ORIGINAL RESEARCH

Evaluation and comparison of anti-*Candida* effect of heat cure polymethylmethacrylate resin enforced with silver nanoparticles and conventional heat cure resins: An *in vitro* study

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ABSTRACT

Recent years have been dominated by research in nano science. Dentistry is no exception and there is increased research on nanoparticles in dentistry. Complete dentures increase the carriage of *Candida* in healthy patients, and the proliferation of *C. albicans* can be associated with denture-induced stomatitis.

Purpose: To evaluate the anti-*Candida* effect of heat cure denture base resins reinforced with Ag° in the ratio of 4:1, 3:1, 2:1 (Groups B, C, and D, respectively) to the weight of denture base resins.

Materials and Methods: Ag° were synthesized by chemical reduction method, incorporated into the polymer powder according to the ratio for each group, subjected to polymerization and microbial assay was calculated for the reference *C. albicans* strains by agar diffusion method for the incubation period of 24 h.

Results: Group D showed multifold decrease in the colony-forming units.

Conclusion: The antimicrobial effect of silver could be used vividly in the denture base for immunocompromised and geriatric patients.

Received : 14-05-12
Review completed : 30-01-13
Accepted : 18-04-13

Key words: Ag°-silver nanoparticle, *C. albicans*, denture stomatitis, PMMA-polymethylmethacrylate

Although dentistry has developed new materials and techniques used in rehabilitation of completely edentulous patients, mucosa-supported dentures still act as possible agents of tissue damage.[1,2] In 1885, Black first reported denture-induced pathosis as "sore mouth under plates." He identified acidogenic microorganism from the intaglio surface of dentures and corresponding mucosa as a causative factor of this pathologic condition.[3] In 1936, Cahn first suggested that the acidogenic microorganism responsible for causing "denture sore mouth" were *Monilia albicans*.[4] Denture-induced stomatitis is an inflammatory reaction of the denture bearing mucosa that affects approximately 65% of complete denture wearers.[5] It has a multifactorial etiology, and *C. albicans* is reported as the primary etiologic agent.[6,7] No potential antimicrobial agent that could be incorporated in the denture has been developed.

Over the past few decades, inorganic nanoparticles, whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nanoscale size, have elicited much interest. Nanophasic and nanostructured materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical application.[8,9] Recent studies have demonstrated that specifically formulated metal oxide nanoparticles have good antimicrobial activity.[10] Among inorganic antimicrobial agents, silver has been employed most extensively since ancient times to fight infections and control spoilage.[11-13] Silver has a well-tolerated tissue response and low toxicity profile and it is more toxic than many other metals against a broad spectrum of sessile bacteria and fungi which colonize on plastic surface.[14,15] Such characteristics have led to drawing an attention recently due to the emergence of antibiotic resistant bacteria as a result of overuse of antibiotics and far lower propensity to induce microbial resistance than antibiotics. Silver-containing materials are already used as prosthesis, such as the technology of central venous catheter, vascular graft, and wound dressing.[16,17] The

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Access this article online

Quick Response Code:

Website: www.ijdr.in

DOI: 10.4103/0970-9290.135923

antibacterial activities of silver nanoparticles are related to their size, with the smaller particles having higher activities on the basis of equivalent silver mass content.[18] The nano silver with its rapid and broad spectrum efficacy and its sustained silver cation (Ag+) release[16,17] appears to be more effective antimicrobial than microsized silver powder (μm), which shows lower antimicrobial activity owing to its limited surface.[17,19] This study evaluates the anti-*Candida* effect of heat cure polymethylmethacrylate reinforced with silver nanoparticles. The viable microbial cells are identified by the number of colony-forming units (CFUs), the more the CFU the more virulent is the microorganism.

