



# Anti Microbial Activity of Silanized Silver Nanoparticles Impregnated in PMMA Resin Against Strains of *C.Albicans* – An Invitro Study

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## KEYWORDS

PMMA, Silane coupling agent, Silver nanoparticles, *Candida albicans*, Antimicrobial activity.

## ABSTRACT:

### Introduction:

Poly (methyl methacrylate) (PMMA) is a widely used denture base material owing to its esthetics, ease of fabrication, and cost-effectiveness. However, its susceptibility to microbial adhesion, especially by *Candida albicans*, can lead to denture-induced stomatitis. Silver nanoparticles (AgNPs) exhibit antimicrobial properties, but poor dispersion within PMMA limits their effectiveness. Coating AgNPs with a silane coupling agent, 3-(trimethoxysilyl)propyl methacrylate, enhances their bonding and distribution in the resin matrix.

### Materials

### and

### Methods:

An in vitro comparative study was conducted on four groups of PMMA samples: Control (no AgNPs), 0.75% AgNPs, 1.0% AgNPs, and 1.5% AgNPs. Samples were tested against *Candida albicans* by counting colony-forming units (CFUs) after incubation. Data were analyzed using the Kolmogorov–Smirnov test for normality and Tukey’s Honest Significant Difference (HSD) test for multiple comparisons.

### Results:

A significant reduction in CFU count was observed in all experimental groups compared to the control ( $p < 0.05$ ). The antimicrobial activity increased with higher concentrations of silanized AgNPs, with the 1.5% AgNP group showing the greatest reduction in *Candida albicans* colonies.

### Discussion

### and

### Conclusion:

Silanized silver nanoparticles enhanced the antimicrobial properties of PMMA denture base resin. Improved dispersion through silane coating resulted in effective inhibition of *Candida albicans*. Incorporating silanized AgNPs into denture base materials may reduce microbial colonization and prevent denture-induced stomatitis.

## 1. Introduction

Acrylic polymers closely resemble oral soft tissues due to their shade variations and ability to incorporate fibers. Poly (methyl methacrylate) (PMMA) remains the most widely used denture base material because of its esthetics, ease of processing, repairability, biocompatibility, and durability in the oral environment [1]. Despite these advantages, PMMA readily supports microbial adhesion, leading to denture stomatitis in 50–

70% of denture wearers [2]. The intaglio surface of dentures provides an ideal environment for fungal biofilm formation, predominantly by *Candida albicans*.

Silver nanoparticles (AgNPs) possess potent antimicrobial properties and have been incorporated into denture base resins to reduce microbial colonization. However, their tendency to agglomerate within the PMMA matrix limits uniform dispersion and reduces effectiveness.



Silver, copper, and zinc ions have been used for their fungicidal activity against *C. albicans* [3]. However, studies by Sahebjame et al. and Pachava et al. showed that orally applied antifungal agents were ineffective due to rapid dilution in saliva. A more reliable approach is the incorporation of antimicrobial macromolecules into the PMMA denture base via polymer interaction [4]. Consequently, nanoscale modifications of PMMA have been developed to enhance its antimicrobial performance [5].

Silver nanoparticles disrupt fungal cell wall and membrane integrity, interfere with intracellular structures, and alter genome-wide transcription, thereby inhibiting growth. Their high surface area-to-volume ratio further enhances their microbicidal effect. By attaching to the cell membrane, they increase permeability and induce structural damage that leads to cell death. Traditionally, these nanoparticles have been incorporated into denture base resins through physical mixing without chemical bonding.

The problem associated with the incorporated silver nanoparticles is its incapability to homogeneously disperse within the polymer (PMMA) powder causing agglomeration of nanoparticles thus preventing its high surface area being realized for antibacterial or antifungal action. The response to this problem can be provided by incorporation into the polymeric matrix 3-(trimethoxysilyl) propyl methacrylate (TMSPM). It contains tri-alkoxy groups and can be supportive in adhesion promotion between dissimilar materials. The Si-O-CH<sub>3</sub> can work as the bonding agent between polymers and metals [6].

