant role in the deposition of biofilms[6]. The irregular surfaces favour the adhesion and colonization of microbes, causing diseases such as candidiasis, burning Mouth and glossodynia[7]. Studies show that the addition of herbal extracts like that of Neem to PMMA is therapeutic due to its immunomodulatory, anti-inflammatory, anti-microbial, antioxidant and antimutagenic effects[8]. Neem as powder or extract in PMMA has shown improved antibacterial, antifungal, immunomodulatory properties. Neem can aid in reduction of adherence of microorganisms based on its surface characteristics. We are adding neem to check for surface characteristics and color change as dentures tend to remain in the oral cavity for a long duration.

As these studies done previously show the above mentioned therapeutic effects, it was done with neem powder and neem extracts[8,9]. We have chosen neem oil, as this is the first of its kind because, powder tends to get incorporated and aggregated in clusters in PMMA when incorporated with polymer or monomer of the PMMA resin.

The aim of this study is to assess the surface roughness, hardness and colour stability of PMMA denture base resin with and without the addition of neem oil. The null hypothesis is that, there is no difference in the surface roughness, hardness and color stability in PMMA denture base resin with and without the addition of neem oil. The objective of this study is to analyze and compare the effect of neem oil on Surface roughness, Hardness and color stability on PMMA denture base resin.

2.MATERIALS AND METHODS

In this in vitro study, commercially available materials such as Heat Cure Denture base resin (Dental Products of India), Neem Oil (Aroma Treasures) and Coffee (Nescafe) were utilized. A total of 40 samples were prepared. The total amount of estimated samples were derived using G-Power software in accordance with previous literature[10]. The samples were fabricated according to the International Organization for Standardization, ISO 1567: 2020 with a dimension of 25mm x 25mm x 3mm.

For the purpose of this study, Samples were categorized into two groups of 20 samples each. Group A consisted of conventional PMMA polymer mixed with methyl methacrylate (MMA) monomer. Group B consisted of 1% of neem oil incorporated in the monomer (MMA) component and this modified monomer was manipulated with the polymer. The manipulation of both groups of PMMA resin were done according to the instructions specified by the

manufacturer, and the ratio in which they weremanipulated was 3:1 in polymer to monomer ratio. A summary of the study groups and their sample fabrication is listed as follows,

Group A (Control group): 20 samples of conventional / unmodified polymer and monomer of PMMA resin

Group B (Experimental group): 20 samples of 1% of neem oil incorporated in the monomer of PMMA resin.

Prior to fabrication of samples, the wax patterns were fabricated. The wax pattern was of the following dimension, 30mm x 30mm x 5mm and was flasked following which dewaxing was done. Once the dewaxed mold was obtained, it was used for packing of heat cure denture base PMMA resin for the fabrication of samples.

For Group A samples, conventional PMMA polymer and monomer were mixed in 3: 1 ratio and once it reached dough stage, it was kneaded and placed into the mold. For Group B samples, monomer was incorporated with 1% neem oil and was utilized for manipulation with PMMA polymer, this was mixed in 3:1 ratio, once the mixture reached dough consistency, it was kneaded and then placed in the fabricated mold. Once all samples of both groups were in the mold, their flasks were closed and positioned in hydraulic press. A pressure of 1400 psi was applied for 30 minutes for the purpose of bench curing. Subsequently, the clamped flask was transferred to a water bath of acrylizer unit. The temperature in the acrylizer was maintained at 72°C for 90 minutes and then increased to 100°C for 60 minutes. After complete curing occurred, the samples were left in the water bath and allowed come down to room temperature. Once cooled, the samples were removed from the mold, cleaned of residual stone particles, trimmed to the required dimension of 25mm x 25mm x 3 mm and polished sequentially using 80, 120, and 150 grit sandpapers. Following this, 180 grit emery paper was used for finishing. For the purpose of polishing, a pumice slurry was used. Once the samples were fabricated, they were subjected to testing.

