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Evaluation of the Impact of Nanoparticle Additives on the Shear Bond Strength between Soft Liners and Acrylic

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Abstract

Objectives: The study aimed to evaluate the antifungal properties of incorporating two types of nanoparticles into the denture soft lining material, and shear bond strength between the reinforced soft liner and acrylic resin.

Materials and Methods: Ninety specimens were fabricated and grouped into three distinct groups named group A which is the control, group B involved cerium oxide nanoparticles infused soft liner, and group C involved zinc oxide nanoparticles infused soft liner specimens. Specimens for shear bond strength consist of two acrylic parts and one soft liner part occupying the space between the acrylic specimens. Antifungal properties were tested by counting the number of viable cells visually, and shear bond strength was tested using a universal testing machine at speed of 0.5 mm per minute. Statistical tests involved one-way ANOVA and post hoc tests at $P \leq 0.05$.

Results: Cerium oxide nanoparticles infused specimens revealed higher antifungal properties compared to zinc oxide infused specimens and control specimens. Also, higher shear bond strengths (0.419) followed by those infused with zinc oxide nanoparticles (0.342), while the lowest values were for the control group (0.305).

Conclusions: The observed enhancements in antifungal and mechanical properties resulting from cerium oxide and zinc oxide nanoparticles infusions underscore their considerable potential as effective additives for improving performance in various applications.

Keywords: Acrylic denture base; Bond Strength; Microbial Colonization; Removable Prostheses.

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Introduction

Soft lining materials are commonly utilized to address oral mucosa injuries caused by persistent ridge resorption, damage from detachable dentures, or surgery. These materials promote tissue healing and improve patient comfort when in contact with underlying tissues [1]. The soft lining material absorbs chewing pressures on the residual tissues, minimizing energy delivered to periprosthetic tissues. Soft lining materials are difficult to clean due to their softer texture and lower resistance to brushing compared to traditional acrylic resins [2]. Soft lining materials, particularly temporary ones, are quickly degradable and prone to microbial colonization. This can lead to pathological processes and hinder treatment of existing infections [3].

Antimicrobial drugs may be effectively incorporated into soft denture materials, as demonstrated in in vitro and in vivo investigations. Incorporating antibacterial compounds into soft denture materials can improve clinical lifespan and minimize plaque buildup [4]. This combination may be effective in treating denture stomatitis for numerous reasons such as reducing trauma from the internal denture surface, eliminating contact with contaminated oral tissues, interrupting the cycle of re-infection, and incorporating antimicrobial agents into the material to target infected tissues [5].

A variety of antifungal agents can be effectively added to denture soft liners, including herbal extracts, conventional antifungal drugs such as fluconazole and nystatin, and various nanoparticles including AgNPs, SZZ-NPs, CeO2 NPs, and nano-ZnO [6]. Each of these agents offers unique benefits and potential for enhancing the antifungal efficacy of denture soft liners, providing valuable options for managing denture-induced stomatitis [7]. A previous study evaluated the addition of silver nanoparticles into denture soft liner. The results demonstrated that silver nanoparticles decreased candida albicans effectively, with antifungal efficacy ranging from 16.3% to 52.5% depending on AgNP concentration [8]. In addition, a recent study had evaluated the addition of silicone dioxide on antifungal properties, roughness, an wettability of soft liner. It was concluded that 0.25% and 0.5% had effectively reduced candida albicans. Conversely, higher concentrations were found to diminish antifungal performance and increase surface roughness [9]. However, incorporating antifungal agents into soft lining materials can impact structural characteristics and tensile strength [10]. To work properly, soft lining materials must adhere to the acrylic denture bases of removable prostheses. Clinical usage of soft lining materials might cause peeling and fluid penetration, potentially leading to bacterial growth and biofilm development [11]. Clinical failures due to loss of bonding with acrylic-based resins highlight the need for bond strength assessments. To effectively heal traumatized tissues, the soft denture liner should be connected to the removable denture's acrylic foundation. Previous research has focused on the bonding between soft denture materials and denture foundation acrylic resins, since peeling from

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.848

the base has been linked to clinical failure. Several investigations have evaluated the connection between soft lining materials and denture base resin [12,13,14]. However, little work has investigated the shear bond strength of soft lining materials after adding antifungal agents at lower doses that effectively inhibit Candida albicans.

Materials and Methods

Materials used in the study

This study involved the use of Vertex-soft heat cured acrylic-based soft-liner material (Vertex, Netherlands), Polymethyl methacrylate, cerium oxide nanoparticles, and zinc oxide nanoparticles as indicated in (Table 1).