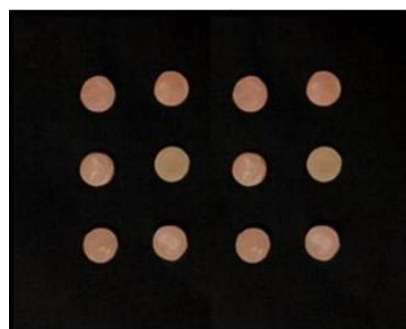
## 2. Methodology

This in-vitro study evaluated and compared the antifungal activity of silver-nanoparticle (AgNP)-impregnated polymethyl methacrylate (PMMA) denture base resin against an unmodified PMMA control. Four groups were prepared, each consisting of 12 acrylic resin specimens:

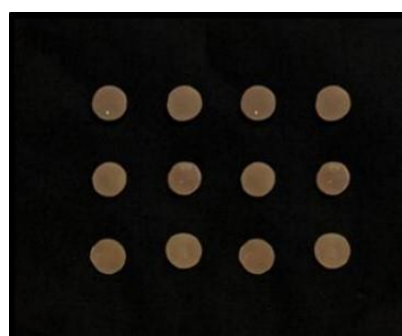
- **Group I (Control):** PMMA without AgNP (n = 12) Fig 1
- **Group II:** 0.75% AgNP impregnated PMMA (n = 12) Fig 2
- **Group III:** 1.0% AgNP impregnated PMMA (n = 12) Fig 3

- **Group IV:** 1.5% AgNP impregnated PMMA (n = 12) Fig 4

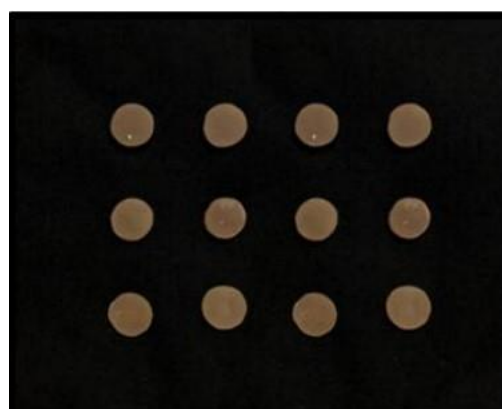
**Figure 1: Control group PMMA resin discs**



**Figure 2: 0.75% AgNP impregnated PMMA resin discs**

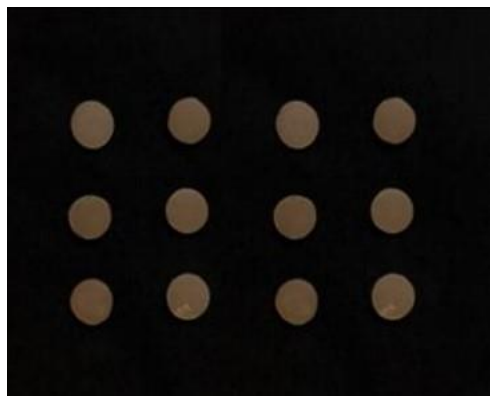


**Figure 3: 1.0% AgNP impregnated PMMA resin discs**





**Figure 4: 1.5% AgNP impregnated PMMA resin discs**



#### Sample Size determination:

Using statistical power analysis G\*Power 3.1 software and considering ANOVA Repeated measures, the sample size for the current study was estimated to be 48 by maintaining  $\alpha$  error as 0.05 at 95% CI,  $\beta$  error as 0.2, Power of the test (1- $\beta$  error) as 80%, effect size (Cohen's f statistic) as 0.25, number of groups as 4 and number of measurements as 2. The final sample size was approximated to 80 for assessing bonding property and antifungal efficacy. To allow for unanticipated deviations from statistical assumptions, equal number samples were randomly distributed in each of the groups i.e. 12 samples in each group with an allocation ratio of 1:1 in the control and test groups.

#### Preparation of AgNP

Silver nanoparticles of size 10–20 nm and a coupling agent 3-(Trimethoxysilyl) propyl methacrylate TMSPM was used. One hundred milliliter of ethanol aqueous solution (70 vol %) was prepared using 99.8 vol% ethanol and de-ionized water (30 % vol), The pH of ethanol was maintained at 4.8. 5 ml of ethanol was added to 5 ml of coupling agent and this mixture was then added to 5 gm of silver nanoparticles. The mixture was then placed in magnetic stirrer for 20 mins and sonicated in probe sonicator apparatus for 20 mins, then the solution was left to dry at room temperature for 14 days. For the fabrication of experimental groups, the specified percentage of AgNP (0.75%, 1.0%, 1.5%) is added to polymer in Group II, III and IV respectively and it is ball milled for one hour to achieve homogenous mixing.

