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RESEARCH PAPER

Inhibitory Effect of ZrO_2NPs on *Candida Albicans* in Heat-Cured Acrylic-Based Soft Lining Material

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ABSTRACT

The most prevalent issue that can contribute to persistent mucosal inflammation is the development of microorganisms and fungi, particularly *Candida Albicans* (*C. albicans*), on soft denture lining material. The objective of the current study is to identify the inhibitory effect of Zirconium Oxide Nanoparticles (ZrO_2NPs) on *C. albicans* in heat-cured acrylic-based soft lining material and the quantity of zirconium ions (Zr) released by the composite of soft liner and ZrO_2NPs. Soft denture liners made of acrylic were given varied ratios of ZrO_2NPs. According to the test that will be conducted, 200 samples were separated into two groups (experimental and control). The soft liner/ZrO_2NPs composite's antifungal activity was evaluated utilizing two techniques over three distinct time periods (disk-diffusion test and viable count of *C. albicans*). Atomic Absorption Spectroscopy (AAS) was used to measure the amount of Zr emitted in synthetic saliva over two separate time periods. The strength of the soft liner's shear bond to the denture base material made of acrylic was evaluated using an Instron testing equipment. Compared to the control group, there was a highly significant drop in *C. albicans* colony forming units in the experimental groups (1.5% and 2% ZrO_2NPs). Once ZrO_2NPs were added to soft liners at a rate of 2%, the shear bonding strength's mean value increased in a manner that was highly significant. As a result of this research, ZrO_2NPs could be incorporated into acrylic-based soft denture lining materials to give them antifungal qualities, which lower the risk of developing denture-induced stomatitis.

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INTRODUCTION

In both full and partial removable dentures, soft denture liners are utilized to evenly disperse functional demands on the tissues supporting the appliance [1,2]. These materials are suggested for patients who have xerostomia and bruxism, immediate prostheses, thin atrophic mucosa, and bony undercuts [3,4]. Silicone elastomers or acrylic resins that have been plasticized are two types of resilient lining materials [5–7]. In order to reduce the traumatic transfer of occlusal forces to severely resorbed alveolar ridges and areas healing from surgical procedures, soft denture liners are used to produce a convenient interface across oral tissues and the denture [8–10]. However, soft liners have a porous surface due to their characteristics, composition, and structural form serving as microbial reservoirs as a result [11]. In addition to systemic diseases including respiratory infections, esophageal, oropharyngeal, chronic obstructive pulmonary disease, pneumonia, and bacterial endocarditis, liner contamination may result in serious local problems such as discomfort, pain, delayed implant osseointegration, peri-implant infections, and implant loss. Denture users have found it to be comfortable since it acts as a pad between the oral mucosa and the base of the denture [12,13]. For instance, it's been discovered that the chemical makeup of both denture base and reline materials has an impact on a parameter called shear bond strength [14–18]. High stress concentration during function may negatively impact the health of the tissues supporting dentures. To distribute the stresses placed on soft tissues while they function, chairside hard and soft denture reline materials are utilized. Bacteria might be harbored by a weak bond, which would also encourage discoloration and lining material delamination [19]. Additionally, it is suggested that the reline denture base mechanical strength