As this study is done for the purpose of checking the color stability, a staining solution (Coffee) is used. The immersion in the coffee solution was done for a period of 48 hours. The preparation of coffee staining solution was done using 3.6 grams of coffee powder (Nescafe Classic) mixed in 300ml of distilled boiling water. This was according to manufacturer instructions. Once the coffee powder was mixed thoroughly for about 10 minutes ensuring complete dissolving of coffee, the solution was filtered using a filter paper and used for staining of PMMA resin samples. A period of 48 hours was chosen as per manufacturer's claim that this time frame was used to simulate the cumulative exposure of oral cavity to coffee over approximately two months[11]. The prepared samples of both groups were first subjected to recording of baseline values for colour stability, surface roughness and hardness then immersed into 100ml of the prepared solution separately for staining (Figure 1).

Figure 1. Samples immersed in staining solution

After 48 hours, the samples were removed from the staining solution and gently rinsed before recording post immersion values.

To record the baseline values prior to immersion, for color stability, a UV - Vis Spectrometer (Spectrophotometer CM-5, Konika Minolta, Japan) was used and the respective L*a*b* values were taken (Figure 2).

Figure 2. Recording of the color

All color change measurements (ΔE) were determined using the CIE-Lab (L*, a*, b*) colorimetric system established by the Commission Internationale de l'Eclairage, a widely accepted method for numerically assessing color differences. The values L* indicate brightness, a* indicate red-green proportion and b* indicates yellow-blue proportion. After immersion of 48 hours, the post immersion values of L*a*b* were recorded. The colour change was measured using CIE lab colour space and Delta E (ΔE) value of both groups calculated using the following equation,

$\Delta E^* = [(L_1^* - L_0^*)^2 + (a_1^* - a_0^*)^2 + (b_1^* - b_0^*)^2]^{1/2}$ [12]

Similarly the tests of surface roughness and hardness were done to record both baseline (pre-immersion) and post immersion values. For recording surface roughness, a Profilometer (Surface Profilometer SJ 310, Mitutuyo, Japan) equipped with a diamond stylus with a measuring force of 4mN and with a tip radius 5µm, cut off length of 0.1mm with Gaussian filter, tip angle 90 which records minor surface variations when brought in contact with the surface of PMMA resin was used. An area of 50mm x 50mm was chosen to record rough and 5 different sites on one side of the sample were checked for baseline and 5 sites on the other side were chosen for post insertion. (Figure 3).

Figure 3. Recording of Surface Roughness using a Profilometer

The surface roughness of the samples were checked for baseline and post immersion values and an average of R_a was obtained. For recording hardness, Vickers's hardness tester (Shimadzu HMV - G31-FA Series, North America) was used. Micro Vickers hardness tester with a micro Vickers indenter with a force of 2.94 N and a holding time of 15 seconds. The number of indentations were 15, made on the surface of the samples on one side for baseline and similarly 15 indentations on the other side were made post immersion, and Vickers's hardness number was assessed(Figure 4).

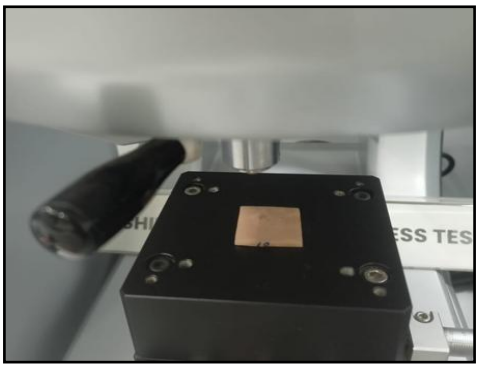


Figure 4. Hardness test done Vicker's Hardness tester

All the data obtained from the tests were tabulated and statistical analysis was done.