Table 1 .Materials used in the study.

No.	Material	Manufacturer
1	Vertex-soft heat cured acrylic-based soft-liner	Vertex, Netherlands
2	Polymethylmethacrylate (PMMA)	New static S.A,Antioquia, Colombia
3	Ceriumnanoparticles (Average particle size of 25 nm)	Nanoshel, USA
4	Zinc oxide nanoparticles (Average particle size of 50 nm)	Nanoshel, USA

Study design

A total of 90 specimens were fabricated and divided into two main groups according to the conducted test. Each group was divided into three subgroups: 15 specimens for each subgroup. The subgroups were as follows: Group A (control) with no addition, Group B (experimental) with 2 wt. % CeO2, and Group C (experimental) with 2 wt. % ZnO. The percentages of nanoparticles were chosen based on the results of a previous pilot studies. Two tests were conducted: Viable count test, and shear bond test.

Specimen fabrication

A custom-made acrylic blocks with dimensions of 10 mm length×10 mm width×2.3mm thickness for fungal test, and 25mm width × 75mm length × 5mm depth, 3mm depth stopper, and 13mm handle thickness for shear bond test were prepared [15]. Each specimen for shear bond test had two blocks of heat-cured acrylic material that created a gap of 25mm width × 25mm length × 3mm depth for soft lining material placement.

A replicating additional silicone (Zhermack, Italy) was used to invest plastic models that were cut from clear acrylic plates (Perspex Cell Cast Acrylic, Clairvaux les Lacsrance, France) specifically for the test. After setting the silicone material, the dental flask (HANUA, Engineering Crop., USA) was filled with a layer of dental stone (Durux, Spain) mixed according to the manufacturer's instructions (W/P ratio: 50mL/100g). Next, a silicone mold with a plastic pattern were placed inside the flask over the gypsum layer. Finally, dental stone mixed according to the manufacturer's instructions was poured into the entire lower portion of the flask. Brush a coating of separating media (BMS dental, Italy) over the dental stone layer and allow to dry. Then, the upper section of the dental flask was placed over the bottom piece and filled with dental stone. Then, the dental flask was covered and waited for it to set completely. After setting it was opened and the plastic templates were removed to make space for the PMMA to be packed inside (Figure 1). Heat-cured acrylic soft liners are available in both powder and liquid form. The manufacturer recommends a mixing ratio of 1mL liquid to 1.2g powder. A glass beaker was well cleaned and dried before adding powder and liquid and mixing for 30 seconds. The container was then firmly sealed and allowed for around 15 minutes to reach dough stage. The two parts of the flask were painted with the separating medium and allowed to dry. When the dough time was over, the material was ready to pack. An amalgamator equipment (Perfect Plus, United Kingdom) was used to mix nanoparticle powder with soft liner powder for 40 seconds [15]. To get an exact powder-to-liquid ratio, the weight of nanoparticle powder was subtracted from the overall weight of the acrylic-based heat-cure soft liner powder. According to the manufacturer's instructions, the curing cycle involved heating the water to 70°C for 90 minutes and then increasing the temperature to 100°C for 30 minutes. Soft-liner specimens for fungal test were then retrieved and finished.

Figure 1. Silicone mold space inside dental stone after removing the plastic pattern.

To make the acrylic dough, mixing of heat-cured acrylic resin powder with monomer liquid in a clean, dry glass container. Sealing the container to avoid monomer evaporation. Once the acrylic resin reached the dough stage, it was packed into a silicon mold covered with separating media for easy removal after curing. To ensure uniform material distribution inside the mold, a layer of polyethylene sheet (Amalgamated dental T.D., England) was wrapped over the acrylic, and the upper portion of the flask was assembled to the lower section before being placed under a hydraulic press (BegoHydrofix, Germany). After removing the polyethylene film, the flask was opened, and surplus material was removed using a sharp knife. A new coating of separating medium was placed and allowed to dry. After securing the lids of the flasks, a hydraulic press at 100 kg/cm2 for five minutes was used to achieve metal/metal contact. The clamped flask was secured before immersing in a water bath (Memmert, Germany) to cure. Acrylic specimens were cured using a digital thermostatically controlled water bath. The temperature was set to 70°C for 30 minutes, then increased to 100°C for another 30 minutes, following the manufacturer's recommendations. After removing the clamped flasks, they were allowed to cool for 30 minutes at room temperature, followed by 15 minutes of tap water chilling. Once completely cooled, the flasks were opened, and acrylic specimens retrieved and finished (Figure 2).

