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Comparative Evaluation of the Flexural Strength and Antimicrobial Properties of Heat Cure Denture Base Resins by Addition of TiO2 and Methacrylic Acid: An in-Vitro Study.

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Abstract-

- ***Aim:***

To evaluate and compare the flexural strength and antimicrobial properties of heat cure denture base resins by addition of TiO2 nanoparticles and methacrylic acid at different concentration.

- ***Materials and Method:***

In the present study 180 samples were prepared. 30 samples for each group i.e. conventional non altered denture base acrylic resins (group I), altered heat cure acrylic resin with 0.5% and 1% TiO_2NPs (group IIA and IIB), PMMA altered with 20% and 25% MAA (group IIIA and IIIB) and 1% TiO_2NPs+25 % MAA incorporated in PMMA (group IV). Specimens were prepared. These specimens were stored at 37˚C in distilled water for 1 day. Specimens were incubated in BHI broth containing *S. aureus* and *C. albicans* in different test tubes at 37˚C for 18 hrs before the evaluation of microorganism adhesion and flexural strength. CFUs were evaluated of *C. albicans* and *S. aureus* when incubated at 37˚C on blood agar. Flexural test was measured by using universal testing machine at speed of 5mm/min. Data were subjected to static analysis (ANOVA, Tukey's HSD test).

- ***Results:***

Reduction in CFU counts of *S. aureus* and *C. aureus* were found more significant for group IV as compared to group I and there was non-significant reduction in flexural strength in group II. Other groups showed significant reduction when compared to control group.

- ***Conclusion:***

Group IV had better antimicrobial property and acceptable flexural strength hence this information can be used clinically as per patient requirement.

Key words: Antimicrobial property, flexural strength, TiO_2NPs, MAA, PMMA

I. INTRODUCTION

The increase in average life span of human beings, as a result of various treatment and cure modalities, enhances the need of rehabilitative procedures for elderly; including edentulous. In spite of preventive and restorative measures, the change in dietary habits from raw food to cooked soft and fast food is leading to tooth decay and gum diseases or even edentulism. Any loss to the non-healing dental tissues compels dentistry to replace it with restorative or prosthetic means. Prosthodontists have an obligatory commitment towards society for rehabilitating the edentulous / semi-dentulous population with denture prosthesis to enable the edentulous people lives a healthy life.

Even with the presence of implants around, the conventional complete dentures are still most viable option for rehabilitation of edentulous in countries like India.

Poly (methyl methacrylate) had been material of choice because of innumerable reasons [1]. But comparatively it has poor mechanical properties like impact, bending, and fatigue strength, thermal conductivity and is also prone to adhere with bio film, plaque and microbial flora [2, 3] on its intaglio surface and remains in contact with oral mucosa.

Poorly nourished elderly are mostly on varieties of medication and at times immune-compromised, in whom

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normal oral bacterial and fungal flora[6] may turn pathogenic[4] or fatal[5].

Along with various disinfectant solution for immersion, additives like nanoparticles of TiO_2[7], SiO_2, ZnO_2, Ag, and Methylacrylic acid[5]are also suggested to enhance antibacterial property of resins, but with associated effects on physical and mechanical properties like flexural and impact strength[9], micro-hardness[7], elastic-modulus and glass-transition temperature[8] of PMMA.

In this study there will be evaluation of antimicrobial activity and flexural strength of denture base resin by adding TiO_2NPs and MAA at different concentration.

II. MATERIAL AND METHOD

➢ *Sampling Method*

In this study a total of 180 specimens were made of resin and resin containing TiO_2NPsor/and MAA. The specimens were of 65×10×3.3 mm dimensions according of ADA specification no-12 to evaluate and compare the flexural strength and antimicrobial properties against *S. aureus* and *C.albicans* of heat cure denture base resin (Trevalon) by addition of Titaniun dioxide NPs Anatase phase (SRL) 0.5% and 1% by wt and Methacrylic acid extraoure stabilized with Hydroquinone monomethylether (SRL) 20% AND 25% by volume.

Table 1 Distribution of Samples and Allocation of Groups

	Group-I	**Group-II Contains TiO_2NPs**		**Group-III Contains MAA**		**Group-IV Contains TiO_2NPs+MAA**
	Control group (N=30)	Subgroup-IIA(N=30) (0.5%TiO_2NPs)	Subgroup-IIB(N=30) (1%TiO_2NPs)	Subgroup IIIA(N=30) (20%MAA)	Subgroup IIIB(N=30) (25%MAA)	(N=30) (1%TiO_2NPs +25%MAA)
For flexural strength	10	10	10	10	10	10
For *Staphylococcus aureus*	10	10	10	10	10	10
For *Candida albicans*	10	10	10	10	10	10

To prepare these, four stainless steel metal dies of same dimension were fabricated (Figure-1).