MATERIALS AND METHODS

Preparation of silver nanoparticles

Silver nanoparticles were synthesized by the chemical reduction method. The synthesis of silver nanoparticles was made by dissolving 2.52 g of silver nitrate (Merck Specialities Pvt Ltd, India) in 15 mL of deionised water to get a solution of 1 mole. Then 2.23 g of sodium borohydride (Sisco Laboratories Pvt Ltd, India) mixed in 30 mL of deionised water in a conical flask and mixed with magnetic stirrer to get a solution of 2 mole. The crystals were measured using an electronic weighing machine. The silver nitrate solution is transferred to a clean burette and is then allowed to drop slowly into sodium borohydride solution in a conical flask and mixed with magnetic stirrer to prevent agglomeration. The temperature around the conical flask was controlled by placing ice cubes. As the stirring continues, the agglomerates of silver nanoparticles starts forming. After completion of stirring process, the agglomerates of silver nanoparticles were washed with deionized water, centrifuged, and dried in incubator at 65°C overnight. It is then ground with a mortar and pestle. The purity of nanoparticles was confirmed under "X" ray diffraction. The instrument used for X-ray diffraction is Panalytical "X" pert pro. The synthesized nanoparticles contained 90% pure silver. The size of the nanoparticles was 20-100 nm [Figure 1] that was determined by atomic force microscopy (AFM) (nanosensor).

Sample fabrication (Ag° heat cure resin)

The heat cure polymethylmethacrylate selected in the study was DPI heat cure material (DPI Company, India). The Ag° was added to the polymer powder. The amount of Ag° required to get the ratio of 4:1, 3:1, 2:1 by weight with conventional denture base resin is tabulated and is grouped as Groups B, C, and D, respectively [Table 1]. Forty wax patterns were fabricated with modelling wax (Hindustan modelling wax no. 2, India), invested in a dental flask and processed with acrylic containing Ag° corresponding for each group. Each group contained 10 sample. In order to fabricate sample with regular shape, a metal die of 5 mm diameter and 1 mm thickness was prepared, duplicated with addition silicone putty material (Aquasil, Dentsply, India). The amounts of silver nanoparticle required to get the proportion for each sample is given in table [Table 2].

Before microbial assay, all samples were subjected to U-V ray sterilization for 15 min.

Microorganisms

One standard strain organism was used (*C. albicans*). The samples were inoculated in brain heart infusion broth [Figure 2] containing *Candida albicans* (Hi Media Laboratories Pvt Ltd, Mumbai) for 24 h. The inoculated samples were sonified with normal saline and inoculated into sarbastose dextrose agar medium [Figure 3] for 24 h and the agar diffusion of *C. albicans* and the colonies were counted [Figure 4].

RESULTS

The CFU per 1 mL of broth against *C. albicans* was demonstrated as the mean viable cells (CFU) after 24 h incubation [Table 3]. When compared the control group (0% Ag°) did not show any *Candida* inhibitory effect.[Figure 4] The bar diagram shows the CFU per mL of broth [Figure 5].

DISCUSSION

In the present study, the addition of Ag° to heat cure polymethylmethacrylate yielded fungicidal effect for the reference strain *C. albicans*. *C. albicans* occurs as a commensal in the gastrointestinal tract. Under predisposing conditions, *Candida* can produce a broad array of infections, from superficial mild infections to deep-seated fatal infections. Oral candidiasis is one of the most common forms of candidiasis, which presents a mucocutaneous infections

Table 1: Classification of the experimental groups

Group A	Conventional denture base resin
Group B	2.5% Ag nP reinforced 4:1 ratio with conventional denture base resin
Group C	3% Ag nP reinforced. 3:1 ratio with conventional denture base resin
Group D	5% Ag nP reinforced 2:1 ratio with conventional denture base resin

Table 2: Amount of silver nanoparticle required to obtain the ratio

Group	Silver nanoparticle
Group B	0.11 g
Group C	0.16 g
Group D	0.25 g

Table 3: *In vitro* antimicrobial properties of heat cure denture base resins with silver nanoparticles against *C. albicans*. Results are expressed in CFU count

Group	Colony forming unit (CFU)/mL of broth
Group A	10^5
Group B	10^4
Group C	10^2
Group D	10