3.RESULTS

The data recorded were transferred and tabulated to the computer in Windows Microsoft Excel (2007). Statistical Package of Social Science (SPSS; IBM Chicago Inc., USA) was used. The total data was subdivided, distributed and presented as individual tables along with graphs. As the data was normally distributed parametric tests of significance were used. Independent Sample t test was used to analyze the difference in the means of continuous variables. For all comparisons, p value of < 0.05 was considered to be statistically significant. The results obtained from the study indicate that there is a significant difference in the surface roughness of the experimental group of mean 0.0718 was found to be statistically significant ($p < 0.05$) than the control group at baseline with a mean of 0.5652. The post immersion mean of Group B for surface roughness was also lesser than that of Group A(Table 1, 2).

Table 1. Descriptive analysis of surface roughness among the samples.

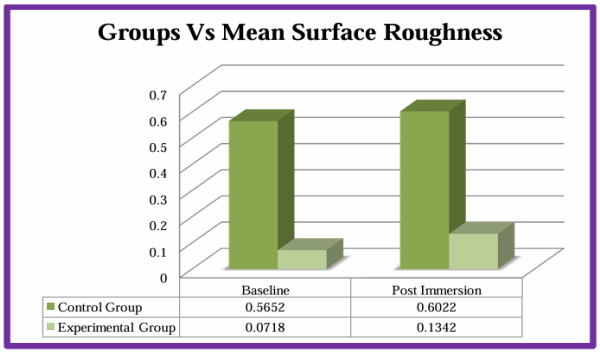
STATISTICAL ANALYSIS OF THE FREQUENCY OF SURFACE ROUGHNESS AMONG THE TWO STUDY GROUPS AT BASELINE AND POST IMMERSION USING INDEPENDENT SAMPLE T TEST

Variable		Levene's Test for Equality of Variances		t-test for Equality of Means						
			Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Baseline	Equal variances assumed	30.27	**<0.001****	2.220	38	**0.032***	0.49340	0.22225	0.04348	0.94332
	Equal variances not assumed			2.220	19.104	**0.039***	0.49340	0.22225	0.02840	0.95840
Post Immersion	Equal variances assumed	170.58	**<0.001****	5.571	38	**<0.001****	0.46800	0.08400	0.29794	0.63806
	Equal variances not assumed			5.571	19.983	**<0.001****	0.46800	0.08400	0.29276	0.64324

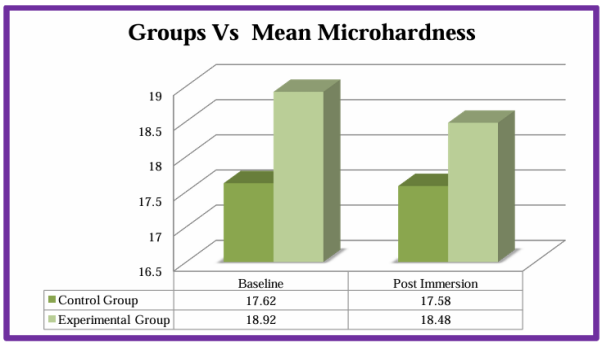
N indicates total number of samples in a group. Baseline values indicate pre immersion values and post immersion values are recorded after staining of samples

Table 2. Statistical comparative analysis of the frequency of surface roughness among the study samples

Surface roughness		N	Mean	Std. Deviation	Std. Error of Mean	Median	Minimum	Maximum	Range
Control Group	Baseline	20	0.5652	0.99257	0.22195	0.08	0.07	2.5	2.43
	Post Immersion	20	0.6022	0.3709	0.08294	0.803	0.11	0.96	0.85
Experimental Group	Baseline	20	0.0718	0.05194	0.01162	0.048	0.04	0.17	0.14
	Post Immersion	20	0.1342	0.05967	0.01334	0.117	0.07	0.24	0.17



Graph 1



Graph 2

Graph 1, 2: Shows the mean surface roughness and microhardness among the study groups respectively

With regards to micro hardness, Group A had significantly higher micro hardness than group B both at baseline mean of 17.62 and 18.92 and at post immersion mean of 17.58 and 18.48 but this difference was not statistically significant post immersion (P >0.05) but was significant at base line (P <0.05) (Table 3,4).