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.848

Figure 2. Finished acrylic specimens.

For each shear bond strength test, two acrylic specimens were arranged facing each other. A 3 mm depth, 25 mm length, and 25 mm breadth gap were filled with wax to hold the two blocks together. Acrylic blocks were pushed into a silicone mold (putty type) in a specialized flask (Broden, Sweden) and the silicone was allowed to completely solidify (Figure 3).

Figure 3. Specialized flask for packing the soft liner into the gaps between acrylic specimens.

The silicone molds with acrylic specimens were placed in a custom-made flask facing each other and bonded by wax. The flask was filled with newly mixed dental stone. The flask was then covered with a petroleum gel coating. After setting the stone, it was dewaxed and washed with hot water and soap. The flask and acrylic blocks were then allowed to dry completely (Figure 3). Soft liner powder and liquid were mixed as previously mentioned.

To avoid air bubbles, soft lining material was gradually injected and condensed in the gap using a condenser. The flask was overfilled, covered under pressure of 2 kg/cm2, and securely tightened until edge-to-edge contact was obtained. The flask was cured using a digital water bath, as described earlier. All samples were finished by removing surplus soft lining material with a sharp blade. Samples to be utilized after 24 hours were kept in distilled water at 37 degrees Celsius (Figure 4).

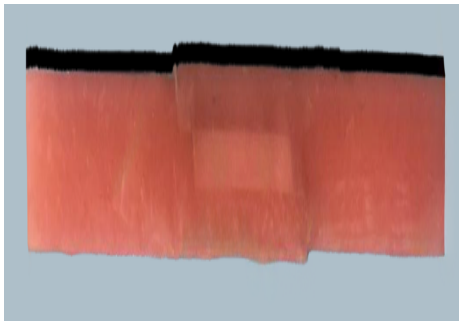

Figure 4. Finished shear bond test specimens.

Candida albicans viable count test

Candida albicans isolates were obtained from the oral cavity of 15 patients were medically fit denture users aged 50-65 with denture stomatitis. A sterile cotton swab was used to obtain the isolate by gently stroking the palatal mucosa. The swab was promptly inoculated in sabouraud dextrose agar (SDA) as a primary isolation medium.

To prepare the culture media, a clean, dry beaker was filled with 1000 ml of distilled water. Then, 62 g of sabouraud dextrose agar was weighed using an electronic balance and added to the water. The beaker was sealed with sterile cotton and sterilized in an autoclave for 15 minutes at 121 °C/15 psi. Finally, the media was left to cool to 47 °C on the counter. Candida albicans colonies appeared pearl-shaped, pasty, creamy, smooth, and slightly convex on SDA under microscope [16].

To prepare the sabouraud dextrose broth, weigh 30 g and add 1000 mL of distilled water to a glass beaker. Autoclave at 121 °C/15 psi for 15 minutes, then cool to 47 °C on the counter. To examine the antifungal efficacy of nanoparticles-loaded materials, a Candida albicans suspension of about 107CFU/mL (equivalent to 0.5 McFarland standards) was prepared. This suspension was made by diluting a tiny amount of inoculums in a test tube with normal saline and measuring the solution with a McFarland densitometer instrument. A micropipette was used to transfer 0.1% of the suspension to a test tube containing 0.9% Sabouraud dextrose broth. The loaded samples (2% Cerium oxide and 2% +zinc oxide nanoparticles) were then inserted in the tube and incubated for 24 hours at 37°C. After incubation, 0.1% of the broth mixture was transferred to a test tube with 0.9% normal saline. Serial dilution was performed. Around 0.1% of the third dilution was collected and distributed over the surface of SDA with a glass spreader, and the plates were incubated at 37 °C for 48 hours. The third dilution was chosen because it

showed a countable range of 30-300 CFU.

All viable counts on the SDA surface were counted visually and analyzed statistically. The antifungal efficiency (AFE) was estimated using the following formula:

AFE[%]=(Vc-Vt)/Vc×100%

According to Chladek et al. (2011), the number of viable colonies in control samples was denoted as Vc, whereas the number of viable colonies in experimental samples was represented as Vt [11].

