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EVALUATION AND COMPARISON OF ANTICANDIDAL EFFECT OF HEAT CURE POLYMETHYLMETHACRYLATE RESIN REINFORCED WITH DIFFERENT CONCENTRATIONS OF TITANIUM DIOXIDE NANOPARTICLES – AN INVITRO STUDY

Dental Science

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ABSTRACT

Most of the fungal infections in denture wearers are caused by Candida species which colonizes the denture surfaces forming a biofilm which is critical in the development of denture stomatitis. TiO_2 nanoparticles has gained popularity in dentistry as it is nontoxic, chemical inactive, cost efficient, corrosion resistant and possesses high microhardness. Since antimicrobial studies done in the past were with titanium coatings, titanium dioxide nanoparticles in different concentrations has been used in this study. A total of 44 acrylic resin specimens with concentrations of 0%, 0.5%, 1% and 1.5% of TiO_2 nanoparticles were tested for anticandidial activity and the colony forming units were counted. It was found that the addition of TiO_2 nanoparticles had an inhibitory effect on candidal growth and a decrease in the colony forming units were noted with an increase in the concentration of the nanoparticles.

KEYWORDS

TiO_2 nanoparticles, anticandidal, acrylic resin

INTRODUCTION

Candida species are normal commensal present in the oral cavity and predominantly seen in 60-100% of denture wearers and are the most common cause of opportunistic fungal infections.[1,2] These organisms colonize the denture surfaces forming a biofilm which is an important factor in the development of denture stomatitis.[3,4] This infection is prevalent among individuals under continuous use of immunosuppression drugs, nutritional deficiencies and poor denture hygiene.[1] Generally, proper denture care and oral hygiene can prevent this lesion. However, for older or hospitalised patients maintenance of adequate denture hygiene might be compromised owing to cognitive impairment, reduced motor dexterity and memory loss. In the past, systemic or local antibiotic agents have been used to reduce the fungal population but owing to microbial resistance and healthcare costs, research on denture base materials that has antimicrobial property is the need of the hour for its prevention and care.[5]

With the evolution of nanoscience, nanoparticles such as Al_2O_3, ZrO_2, Silver, TiO_2, nano gold have been added to polymethyl methacylate resin to overcome its mechanical properties such as low thermal conductivity, high coefficient of thermal expansion and relatively low modulus of elasticity and has been found to significantly improve its wear and tear resistance, anti-corrosion ability, fracture resistance and impact strength.[6,7] But the anticandidal effect of acrylic resin has been investigated only with silver nanoparticles where silver is known to have a wide antimicrobial spectrum.[8]

TiO_2 is widely used in dentistry as it is nontoxic, chemical inactive with high refractive index, cost efficient, corrosion resistant and has high microhardness.[9] Antibacterial studies have been done with titanium coatings.[10] Hence in this study, different concentrations of titanium dioxide nanoparticles were added to heat polymerized acrylic resin to evaluate its anticandidal activity.

MATERIALS AND METHODS:

I. FABRICATION OF TEST SAMPLES:

According to ISO specification no.1567[11], a metal die of dimensions 65mm × 10mm × 2.5mm was used to prepare wax patterns for the fabrication of acrylic resin specimens (Figure 1). The metal die was placed on a glass slab into which modelling wax was melted & poured and the procedure was repeated until 44 wax patterns were obtained (Figure 2). All the wax patterns were invested in dental flask using dental plaster using two stage pour technique where 3 wax patterns were invested in each flask following which dewaxing was done at 100ºC for 10 minutes to obtain the final moulds to fabricate the test samples. A thin layer of separating medium was then carefully painted on both halves of the flask and left to dry.