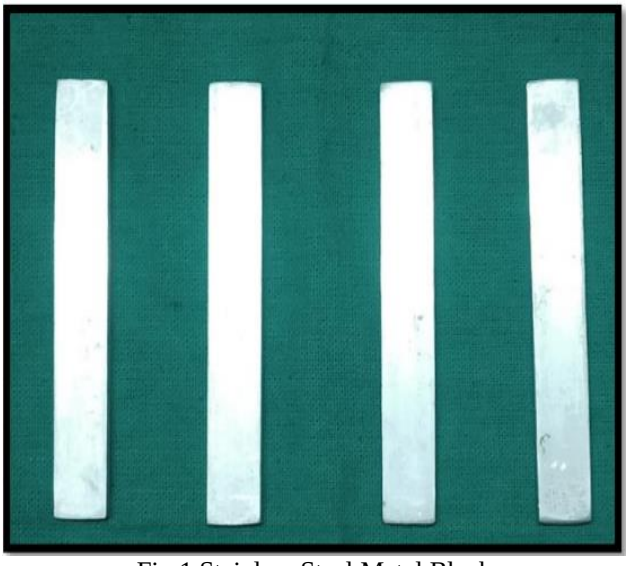

Fig 1 Stainless Steel Metal Block

Petroleum jelly (Vaseline) was applied to the all four dies and dies were invested in lower half of dental flask(VarsityFlask)filled with type 1 dental plaster(Dentco, Neelkanth Minechem,jodhpur) (figure-2).

Fig 2 Flasked Metal Block

After it set, a thin layer of separating media (Pyrax) was applied. Then, the plaster was poured in the counterpart of the flask which was placed over the base part. A metal to metal intimate contact was ensured. Then, the flask was tightened in the clamp, after the plaster was set the flask was then opened, the dies were retrieved and a negative mold obtained (figure-3).

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Fig 3: Negative Mould for Sample Fabrication

For all group's specimen polymer powder was measured in a digital weighing machine (Shimadzu AUX220) (figure-4). Monomer was measured using a syringe and measuring jar.


Fig 4 TiO2NPs Being Weighed on Digital Weighing Machine

For group I measured amount of powder and liquid for 30 samples were mixed in silicon cup.

For modified group II and III, part of measured powder of resin for specimens were substituted with same weight of TiO_2 as required to bring it 100% powder and Parts of measured liquid of resin for specimens were substituted with same volume of Methacrylic acid as required to bring 100% liquid. For example, in subgroup IIA, 0.5% w/w (0.5 g) TiO_2 were added to 99.5% (99 g) PMMA polymer to bring polymer powder to 100% (100 g) and in subgroup IIIA, 20%v/v (20ml) MAA were added to 80% (80ml) PMMA liquid to bring PMMA liquid to 100% (100ml) then mixed with liquid and powder as per manufacturer's recommendation respectively. Same was done for subgroup IIB and IIIB.

For group IV, 1% w/w TiO_2NPs added powder and 25% v/v MAA added liquid (addition of TiO_2NPs and MAA have been already explain in above methods)were mixed in a silicon cup as per manufacturer's recommendation.

When dough stage was reached, mixture was kneaded properly and packed into the mold space of the dental flask. The flask was left under the hydraulic bench press for bench curing for 30 minutes, then flask was kept in heat cured acrylic curing unit as recommended by the manufacturer. Slow bench cooling was done till room temperature, and the specimens were retrieved carefully (figure-5).

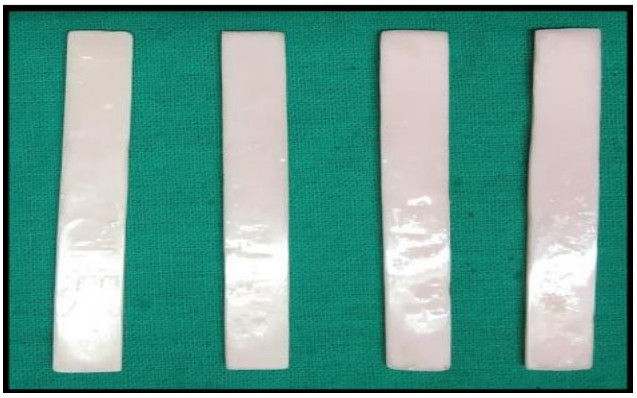
Fig 5 Example of Acrylic Samples

III. TESTING THE SPECIMENS

➢ *Flexural Strength Testing*

10-10 specimens from each groups and subgroup were subjected to flexural strength testing under 3-point loading[8]. Prior to flexural strength test, finished and polished samples were stored in distilled water for 1 day at 37˚C (figure-6.A&B) to reduce residual monomer[8,13].