Shear bond strength

The Instron testing machine was used to determine soft liner shear bond strength. Specimens were exposed to a load cell capacity of 100 kg and a crosshead speed of 0.5 mm per minute. The machine readings indicate the maximum load of failure (Figure 5). ASTM standard D-638 (1986) was used to calculate bond strength by dividing the maximum force of failure by the cross-sectional area of each sample (25mm×25mm=625mm). The shear bond strength was determined using the following equation:

Shear strength = F/A

Where F is the maximum load (N),

and A is the cross-section area (mm2)

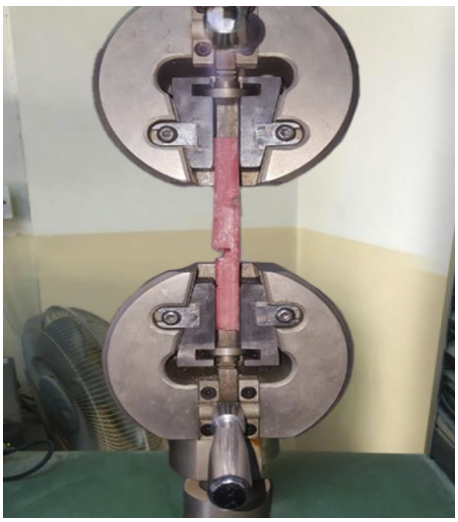

Figure 5. Shear bond strength test.

Results

Candida viable count

After 48 hours of incubation, the lowest number of viable counts was recorded for group B (Cerium oxide nanoparticles infused

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.848

specimens), followed by group C (Zinc oxide nanoparticles infused specimens), and the highest viable counts was recorded for group A (Control group). There was a highly significant difference between all of the groups, as revealed in (Table 2) and (Figure 6).

Table 2. Statistical test results for viable counts.

Group	Min	Max	Mean	±SD	F	P value	Groups	P value
Group A	105.00	124.20	114.7750	5.54398	1103.545	.000	A B	.000
Group B	29.90	38.10	33.6400	3.06936			A C	.000
Group C	45.00	55.10	50.0900	3.13898			B C	.000

Viable cell counts ANOVA Tukey HSD

Levene statistics=1.727, p value=0.197 [HS]

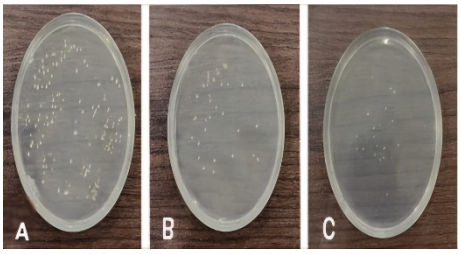

Figure 6. Viable counts of Candida albicans; A, Group A specimens. B, Group B specimens. C, Group C specimens.

Shear bond strength

Shear bond strength between cerium oxide nanoparticle infused soft-liner and acrylic resin had the highest values (0.4193), followed those infused with zinc oxide nanoparticles (0.3424), while the lowest value of shear bonding was recorded for the control specimens (0.3054) as revealed in (Figure 7).

Table 3. Statistical test results for shear bond strength.

Group	Min	Max	Mean	±SD	F	P value	Groups	P value
Group A	.302	.309	.30540	.002675	1354.602	.000	A B	.000
Group B	.415	.422	.002263	.41930			A C	.000
Group C	.325	.352	.34240	.007905			B C	.000

One-way ANOVA revealed highly significant differences between the group (P<0.01). Post hoc test revealed highly significant differences between all of the three groups (Table 3).

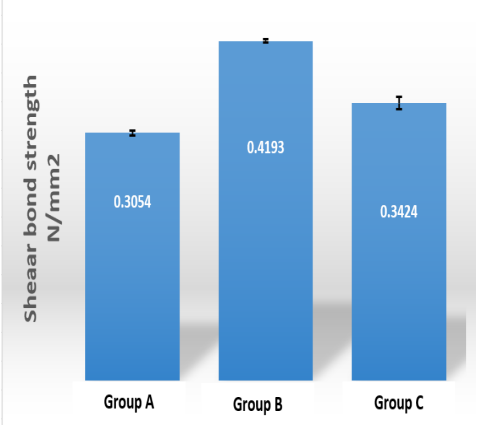

Figure 7. Bar chart representing the means and standard deviation of the three groups.

Discussion

One of the most frequent issues with applying soft denture liners are the bonding with acrylic base and the colonization of different microbes, especially Candida albicans, which can cause pain, infection, and material failure. More attention has recently been paid to employing nanoparticles to combat fungal resistance to pharmaceuticals [8,9,17].