Fig 1: Metal trough of dimension 65mm × 10mm × 2.5mm

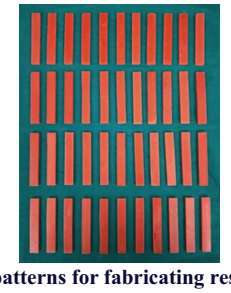


Fig 2: Wax patterns for fabricating resin specimens

The nanoparticles were treated with silane coupling agent to ensure its uniform dispersion in the resin matrix following which the surface treated nanoparticles were added to the polymer in different concentrations of 0%, 0.5%, 1% and 1.5% respectively. Eleven samples of each group were fabricated according to manufacturer's instructions by mixing polymer and monomer in the ratio of 3:1 in porcelain mixing jar for 30 seconds. When it reached dough stage, it was packed into the mould space and the flasks were closed and kept under pressure of 2000 psi for bench curing for 30 minutes. Once bench curing was done, the flasks were clamped and polymerized using short curing cycle of 74ºC for 90 minutes and then boiled for 1 hour. After curing, the flasks were allowed to bench cool to room

temperature and the specimens were retrieved. Excess material was trimmed off and the specimens were polished using sand paper and pumice. They were stored in distilled water at room temperature for 2 weeks and subjected to their respective testing (Figure 3).

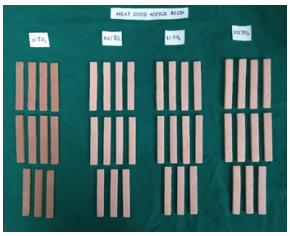


Fig 3: PMMA specimens reinforced with different concentrations of TiO_2 nanoparticles

II. CULTURE PREPARATION:

Sabouraud Dextrose Broth (Glucose 40g, Peptone 10g and distilled water 1000mL) was used. 30mL of the broth was prepared and autoclaved at 121°C for make it 15 minutes. *Candida albicans* from stock culture was inoculated into the sterile broth and incubated at 25°C for 72 hours. After incubation, the inoculum was adjusted 0.5 spectrophotometrically at 600nm using saline (1% sodium chloride $^{w}/_{v}$).

III. ACRYLIC SPECIMENS TREATED AGAINST CANDIDA ALBICANS:

The specimens (0%, 0.5%, 1% and 1.5%) were added respectively into test tubes containing 10mL of Sabouraud dextrose broth (Figure 4). Later, 100µL of *Candida albicans* was inoculated into the respective specimen tubes and incubated at 37°C for 24 hours.

Fig 4: Specimens in Sabouraud dextrose broth

IV. COLONY COUNTING:

The treated specimens were transferred into 10mL of sterile saline (1% sodium chloride w/v) respectively and sonicated for 10 minutes (Figure 5). Later, 100µL of suspension was poured into respective plates and approximately 20mL of sterile Sabouraud dextrose agar was added (Pour plate technique) and allowed to solidify. Later, the plates were incubated for 72 hours at 25°C. After incubation, the colony forming units were calculated (Figures 6-9).

Fig 5: Sonication of the specimens

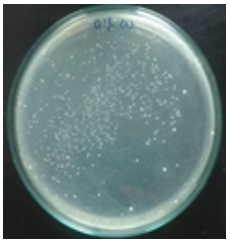


Fig 6: SDA plate of 0% TiO_2 nanoparticles

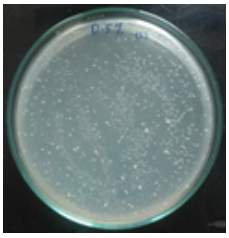


Fig 7: SDA plate of 0.5% TiO_2 nanoparticles

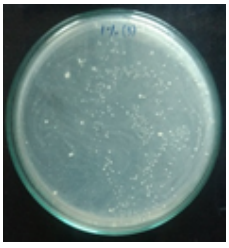


Fig 8: SDA plate of 1% TiO_2 nanoparticles

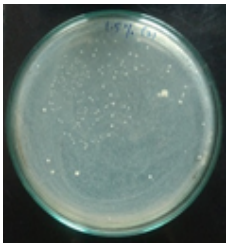


Fig 9: SDA plate of 1.5% TiO_2 nanoparticles

The results were statistically analysed using One way ANOVA followed by post hoc Tukey's test for the comparison between the four groups.