Fig 6 A&B Specimens Immersed in DistilledWater Placed in Incubator at 37º C

The specimens were inserted in universal testing machine which consist a loading wedge and a pair of adjustable supporting wedges. The distance between the centers of the wedges was 50 mm. This dimension represents the space between the maxillary molars in a

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complete denture. The specimens were placed on the supporting wedges in such a way that loading wedge, set to travel at a crosshead speed of 5mm/min (figure-7).

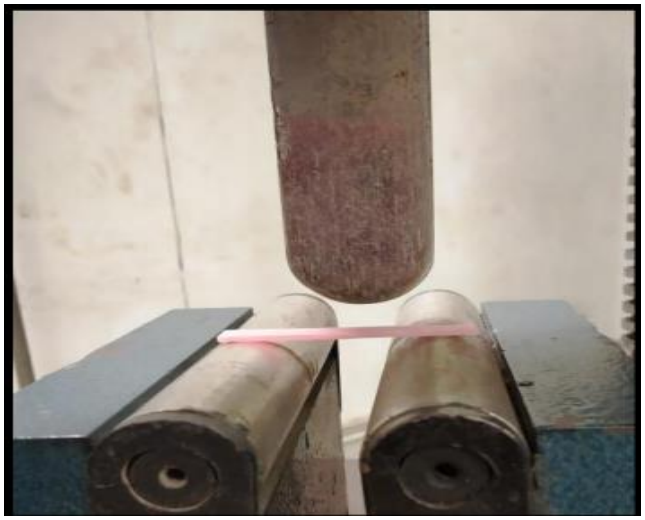

Fig 7 Testing of Flexural Strength on Universal Testing Machine

The initially applied force was zero followed by gradual increase. The force was applied perpendicular to the center of specimen strips until the deviation of the load-deflection curve and fracture of specimen occurred (figure-8). The load was measured at the point of fracture of the specimen in Newton.

Fig 8 Fractured Specimens

The ultimate flexural strength was calculated using the following formula in Megapascal:

$$\text{Flexural strength (MPa)} = \frac{3 \times F \times l}{2 \times b \times h^2}$$

F = Maximum applied load in Newton,
I = Distance between the support wedges,
b = Width of the specimen prior to storage in water,
h = Height of the specimen prior to storage in water

Here values of l, h and b were 50, 3.3 and 10 mm respectively.

➢ *Antimicrobial Testing:*

- *Culture Media used*;
  Sucrose containing blood agar media at 37˚C.

- *Sample Preparation for S. Aureus and C.Albicans Adhesion;*
  The specimens were immersed in distilled water and incubated in incubator for 24 hours at 37˚c (figure-6.A&B); in order to prevent the occurrence of distortion and release of monomer after polymerization when in culture, by promoting the maximum water sorption [8,13]. After 24 hours all the samples were clean and sterilized with chlorhexidine 2% [8,14]. The sterile broth was used to wash them in-order to remove chlorhexidine residue and placed in different sterilized container.

- *Microbiological Test;*
  The BHI broth volume was maintained with a suspension and turbidity equivalent to a McFarland standard of 0.5. 5 ml of BHI broth for each sample was distributed into the individual test tubes and inoculated with *C. albicans* and *S. aureus*. Sterilized specimens were transferred into these test tubes with help of sterile forcep. The test tubes were incubated in incubator for 18 hrs at 37˚C (figure-9&10).

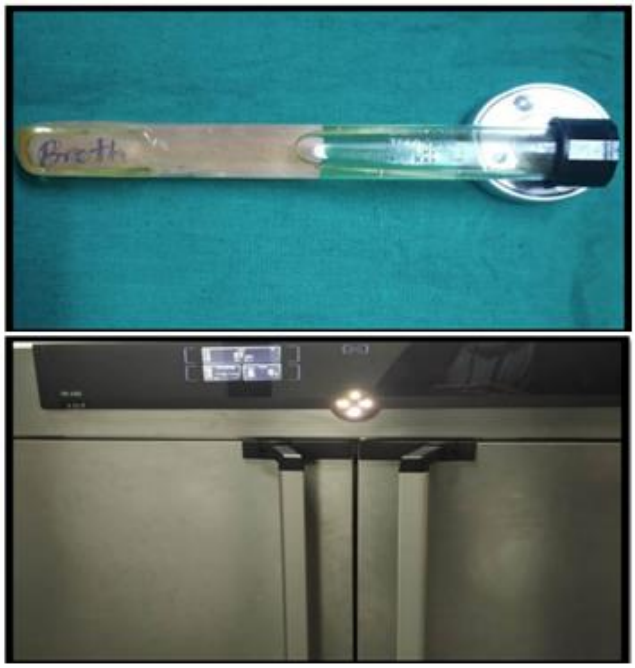

Fig 9&10 Specimens Immersed in BHI Broth Containing Microbial Strains & Incubated at 37º C

After this specimens were removed and cleaned with sterile BHI broth to remove non-adherent cells of *S. aureus* and *C. albicans*. With the help of the sterilized nichrome wire loop (2mm diameter) primary inoculum was spread over the blood agar plate with quadrant streaking method (figure-11).