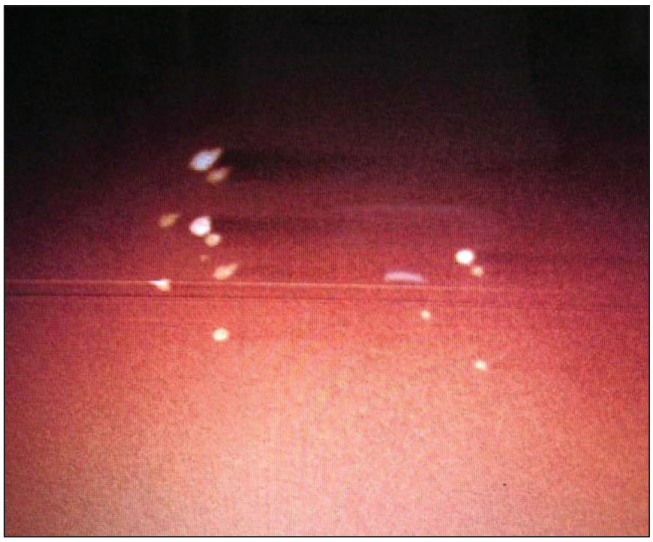

Figure 1: AFM showing the silver nanoparticle

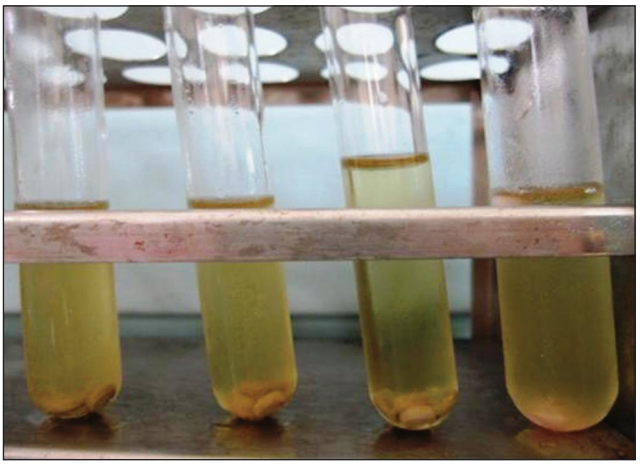

Figure 2: Samples in BHI broth

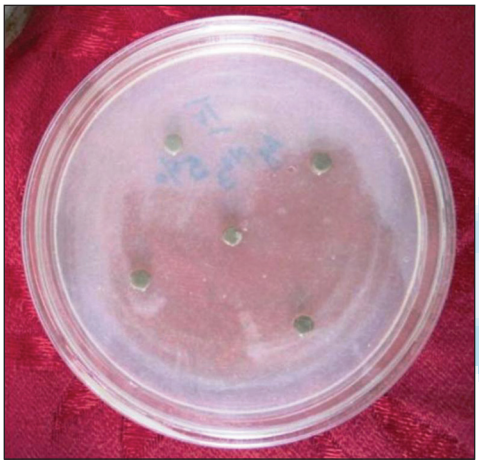

Figure 3: Samples in sarbastose dextrose agar medium

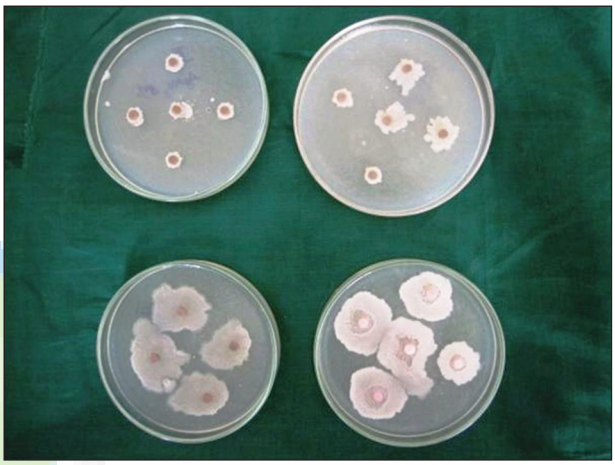

Figure 4: *Candida* growth

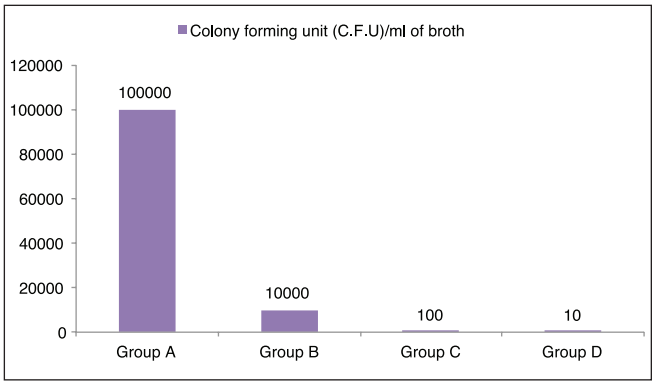

Figure 5: Bar diagram shows the value obtained from the CFU per mL of broth for each group

of the oral cavity involving mainly tongue, palate, gingival, and fissures of the mouth.[20,21] *Candida* infections receive increasing attention, presumably due to the increased prevalence worldwide.[22] There is a large body of evidence that *Candida* is able to adhere to acrylic resin dentures. This is the first step that may lead to the development of infectious process and that may ultimately result in varying degree of denture stomatitis of the adjacent mucosa.[23-25]

Candida adheres directly or via a layer of denture plaque to denture base (polymethylmethacrylate-PMMA).[26-28] In this study, a test tube containing 10 mL of brain heart infusion broth was infused with a single CFU of reference strain *C. albicans*. The samples were immersed for 24 h. The assays tested with samples immersed in a large volume of microbial suspension could not reproduce *in vivo* denture base resin which closely contacts the mucosa.[29] The present microbial assay confirmed that the susceptibility of *C. albicans* to Ag°-PMMA sample (5%) was multifold less than to samples with 2.5% Ag°. It was reported in previous studies that Ag- and silver-based compounds are highly toxic to prokaryotic cell showing strong biocidal effects on as bacteria species[15] but has less effects on eukaryotic cells such as fungi and yeasts.[30] The capacity of certain microbial strains to develop resistance against antibiotics has aroused an increasing interest for the controlled delivery of other antimicrobial agents with a broader activity and low incidence of resistance, such metals like silver or zinc, being an alternative strategy to avoid the formation