#### Sample fabrication

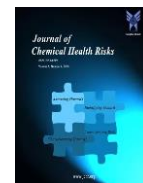
Steel dies of standard ISO size were prepared in the dimension 10\*2mm [7]. Vernier caliper was used to measure the dimension of the dies. These standardized steel dies were used to prepare the mold space for acrylic samples, processed using the compression moulding technique and finished as per standard procedures.

#### Antifungal testing

The Four groups were tested for antifungal property by counting the colony forming units. Sample Preparation: 12 numbers of AgNP impregnated Acrylic samples in each group (Disc) were autoclaved at 121°C for 15 mins before treatment. Pure colony of *Candida albicans* (ATCC 10231) strain was inoculated in potato sabouraud dextrose broth (SDB) (1% yeast extract, 1% peptone, 4% glucose, 1% agar) and incubated at 37°C for 24 hrs. The cell suspension of *C. albicans* was determined using a haemocytometer Neubauer improved chamber and adjusted to  $1 \times 10^6$  CFU/mL in RPMI 1640 medium supplemented with 2% (w/v) glucose. The test materials were kept in a sterile 15 ml centrifuge tube, 1 ml of above adjusted culture was added and incubated for 72 hrs at 37°C. Post incubation, the media was removed and the materials were washed 5 times with sterile PBS. Then, the *C.albicans* adhered to the study material were collected in 1 ml of PBS via vigorous vortex mixing for 2 mins. The above collected *C.albicans* cells were serially diluted (10-3 dilution) and 0.1 ml of sample was cultured on the Mueller Hinton Agar Medium (Himedia M173-500G) by spread plate technique. The test plates were incubated for 24h at 37°C as inverter position, colony formed on next day were counted manually and the results were expressed as colony forming units per disc.

#### Statistical Analysis

Data regarding colony forming units (CFU) of *Candida Albicans* were entered into Microsoft Excel and analysed using IBM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, N.Y., USA). Data was investigated for normality using the **Kolmogorov-Smirnov test and analysed a normal distribution**. The no. of CFU of *Candida Albicans* between and within 4 groups were analysed using One-way ANOVA followed by multiple comparisons with Tukey's Honest



significant difference test ( $\alpha=0.05$ ). Pearson's Correlation of the no. of CFU with the percentage variation in PMMA coated on AgNP was also analysed.

The level of statistical significance was determined at  $p \leq 0.05$ .

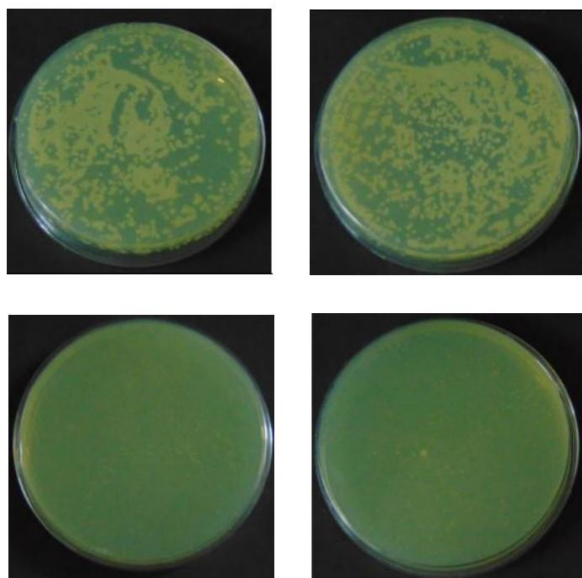
### 3. Results

**Table 1: Mean distribution of CFU counts of *Candida albicans* in control and AgNP-coated PMMA groups (Fig 9)**

Groups (No. of CFUs) (CFU/disc)	n = 48 (n = 12 per group)	Minimum	Maximum	Mean $\pm$ SD
Control (Fig 5)	12	230.00	608.00	396.41 $\pm$ 142.82
0.75% coated AgNP with PMMA (Fig 6)	12	174.00	228.00	193.91 $\pm$ 16.64
1.0% coated AgNP with PMMA (Fig 7)	12	116.00	168.00	135.83 $\pm$ 20.63
1.5% coated AgNP with PMMA (Fig 8)	12	21.00	115.00	63.00 $\pm$ 32.50

Table 1 presents the distribution of *Candida albicans* colony-forming units (CFUs) across the control and AgNP-impregnated PMMA groups. The control group showed the highest CFU counts, while all AgNP-containing groups demonstrated progressively lower values with increasing nanoparticle concentration. The 1.5% AgNP group exhibited the lowest mean CFU count among the four groups. A progressive reduction in CFU count was observed with increasing AgNP concentration.