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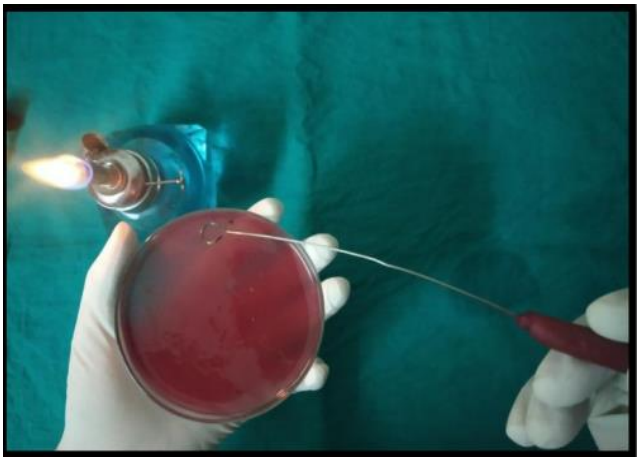

Fig 11 Primary Inoculum Being Spread Over the Blood Agar Plate

Blood agar plates with primarily inoculum with *S. aureus* and *C.albicans* incubated at 37˚C for 48 hrs and for one week respectively. After incubation, the colony forming units (CFU) were noted for all the samples.

On blood agar plate *S. aureus* appeared as grapes like clusters which were golden yellow in colour whereas growth of *C. albicans* on blood agar plate appeared as small, white colonies. (figure-12,13,14,15,16,17).

Fig 12 Growth of Microbes on Blood Agar (Group I) A- S. aureus B- C. albicans

Fig 13 Growth of Microbes on Blood Agar (Subgroup IIA)

Fig 14 Growth of Microbes on Blood Agar (Subgroup IIB) A- S. aureus B- C. albicans

Fig 15 Growth of Microbes on Blood Agar (Subgroup IIIA)

Fig 16 Growth of Microbes on Blood Agar (Subgroup IIIB) A- S. aureus B- C. albicans

Fig 17 Growth Of Microbes On Blood Agar (Group IV) A- S. aureus B- C. albicans

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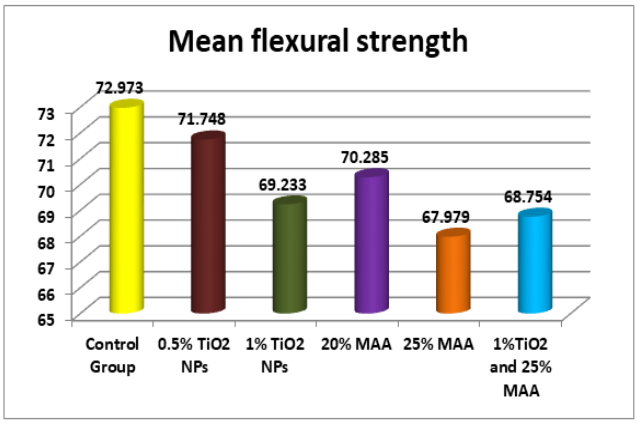
RESULTS AND OBSERVATIONS

➢ *For Flexural Strength*



Graph1 Graphic Representation of Mean Flexural Strength

Comparing the effect of groups and subgroups together on flexural strength, **ANOVA** showed highly significant effect of all four groups and subgroups (**F=9.953, p<0.001**) on flexural strength.

Pairwise comparison using Post hoc Tukey's test showed that significant difference(P≤0.05) was found in the flexural strength of all the groups except subgroup-IIA (P=0.704)when compared with control group.

Significant differences were found in the flexural strength of subgroup-IIA when compare with subgroup-IIB(P=0.50), subgroup-IIIB(P=0.001) and group-IV(P=0.011). Comparison of flexural strength subgroup-IIIB vs subgroup-IIA(P=0.001),subgroup-IIB vs subgroup-IIA(P=0.50) and group-IV vs subgroup-IIA(P=0.011) were also significant.(Table-2)