of adhesive microbial films.[31] The antimicrobial properties of silver at low concentrations over a wide range of pathogens, including the common microbial strains involved in implant-associated infections as well as the lack of toxicity of the mammalian cells, are well-known. The present study could not conclude whether the antimicrobial effect was resulted from release of silver ions from the sample into the agar medium or by direct contact Ag° and microbial cells. Ag+ has been reported to interact with cytoplasmic components and nuclei acids, to inhibit respiratory chain enzymes and to interfere with membrane permeability.[32] The nano-Ag are sensitive to oxygen and convert oxygen into active oxygen by its catalytic action. These active oxygen cause the structural damage of the microorganisms, called as "oligodynamic action" of silver.[17,33]

It is desirable for the denture base resin to have low microbial adhesion and better tissue response. The result of the present study shows that Ag° incorporated denture base resin acts as a potent anticandidal agent and could be used as material of choice for immunocompromised patients and for patients resistant to conventional therapy of denture stomatitis. However, further studies are required to clarify the exact mechanism of action of silver nanoparticle, its long-term effect on tissues and its systemic effects due to ionic release for a safer lineal application. The limitation of Ag° reinforced denture base resin also affects the esthetis due to its color, hence, could be used only for the palatal and the lingual contours.

CONCLUSION

Within the limitations of the present *in vitro* study, the modified denture base reinforced with Ag° showed 10^5 less *C. albicans* adhesion than the control group after 24 and 48 h incubation period. Further studies on molecular biology, cell toxicity, systemic toxicity, and physical stability of Ag° are required for its clinical use.

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How to cite this article: Suganya S, Ahila SC, Kumar BM, Kumar MV. Evaluation and comparison of anti-*Candida* effect of heat cure polymethylmethacrylate resin enforced with silver nanoparticles and conventional heat cure resins: An *in vitro* study. Indian J Dent Res 2014;25:204-7.

Source of Support: Centre for Nanotechnology and Research, SRM University, Chennai (supported in synthesis of silver nanoparticles), **Conflict of Interest:** None declared.