**Figure 5: Group I showing control group CFU**



**Figure 6: Group II showing CFU of 0.75% AgNP impregnated PMMA**

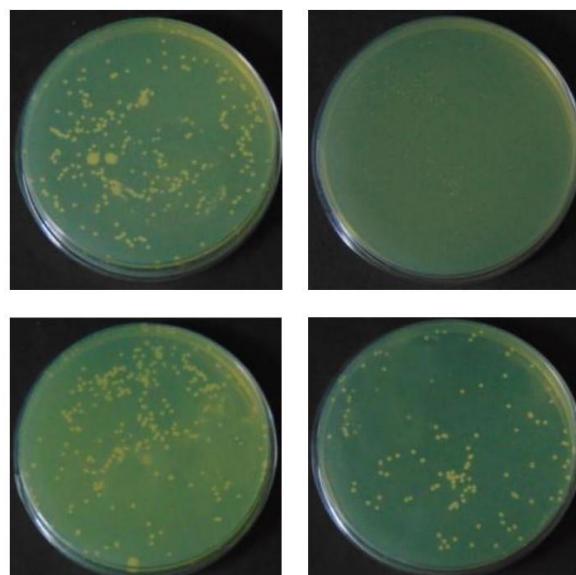




Figure 7: Group III showing CFU of 1.0% AgNP impregnated PMMA

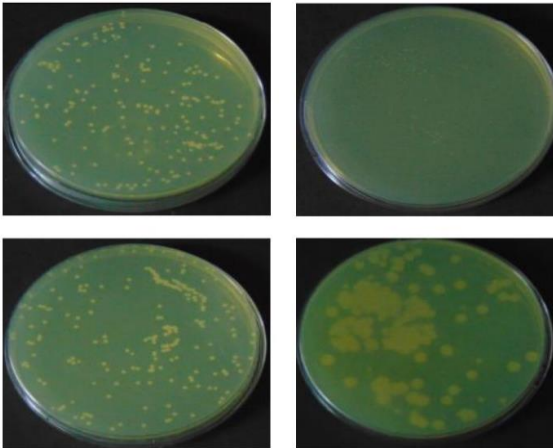


Figure 8: Group IV showing CFU of 1.5% AgNP impregnated PMMA

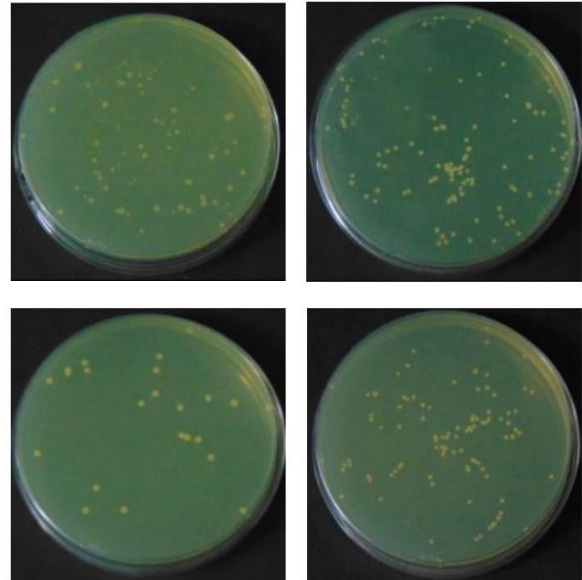
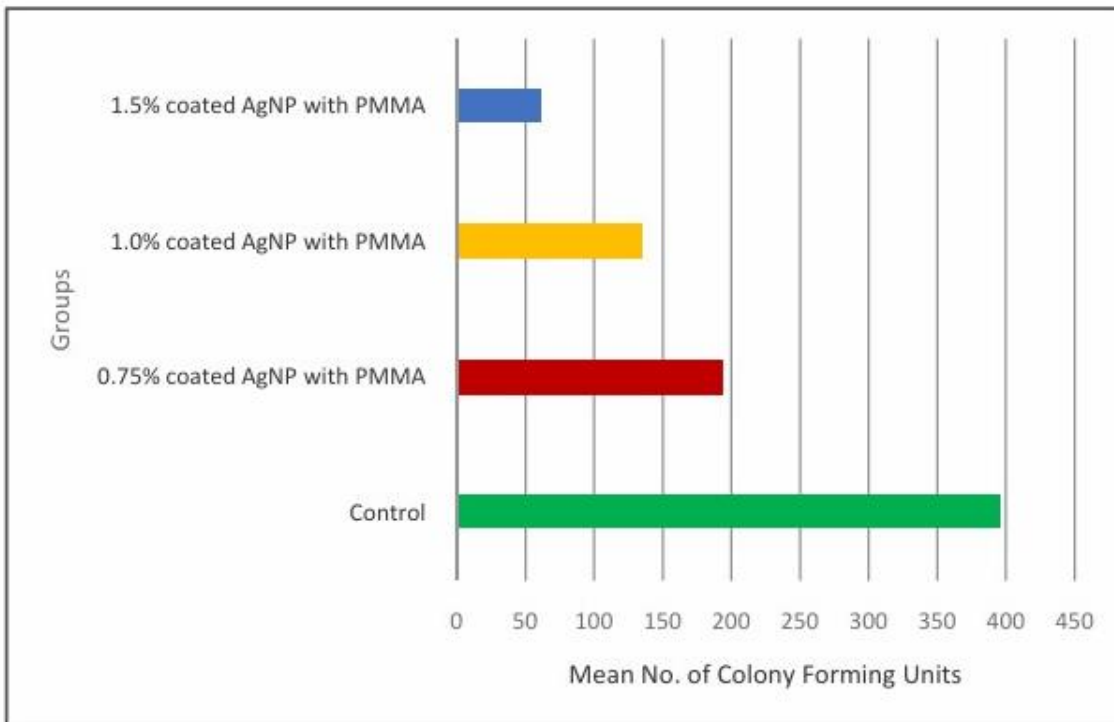