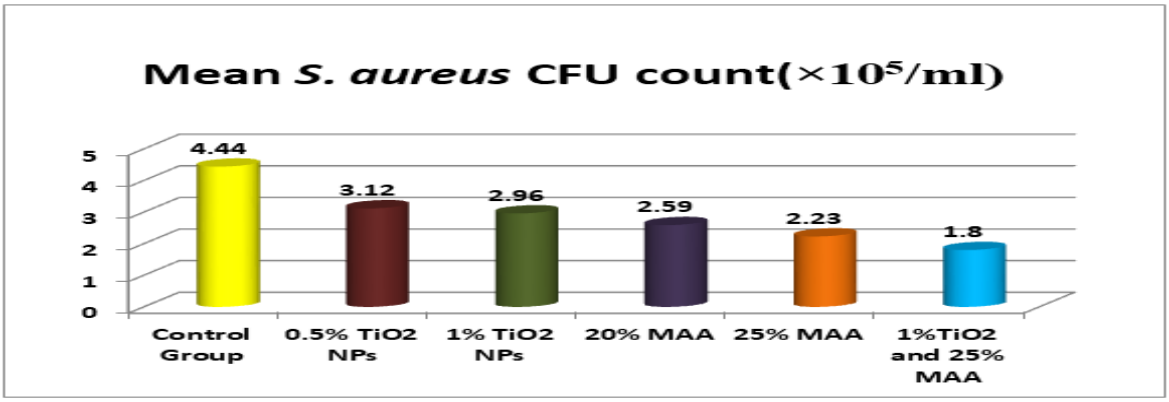
Table 2 Inter Group Comparison of Mean Difference of Flexural Strength Assessed by using TUKEY HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P-value	level
Group-I	Subgroup-IIA	1.22500	.85157	.704	NS
	Subgroup-IIB	3.74000	.85157	.001	***
	Subgroup-IIIA	2.68800	.85157	.030	*
	Subgroup-IIIB	4.99400	.85157	0.000	***
	Group-IV	4.21900	.85157	0.000	***
Subgroup-IIA	Group-I	-1.22500	.85157	.704	NS
	Subgroup-IIB	2.51500	.85157	.050	*
	Subgroup-IIIA	1.46300	.85157	.526	NS
	Subgroup-IIIB	3.76900	.85157	.001	***
	Group-IV	2.99400	.85157	.011	**
Subgroup-IIB	Group-I	-3.74000	.85157	.001	***
	Subgroup-IIA	-2.51500	.85157	.050	*
	Subgroup-IIIA	-1.05200	.85157	.818	NS
	Subgroup-IIIB	1.25400	.85157	.683	NS
	Group-IV	.47900	.85157	.993	NS
Subgroup-IIIA	Group-I	-2.6880	.85157	.030	*
	Subgroup-IIA	-1.46300	.85157	.526	NS
	Subgroup-IIB	1.05200	.85157	.818	NS
	Subgroup-IIIB	2.30600	.85157	.090	NS
	Group-IV	1.53100	.85157	.476	NS
Subgroup-IIIB	Group-I	-4.99400	.85157	0.000	***
	Subgroup-IIA	-3.76900	.85157	.001	***
	Subgroup-IIB	-1.25400	.85157	.683	NS
	Subgroup-IIIA	-2.30600	.85157	.090	NS
	Group-IV	-.77500	.85157	.942	NS
Group-IV	Group-I	-4.21900	.85157	0.000	***
	Subgroup-IIA	-2.99400	.85157	.011	**
	Subgroup-IIB	-.47900	.85157	.993	NS
	Subgroup-IIIA	-1.53100	.85157	.476	NS
	Subgroup-IIIB	.77500	.85157	.942	NS

NS=Non-significant (p>0.05);*=Significant (p<0.05);**=Very significant (p<0.01); ***=Highly significant (p<0.001)

➢ *Microbial Tests*

- *For Staphylococcus Aureus:*



Graph-2 Graphic Representation of Mean CFU Count (×10^5 /Ml) of *S. Aureus*

Comparing the effect of groups and subgroups together on CFU count (×10^5 /ml) of *S.aureus*, **ANOVA** showed highly significant effect of all four groups and subgroups (**F=122.502, P=00.0001**) on CFU count of *S. aureus*.

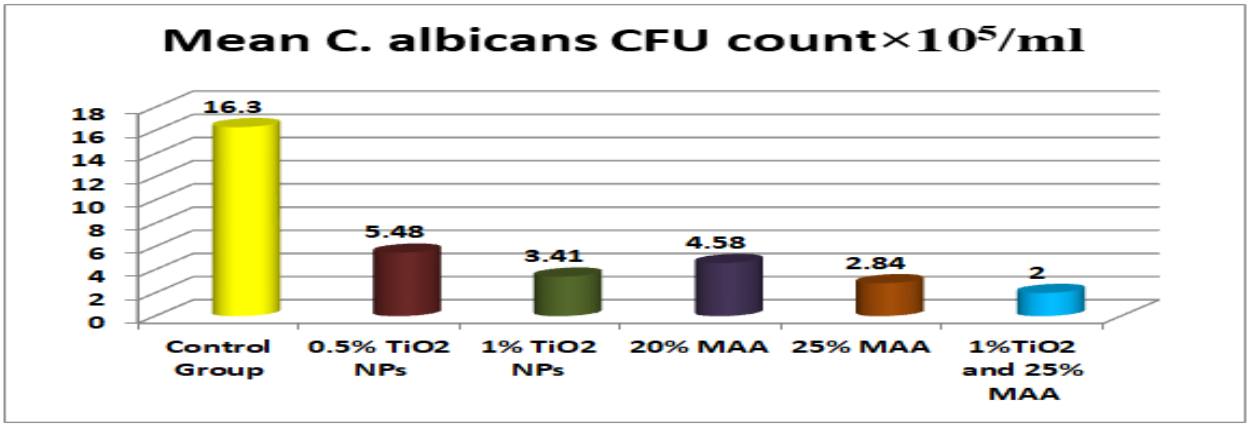
Pairwise comparison using Post hoc Tukey's test showed that significant difference(P≤0.05) was found in the *S.aureus* colony count of all the groups except subgroup IIA when compared with subgroup IIB(P=0.743)(Table-3)