The observed decrease in viable fungal cell counts is related to the ability of CrO2 and ZnO nanoparticles to generate reactive oxygen species (ROS) [18]. CeO2 nanoparticles with their high surface energy and catalytic properties, can facilitate the conversion of molecular oxygen and water into superoxide anions (O2-) and hydrogen peroxide (h2O2) [19]. Such highly reactive species cause oxidative damage to the fungal cell by attacking lipids, proteins, and nuclic acids [20]. ZnO nanoparticles also initiate ROS such as hydroxyl radicals (OH) and superoxide anions (O2-) which can damage the fungal cells [21]. In addition, the high surface energy and surface area of CrO2, similarly ZnO, enhances the interaction of nanoparticles onto the fungal cell walls, disrobing the integrity by causing leakage of vital components [22]. CrO2 and ZnO nanoparticles can also release metal ions such as Ce3+, Ce4+, and Zn2+ from CrO2 and ZnO respectively. These metal ions can interfere with enzymatic activities, disruption of metal ion hemeostasis, and interfere with cellular singling and transport mechanisms by altering the function of singling proteins [23]. Moreover, these nanoparticles can exhibit photocatalytic actions when exposed to light by generating ROS and damage the fungal cells as mentioned [24].

Shear bonding between denture soft liners and acrylic denture bases is one of the important factors to ensure durability and efficiency of the denture prosthesis [25]. The incorporation of antifungal agents into soft liner materials can significantly maintain oral health by providing fungicidal and fungistatic effects. However, the presence of such agents can also affect the adhesive characteristics between the denture parts. Depending on the concentration and formation of these agents, surface energy, wettability, and chemical interactions at the interface can be affected [12, 14].

The results revealed that CeO2 incorporated specimens had stronger shear bonding compared to control and zinc oxide incorporated specimens. CeO2 nanoparticles are known for their high surface energy due to

Dentistry 3000
Vol 13 No 1 (2025) DOI 10.5195/d3000.2025.848

their large surface area that can facilitate the adsorption of the CeO2 nanoparticles onto the surface of polymer matrix which improves the intimate contact and bonding [26].

In addition, hydrogen bonding between hydroxyl groups on the CeO2 surface and functional groups of the polymer such as carbonyl (-C=O) and hydroxyle groups (-OH) further increases the adhesion at the interface between the nanoparticles and the acrylic resin [27].

In contrast, zinc oxide nanoparticles display a dissimilar surface chemistry and structure that result in weaker interactions with the PMMA matrix. The potential lack of suitable functional groups on zinc oxide nanoparticles means there will be no hydrogen bonds with the functional groups of the PMMA (e.g. Carbonyl groups) [28]. Such absence of functional groups can reduce the affinity between zinc oxide nanoparticles and PMMA. In addition, low surface energy results in poor wetting properties of the acrylic polymer around the zinc oxide nanoparticles. This effect led to gaps or spaces that weaken the bond between the materials [29]. Furthermore, zinc oxide nanoparticles may have a crystal lattice that does not provide effective mechanical interlocking [30].

Physically, CeO2 nanoparticles can induce surface roughness at nanoscale levels due to their crystalline structure and high surface energy. The increased irregularities and protrusions on the CeO2 nanoparticles can effectively interlock with the polymer chains and increases the cohesion between the materials [31]. The increased roughness can also increase the number of sites for the chemical bonding to occur. Therefore, CeO2 nanoparticles not only increase the physical interlocking but also increase the facilitate stronger chemical bonds between with the acrylic resin matrix [32].

Furthermore, CeO2 nanoparticles can serve as stress transfer agents inside the soft liner which act by dispersing mechanical stresses and reduce localized areas of stress concentration at the interface between soft liner and PMMA [33]. ZnO nanoparticles typically demonstrate a hexagonal wurtzite or cubic zinc blende structure. While ZnO nanoparticles can have several morphologies, including smooth surfaces, they do not inherently have a porous structure with interconnected channels. The smooth morphology of ZnO nanoparticles may affect their stress distribution and bonding efficiency in composite materials, potentially leading to weaker adhesion due to fewer surface irregularities that contribute to mechanical interlocking [34].

Conclusions

In conclusion, it was possible to successfully include two types of nanoparticles into the heat-cured denture soft lining material, both have antifungal properties and can be used as a medication delivery method to combat Candida albicans. The most effective nanoparticles against fungus seemed to be 2% cerium oxide nanoparticles which also increased shear bond strength with acrylic base material.

Conflict of interest

No conflict of interest.

Source of funding

Completely self-funded study.

Ethical clearance

An in vitro study.

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Dentistry 3000
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