Figure 9 – Bar diagram showing Mean distribution of the No. of colony forming units of *Candida albicans* in control and experimental groups



**Table 2: Post hoc pairwise comparisons following one-way ANOVA**

(I) Groups	(J) Groups	Mean Difference (I-J)	p value	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	0.75% coated AgNP with PMMA	202.50*	.000*	121.37	283.63
	1.0% coated AgNP with PMMA	260.58*	.000*	179.45	341.71
	1.5% coated AgNP with PMMA	333.42*	.000*	252.29	414.55
0.75% coated AgNP with PMMA	Control	-202.50*	.000*	-283.63	-121.37
	1.0% coated AgNP with PMMA	58.08	.238	-23.05	139.21
	1.5% coated AgNP with PMMA	130.92*	.001*	49.79	212.05
1.0% coated AgNP with PMMA	Control	-260.58*	.000*	-341.71	-179.45
	0.75% coated AgNP with PMMA	-58.08	.238	-139.21	23.05
	1.5% coated AgNP with PMMA	72.83	.093	-8.30	153.96
1.5% coated AgNP with PMMA	Control	-333.42*	.000*	-414.55	-252.29
	0.75% coated AgNP with PMMA	-130.92*	.001*	-212.05	-49.79
	1.0% coated AgNP with PMMA	-72.83	.093	-153.96	8.30

\*Mean difference is significant at the 0.05 level

\* indicates significance at  $p < 0.05$ .

Post hoc comparisons using Tukey's HSD test (Table 2) demonstrated a statistically significant reduction in the mean number of *Candida albicans* CFUs in all AgNP-impregnated groups when compared with the control group ( $p < 0.001$ ). The 1.5% AgNP group

exhibited the greatest antifungal effect, with significantly lower CFU counts than both the 0.75% ( $p = 0.001$ ) and 1.0% ( $p = 0.093$ ; not significant) groups. The difference between the 0.75% and 1.0% AgNP groups was also not statistically significant ( $p = 0.238$ ). These findings indicate a concentration-dependent trend in antifungal activity, with 1.5% AgNP-incorporated PMMA showing the most substantial reduction in *C. albicans* adhesion.

**Table 3: Pearson correlation between AgNP concentration and CFU count.**

		No. of Colony forming units
Variable	Pearson Correlation (r)	-0.828**
	Sig. (2-tailed)	0.000**
	n	48

\*\*Correlation is significant at the 0.01 level (2-tailed).

Pearson correlation analysis showed a strong negative correlation between AgNP concentration and CFU count ( $r = -0.828$ ,  $p < 0.001$ ), indicating that increasing the percentage of AgNPs incorporated into PMMA is associated with a substantial reduction in *C. albicans* colonization.