Table 3. Descriptive analysis of microhardness among the study samples.

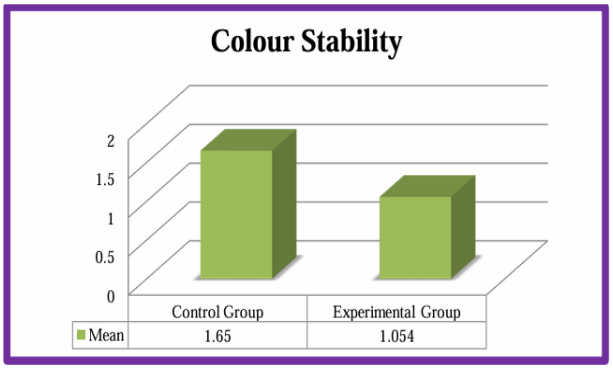
Micro hardness		N	Mean	Std. Deviation	Std. Error of Mean	Median	Minimum	Maximum	Range
Control Group	Baseline	20	17.62	1.92753	0.43101	16.3	15.8	20.2	4.4
	Post Immersion	20	17.58	1.77634	0.3972	16.8	15.5	20.1	4.6
Experimental Group	Baseline	20	18.92	0.74382	0.16632	18.6	18.2	20.1	1.9
	Post Immersion	20	18.48	1.04509	0.23369	18.1	17.4	20.3	2.9

For color stability, control group was statistically significant when compared to the experimental group having a mean of 1.65 and 1.054 respectively (P <0.05) (Table 5, 6).

Table 4. Statistical comparative analysis of the frequency of micro hardness among the study samples

STATISTICAL ANALYSIS OF THE FREQUENCY OF MICRO HARDNESS AMONG THE TWO STUDY GROUPS AT BASELINE AND POST IMMERSION USING INDEPENDENT SAMPLE T TEST

Variable		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Baseline	Equal variances assumed	88.980	**<0.001****	-2.814	38	**0.008**	-1.30000	0.46199	-2.23524	-0.36476
	Equal variances not assumed		**<0.001****	-2.814	24.536	**0.009**	-1.30000	0.46199	-2.25239	-.34761
Post Immersion	Equal variances assumed	15.882	**<0.001****	-1.953	38	**0.058**	-.90000	.46085	-1.83293	.03293
	Equal variances not assumed			-1.953	30.746	**0.060**	-.90000	.46085	-1.84022	.04022



Graph 3: Graph exhibiting the mean colour stability among the study population

Table 5. Descriptive analysis of colour stability among the study groups

Colour Stability	N	Mean	Std. Deviation	Std. Error of Mean	Median	Minimum	Maximum	Range
Control Group	20	1.65	0.61088	0.1366	1.38	1.23	2.82	1.59
Experimental Group	20	1.054	0.11673	0.0261	1.08	0.84	1.18	0.34

N indicates total number of samples in a group.

Table 6. Statistical comparative analysis of the frequency of color stability among the study groups.

Statistical analysis of color stability among two groups

Variable		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Colour Stability	Equal variances assumed	19.689	**<0.001****	4.286	38	**<0.001****	.59600	.13907	.31447	.87753
	Equal variances not assumed			4.286	20.386	**<0.001****	.59600	.13907	.30626	.88574

In the above F - frequency, sig - Significance, t – test for equality of means, df – degree of freedom.

N indicates total number of samples in a group. Baseline values indicate pre immersion values and post immersion values are those recording after staining of the samples.