RESULTS

The colony forming units of resin specimens reinforced with different percentages of titanium dioxide nanoparticles (0%, 0.5%, 1% & 1.5%) is depicted in Table 1. One-way ANOVA analysis and Post hoc Tukey's test both showed significant difference in the anticandidal effect between the groups containing different concentrations (0%, 0.5%, 1% & 1.5%) of TiO_2 nanoparticles ($P<0.05$). (Tables 2 & 3) It was also noted that with an increase in the concentration of TiO_2 nanoparticles, the anticandidal effect also increased. The same is been graphically represented in **Graph 1.**

Table 1: Colony forming units (cfu) for 100µL of suspension

Specimen percentage	Colony forming units										
	1	2	3	4	5	6	7	8	9	10	11
0%	385	492	448	463	398	471	489	427	446	452	411
0.5%	392	423	416	429	409	385	424	394	427	389	407
1%	367	358	394	379	388	364	337	298	403	372	341
1.5%	296	242	194	253	265	234	304	273	249	281	277

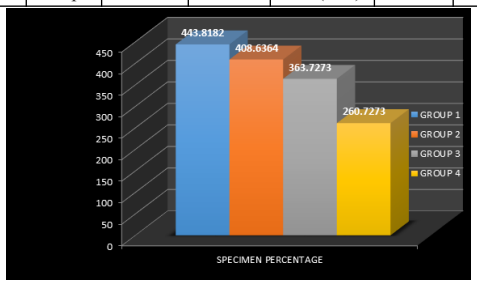
Table 2: Comparison of anti-candidal effect between different groups (One way ANOVA)

	Mean	Standard deviation	F	Significance
Group 1	443.8182	35.32370	82.133	0.000 (H.S)
Group 2	408.6364	16.39068		
Group 3	363.7273	29.90683		
Group 4	260.7273	31.10656		

Table 3: Multiple comparison of anticandidal effect between the different groups (Post hoc Tukey's test)

		Mean difference	Standard error	Significance	95% Confidence Interval	
					Lower bound	Upper bound
Group 1	Group 2	35.18182	12.39218	.034 (S)	1.9656	68.3981
	Group 3	80.09091	12.39218	.000 (H.S)	46.8747	113.3071
	Group 4	183.09091	12.39218	.000(H.S)	149.8747	216.3071

Group 2	Group 3	44.90909	12.39218	.004(H.S)	11.6929	78.1253
	Group 4	147.90909	12.39218	.000(H.S)	114.6929	181.1253
Group 3	Group 4	103.0000	12.39218	.000(H.S)	69.7838	136.2162



Graph 1: Anticandidal effect of resin specimens reinforced with different percentages of titanium dioxide nanoparticles (0%, 0.5%, 1% & 1.5%)

DISCUSSION:

The results of this study show that TiO_2 had an inhibitory effect on the candidal growth. Similar conclusions were drawn by **Arai et al** in 2009[11] where TiO_2 coating was found to decrease the number of C. albicans cells by nearly 35%, showing an adherence-inhibitory effect. This can be explained on the basis that TiO_2 nanoparticles produce intracellular reactive oxygen species (ROS) which induces destructive effects inside the microbial cells, oxidation of intra cellular Coenzyme A and peroxidation of lipids which decreases respiratory activity and subsequently causes death cell. **Haghighi et al** in 2013[12] showed that TiO_2 not only was able to kill *C.albicans* with increasing concentration but also can inhibit *C. albicans* biofilm formation at the less concentration.

In this study it was also observed that there was a positive correlation between the inhibitory effect and the concentration of TiO_2 nanoparticles which agreed with the findings of **Ahmad et al** in 2019[13] in which TiO_2 NPs at different concentrations (50, 100, and 150 µg/mL) were dosed into *C. albicans* culture and they concluded that the antimicrobial activity of NP was concentration-dependent.[13]

CONCLUSION:

Within the limitations of this study, it can be concluded that TiO_2 nanoparticles exhibited anticandidal effect even at low concentration and that its activity increases as the concentration of the nanoparticle increases.

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