Table 3 Inter Group Comparison of Mean Difference of CFU Count (×10^5 /Ml) of *S. Aureus* Assessed by using TUKEY HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P-value	Level
Group-I	**Subgroup-IIA**	1.32000*	0.11665	0.000	***
	Subgroup-IIB	1.48000*	0.11665	0.000	***
	Subgroup-IIIA	1.85000*	0.11665	0.000	***
	Subgroup-IIIB	2.21000*	0.11665	0.000	***
	Group-IV	2.64000*	0.11665	0.000	***
Subgroup-IIA	**Group-I**	-1.32000*	0.11665	0.000	***
	Subgroup-IIB	0.16000	0.11665	0.743	NS
	Subgroup-IIIA	0.53000*	0.11665	0.000	***
	Subgroup-IIIB	0.89000*	0.11665	0.000	***
	Group-IV	1.32000*	0.11665	0.000	***
Subgroup-IIB	**Group-I**	-1.48000*	0.11665	0.000	***
	Subgroup-IIA	-.16000	0.11665	0.743	NS
	Subgroup-IIIA	0.37000*	0.11665	0.029	*
	Subgroup-IIIB	0.73000*	0.11665	0.000	***
	Group-IV	1.16000*	0.11665	0.000	***
Subgroup-IIIA	**Group-I**	-1.85000*	0.11665	0.000	***
	Subgroup-IIA	-0.53000*	0.11665	0.000	***
	Subgroup-IIB	-0.37000*	0.11665	0.029	*
	Subgroup-IIIB	0.36000*	0.11665	0.036	*
	Group-IV	0.79000*	0.11665	0.000	***
Subgroup-IIIB	**Group-I**	-2.21000*	0.11665	0.000	***
	Subgroup-IIA	-0.89000*	0.11665	0.000	***
	Subgroup-IIB	-0.73000*	0.11665	0.000	***
	Subgroup-IIIA	-0.36000*	0.11665	.036	*
	Group-IV	0.43000*	0.11665	.007	**
Group-IV	**Group-I**	-2.64000*	0.11665	0.000	***
	Subgroup-IIA	-1.32000*	0.11665	0.000	***
	Subgroup-IIB	-1.16000*	0.11665	0.000	***
	Subgroup-IIIA	-0.79000*	0.11665	0.000	***
	Subgroup-IIIB	-0.43000*	0.11665	0.007	**

NS= Non-significant (p>0.05);*=Significant (p<0.05);**=Very significant (p<0.01); ***=Highly significant (p<0.001)

- *For Candida Albicans:*



Graph 3 Graphic Representation of Mean CFU Count (×10^5 /Ml) Count of *C. Albicans*

Comparing the effect of groups and subgroups together on CFU count (×10^5 /ml) of *C.albicans*, **ANOVA** showed highly significant effect of all four groups and subgroups (**F=793.985, P=00.000**) on CFU count of *C.albicans*.

Pairwise comparison using Post hoc Tukey's test showed that significant difference (P≤0.05) was found in the *C.albicans* colony count of all the groups except subgroup IIIB when compared with subgroup IIB(P=0.283).(Table-4)