Table 2 depicts the statistical comparison of the mean surface roughness among the control and the experimental group of the study samples at baseline and post immersion. According to the Levene's Test or Equality of Variances and t-test for Equality for Means it was found to be statistically significant at both baseline and post immersion. Table 4 depicts the statistical comparison of the mean micro hardness among the control and the experimental group of the study samples at baseline and post immersion. According to the Levene's Test for Equality of Variances it was found to be statistically significant at both baseline and post immersion. While performing t-test for Equality of Means, the hardness of the experimental group in comparison with control group at post immersion was found not to be statistically significant

4.DISCUSSION

As per the results of this study, the null hypothesis was rejected. The following study was carried to analyze and compare the effects of neem oil when added to the composition of the PMMA denture base resin and its effects on the mechanical properties such as surface roughness, hardness and color stability. Neem is an omnipotent therapeutic agent which has several properties, such as antioxidant, antimutagenic, immunomodulatory, antiviral, including antimicrobial activity, which reduces the colony formation of bacteria on the denture surfaces[13]. However, an increased surface roughness of PMMA can lead to increased accumulation of biofilm as it gets easily adhered to rough surfaces[14]. There are previous studies done with neem powder and neem extract, which have tested mechanical properties of neem incorporated PMMA. However, addition of “neem oil” is the first of its kind and hence, initially few mechanical properties are studied in this research article.

Based on the results of this study, the surface roughness of the Group B was found to be lesser than the Group A which was statistically significant ($P < 0.05$). The normal surface roughness of PMMA resin used in denture fabrication should be equal to a minimum of 0.2µm, in the present study, the experimental group had a lower surface roughness than this[15]. Since neem oil has been employed in this study, the accumulation of biofilm could be reduced due to its additional antimicrobial activity.

The microhardness values when both groups were compared showed difference in the hardness of neem oil modified PMMA, which could be attributed to the physical properties of the material, as the oil may alter the structure of the polymer. It can cause interference in the polymer structure. Its molecular properties disrupt the uniformity of PMMA's polymer matrix, potentially introducing soft regions that reduce the material's overall hardness hence in this study, the hardness of the experimental group was inferred to be lesser than unmodified PMMA as per a study conducted by Gad et al, who found that addition of reinforcements in the composition of PMMA could alter the polymerization reaction as such[16].

The optical properties of PMMA such as translucency and color influence the appearance of the denture base resin. It is equally important for a denture to be esthetically pleasing in order to have increased acceptance of the denture by the denture wearer. The surface characteristics of PMMA can be easily influenced by the consumable food and drinks which contains color pigments. These pigments are liable to de deposited over the denture surfaces affecting the aesthetics and appearance of the denture.

Color reproduction is crucial in esthetic restorations, as the material's ability to maintain its original shade impacts the overall appearance. Surface texture also plays a significant role in maintaining color stability. Rough surfaces are more prone to accumulating stains compared to smooth surfaces, leading to discoloration over time. In this study, even

though the surface roughness was comparatively lesser in the experimental group, the incorporation of natural oils, such as neem oil, contain green color pigments and other components that can degrade when exposed to light and moisture, potentially leading to yellowing or darkening of the resin[17].

This degradation may be attributed to the inherent instability of natural pigments present in neem oil as on par with a study conducted by Fayed et al[18]. The limitations of the study include that even though the addition of neem oil has shown better surface roughness, the other mechanical properties of PMMA have to be checked with further research. In this study, staining agent had been limited to one, other staining agents for a longer duration of staining can be done as a future scope of the study.

5.CONCLUSION

Within the limitations of this study, it can be concluded that the incorporation of neem oil into polymethyl methacrylate (PMMA) significantly influences its surface properties.The increased surface roughness observed in neem oil incorporated PMMA suggests that the addition of the oil modifies the polymer matrix, potentially affecting the topographical integrity of the material. This alteration in surface texture could influence clinical factors such as plaque accumulation and microbial adhesion, which are critical in prosthodontic applications.Overall, neem oil incorporation into PMMA presents a trade-off between improved mechanical properties and compromised aesthetic stability, which should be carefully considered when designing denture base materials or other prosthetic applications.

DECLARATIONS

1. **FINANCIAL SUPPORT**: Nil

2. **ETHICAL APPROVAL:** Not applicable

3. **ACKNOWLEDGEMENT**: Nil

4. **CONFLICT OF INTEREST**: Nil

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