#### 4. Discussion

Silver is a potent antimicrobial and antifungal element that remains non-toxic to host tissues. PMMA–silver nanoparticle composites have therefore emerged as promising materials for dental applications [8]. In this study, silanized silver nanoparticles significantly inhibited *Candida albicans* growth at 0.75%, 1.0% and 1.5% concentrations, supporting their potential as antifungal additives in denture bases [9]. The strong fungicidal activity likely results from the large surface area–to–volume ratio of AgNPs, enabling membrane attachment, permeability disruption, and structural damage to fungal cells.

The present findings align with Kamikawa et al., who reported reduced adherence of *C. albicans* and *C. glabrata* on AgNP-coated resins, [10] and with Acosta-Torres et al., who observed decreased CFUs and biofilm formation in PMMA–AgNP discs [11]. Contrary to Wady et al. (2012), who found no significant effect—possibly due to nanoparticle agglomeration—this study achieved consistent dispersion, likely attributable to silanization, suggesting surface modification is a key factor influencing efficacy.

A notable critical observation is the dose-dependent but non-linear trend in CFU reduction. Although antifungal activity increased with concentration, the improvement plateaued between 1.0% and 1.5%. This suggests that

beyond a certain concentration, the antifungal effect may not proportionally rise, possibly due to reduced surface availability or particle aggregation at higher loadings. This highlights the need for optimizing—not merely increasing—AgNP concentration.

Siddiqui et al. (2018) reported diminished inhibition zones beyond 2.0 wt% due to agglomeration and observed no significant effect at 0.75 wt% [12]. In contrast, the present study demonstrated significant reduction even at 0.75%, indicating that **silanization with 3-TMSPM may reduce agglomeration more effectively** than uncoated AgNPs. This difference underscores the critical role of surface chemistry in determining biological outcomes.

The enhanced antifungal effect can be attributed to the functional compatibility of TMSPM with PMMA. The organofunctional methacrylate group of TMSPM enables chemical integration during polymerization, improving nanoparticle distribution and stability. FTIR confirmation of new peaks at 1636–1646  $\text{cm}^{-1}$  supports the presence of covalent interactions. However, while FTIR demonstrates chemical changes, it does not directly quantify the uniformity or depth of nanoparticle incorporation—an inherent limitation of the method.

This study adds to the limited literature evaluating AgNPs coated specifically with 3-TMSPM. The confirmation of chemical interaction and demonstrated antifungal activity strengthen the evidence for its suitability. Yet, **in vitro conditions do not entirely replicate intraoral environments**, where saliva, masticatory forces, pH fluctuations, and surface wear may influence nanoparticle release and long-term efficacy. Additionally, factors such as potential



discoloration, mechanical property alterations, and long-term cytotoxicity were not assessed and warrant further investigation.

Particle size has critical implications for cytotoxicity [13]. Very small AgNPs (<5 nm) pose greater risk, as noted by Yen et al.<sup>8</sup>. The use of 10–20 nm particles in this study reduces this concern, but **actual ion release levels and biocompatibility under functional conditions remain unverified**, representing another area for future research.

Overall, this study demonstrates that silanized silver nanoparticles incorporated into heat-cured PMMA provide effective anticandidal activity by reducing fungal adhesion. The findings support their potential as modified denture base materials. However, the results should be interpreted with caution due to the controlled in vitro setting. Integrating long-term clinical simulations, evaluating mechanical properties, and determining optimal nanoparticle concentrations are essential before translating this technology into routine clinical use.

## 5. Conclusion

Thus from the findings of the present in vitro study, in which a silane coupling agent, 3-(Trimethoxysilyl) propyl methacrylate – TMSPM was used to coat silver nanoparticles, it can be concluded that, the silanized silver nanoparticles showed an effective antifungal action against *C.albicans* that had significant difference in the CFU when compared to the non coated silver nanoparticles impregnated PMMA.

### Limitations:

This study is an in vitro study, future studies on in vivo study is recommended. This study was limited to only one strain of fungus.

### Future recommendations:

Research into optimizing the incorporation of antimicrobial agents without compromising the mechanical properties of PMMA could lead to the creation of more effective denture materials.

Long-term studies evaluating the clinical efficacy and safety of these modified materials in real-world settings will be essential.

Mechanical properties of PMMA such as surface hardness and abrasion resistance could be studied.

Antimicrobial study could be done through Fluorescence Activated Cell Sorting (FACS) rather than colony forming units because this gives more information about presence of viable cells.

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