Table 4 Inter Group Comparison of Mean Difference of CFU Count (×10^5 /Ml) of *C.Albicans* Assessed by using TUKEY HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P-value	Level
Group-I	**Subgroup-IIA**	10.82000*	.26628	0.000	***
	Subgroup-IIB	12.89000*	.26628	0.000	***
	Subgroup-IIIA	11.72000*	.26628	0.000	***
	Subgroup-IIIB	13.46000*	.26628	0.000	***
	Group-IV	14.30000*	.26628	0.000	***
Subgroup-IIA	**Group-I**	-10.82000*	.26628	0.000	***
	Subgroup-IIB	2.07000*	.26628	0.000	***
	Subgroup-IIIA	.90000*	.26628	.016	*
	Subgroup-IIIB	2.64000*	.26628	0.000	***
	Group-IV	3.48000*	.26628	0.000	***
Subgroup-IIB	**Group-I**	-12.89000*	.26628	0.000	***
	Subgroup-IIA	-2.07000*	.26628	0.000	***
	Subgroup-IIIA	-1.17000*	.26628	.001	***
	Subgroup-IIIB	.57000	.26628	.283	NS
	Group-IV	1.41000*	.26628	0.000	***
Subgroup IIIA	**Group-I**	-11.72000*	.26628	0.000	***
	Subgroup-IIA	-.90000*	.26628	.016	*
	Subgroup-IIB	1.17000*	.26628	.001	***
	Subgroup-IIIB	1.74000*	.26628	0.000	***
	Group-IV	2.58000*	.26628	0.000	***
Subgroup-IIIB	**Group-I**	-13.46000*	.26628	0.000	***
	Subgroup-IIA	-2.64000*	.26628	0.000	***
	Subgroup-IIB	-.57000	.26628	.283	NS
	Subgroup-IIIA	-1.74000*	.26628	0.000	***
	Group-IV	.84000*	.26628	.030	*
Group IV	**Group-I**	-14.30000*	.26628	0.000	***
	Subgroup-IIA	-3.48000*	.26628	0.000	***
	Subgroup-IIB	-1.41000*	.26628	0.000	***
	Subgroup-IIIA	-2.58000*	.26628	0.000	***
	Subgroup-IIIB	-.84000*	.26628	.030	*

NS= Non-significant (p>0.05); *=Significant (p<0.05); **=Very significant (p<0.01); ***=Highly significant (p<0.001)

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IV. DISCUSSION

Heat cure PMMA resins have been the material of choice for the denture fabrication because of numerous reason like it is biocompatible, dimensional stable, easily available material [17]. Despite of these advantages acrylic resin has potential for microbial adhesion. Therefore, efforts have been made to add some specific antimicrobial agents like nanoparticles and comonomer into the PMMA[12]. In many nanoparticles, TiO_2NPs have gained popularity because of its higher stability, antimicrobial properties, less cost, photocatalytic activity, and safety toward both humans and the environment[10] and comonomer (MAA) has gained importance because of its antimicrobial properties and it is biocompatibility[8]. Also prevents the cross-contamination from laboratory to the patient and dentist.

The addition of NPs and MAA in PMMA may affect the mechanical properties of the denture base resin like flexural strength, impact strength and tensile strength etc. Strength of ensures that the prosthesis serves its proposed functions safely and effectually over the extended periods of time.

Girish Nazirkar et al conducted a study in which they added the TiO_2NPs at different concentration (0.5% and 1%) in denture base resin and assessed the flexural strength of denture base resin. According to this study, the flexural strength of the final prosthesis had been adversely affected with incorporation of TiO_2NPs. Flexural strength was less in 1% TiO_2NPs added denture base resin than 0.5% added TiO_2NPs [10].

A study conducted by Mohamed Ashour Ahmed et al showed decrease flexural strength with increase in concentration of TiO_2NPs; as they had incorporated the TiO_2NPs in heat cure PMMA at 1% and 5% by wt.[11]

All the above studies showed a significant reduction in flexural strength of PMMA with increase in concentration of TiO_2NPs. This present study also showed the significant reduction in Flexural strength of TiO_2NPs incorporated PMMA.

The TiO2NPs additive can act as an impurity and interfere with the polymerization reaction which could affect the internal structure of polymerized PMMA and decreases the flexural strength of polymerized denture base resin. The TiO_2NPs added in PMMA act as a plasticizer which increases the amount of residual unreacted monomer, may cause decrease in the strengths of the denture base resin[10].

Lokendra Gupta et al modified the PMMA by adding MAA to the monomer at different concentration (0, 15, 20, and 25 wt %) to evaluate the effect on flexural strength and on adhesion of *S.aureus*. Result showed a non-significant reduction in flexural strength with increase in concentration of MAA. Present study showed significant reduction in flexural strength with increase in concentration of MAA in PMMA[8].

MAA tends to be a hydrophilic material. When MAA incorporated in PMMA, water sorption by PMMA increased due to presence of hydrophilic radicals. Water is a very complex solvent which creates strong interaction with the polymer of denture base resin, because of its ability to form hydrogen bonds with denture base resin. So it can be assumed that water sorption could be the reason for decrease in flexural strength of denture base resin when altered with MAA[18].

Edwin Tandra et al to evaluate the effects of TiO_2NPs added in denture base [containing silane (coupling-agent)] at different concentration 1% and 3%. Result showed that 1% of TiO_2NPs was able to increase the flexural strength of PMMA and flexural strength decreased with increase in concentration of TiO_2NPs (3%); silane (coupling-agent) distributed the NPs evenly in PMMA[16]. In present study there was significant reduction in flexural strength of 1% TiO_2NPs added PMMA and silane was not added in PMMA.

A study was conducted by Ahmad et al on the antibacterial properties of TiO_2NPs added at 0% and 1 % by wt in PMMA. It was concluded that, as the concentration of TiO_2NPs increased, the antibacterial property of the TiO_2NPs also increased. This study was conducted against the *E.coli* bacteria[19].

Laura S. Acosta-Torres et al by incorporated the metal oxide nanoparticles (TiO_2NPs and Fe_2O_3NPs) to heat cure PMMA to evaluate the physical, mechanical and microbial activity denture base resin.There was significant reduction in adhesion of *C.albicans*, flexural strength, porosity of denture base[2].

Lokendra Gupta et al modified the PMMA by adding MAA to the monomer at different concentration (0, 15, 20, and 25 wt %) and evaluated the effect on adhesion of *S.aureus* and on flexural strength. Addition of MAA to denture base resin significantly reduced the microbial adhesion without significantly affecting the flexural strength[8].

Another study was done by Gupta L et al to evaluate antifungal activity of MAA incorporated in PMMA at different concentration (0, 15, 20, and 25 wt %). The result showed significant reduction in adhesion of *Candida albicans* with increase in concentration of MAA in PMMA[20].

These all studies showed reduced microbial adhesion on PMMA altered with TiO_2NPs and MAA.

The adhesion of microbes to polymerized denture base resin has been correlated with attractive hydrophobic and repulsive electrostatic forces. For hydrophobic surfaces such as heat cure denture base resin, monomer units on the surface of acrylic plate, relate with the hydrophobic provinces on proteins presented in the cell membrane of the microbes by strong hydrophobic bonds. Such interactions would cause adhesion of microbes more readily to hydrophobic surfaces than such as PMMA plate. Since the adherence process takes place even in the presence of

repulsive forces (due to electrostatic interaction), the contribution of electrostatic interaction is secondary to the hydrophobic interaction[13,21,22,23].

C. albicans and *S. aureus* have net negative surface charges, lead to the electrostatic repulsion through the negative–negative charge interactions with the PMMA. Highly negatively charged denture base acrylic resin (PMMA) can prevent/reduce the adhesion of *C. albicans* and *S. aureus*. Conventional PMMA has very less net negative charge. Thus, it can be proposed that the addition of MAA to PMMA increases net negative charge of PMMA, which probably leads to a significant increase in the electrostatic repulsive forces, therefore this may prevents or reduces the adhesion of the microbes to PMMA[8,13,20].

Addition of TiO_2NPs reduces the adhesion of microbes on PMMA by reducing the microbial biofilm formation in mouth and denture base resin and also due to the anti-adherence property of TiO_2NPs[15].

No study was conducted to evaluate the flexural strength and antimicrobial activities of PMMA by incorporation 1%TiO_2NPs and 25%MAA both together. In this present study PMMA is altered with 1% TiO_2NPs+25% MAA in group-IV. Result showed that mean flexural strength of group-IV was less than all groups and subgroups except subgroup IIIB, which showed lowest mean flexural strength.

The TiO_2NPs act as a plasticizer in PMMA. TiO_2NPs increases the amount of unreacted residual monomer which decreases the strengths of PMMA. In contrast, MAA causes controlled polymerization of residual monomers thus leaving a lesser amount of residual monomer in the acrylized resin, resulting in better strength of the material[10,24,25]. Thus it can be assume that when these two materials are combined together, they counteract each other's actions.

Also it was found that mean CFU counts for *S.aureus* and *C.albicans* of group-IV were lower than all other groups and subgroup. It showed minimum adhesion of microbes because TiO_2NPs inhibits the formation of microbial flora and addition of MAA to PMMA increases net negative charge of denture base resin, which leads to increase in the electrostatic repulsive forces between microbes and polymerized PMMA, thus group-IV produced minimum adhesion of the microbes to PMMA.

The limitation of study include that the intraoral conditions could not be simulated while testing of specimens like saliva and repeated masticatory loads on the prosthesis and the lateral forces were also not taken into consideration during this study. These loads lead to fatigue of the denture and causes failure of prosthesis. In future study may be included clinical trials along with other properties like polymerization shrinkage, tensile strength and water sorption etc to help the dentist choose the most ideal denture material for clinical use.

V. COCLUSION

Within the limitation of this study following conclusions were made that addition of TiO_2NPs and MAA both showed significant increase in antimicrobial properties and decrease in the flexure strength of the material. The combination of 1% TiO_2NPs and 25% MAA shows the best antibacterial and antifungal properties amongst all concentrations. Flexural strength decreases gradually on addition of these agents with least reduction in 0.5% TiO_2NPs added PMMA followed by 20 % MAA, 1% TiO_2NPs, 1% TiO_2NPs+25% MAA, and 25% MAA. When antimicrobial properties and flexural strength is considered together the combination of 1% TiO_2NPs+25% MAA provides the best antimicrobial activity with relatively lesser amount of reduction in flexural strength among all. This information can be clinically applied by using the best suited concentration of TiO_2NPs and/or MAA as per the patient requirement.

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