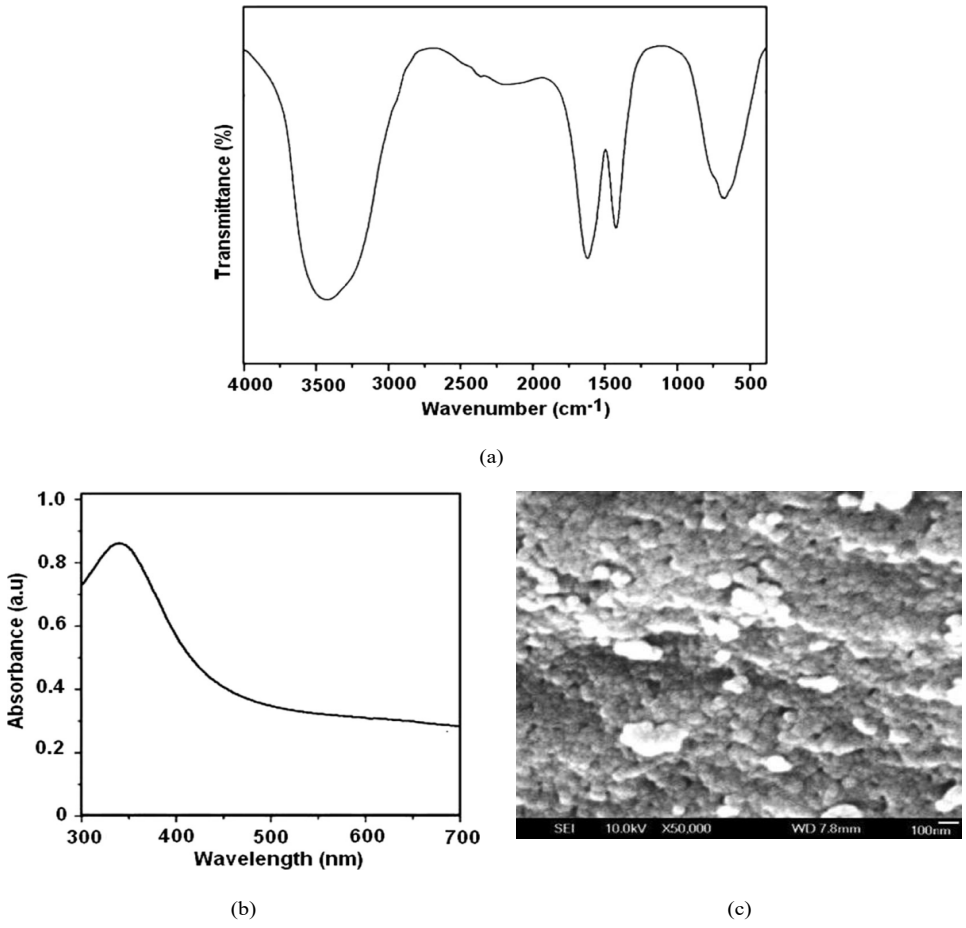

Fig. 1. Analysis results of ZrO_2NPs: (a) FTIR spectroscopy, (b) absorption spectra, and (c) TEM image.

may be affected by the bond strength between the denture base and the resins used to make the denture reline [20,21]. *Candida Albicans* (*C. albicans*) colonization and surface roughness, that might cause denture stomatitis, are another defect [22]. Zirconium Oxide (ZrO_2) is perfect for orthopedic and dental applications due to its minimal thermal conductivity, biocompatibility, chemical stability, and superior mechanical qualities [23,24]. Invasive infections brought on by filamentous fungus have posed a significant hazard to public health during the past few years on a global scale. Human infections can be brought on by the non-filamentous fungus *Candida auris*, *Mucorales* (the most prevalent filamentous fungi), *Coccidioides*, and *Aspergillus* [25]. The innovation of new therapeutic options for the treatment of aspergillosis and infections brought on by *Candida* has employed a variety of tactics, including the incorporation of coating materials, complexes made through green chemistry, and the coupling of polymers. These human infections were chosen as additional targets for the ZrO_2 Nanoparticles (ZrO_2NPs) antifungal efficacy [25–28]. By interfering with cell activity and producing distortion in fungal hyphae, ZrO_2NPs may successfully prevent the formation of fungi [29–31].

To decrease the growth of bacteria called *C. albicans*, ZrO_2NPs have been added to an acrylic-based soft denture liner in the current research. In order to asses Zr release, this addition was also tested to see if it had any significant effects on the material's mechanical and physical characteristics.

MATERIALS AND METHODS

In this investigation, a soft acrylic-based denture liner was used (GC Dental Industrial Corp., Tokyo, Japan). Two hundred specimens in total were prepared, and two groups (experimental and control) were created based on the test. Fig. 1 shows the transform infrared spectroscopy (FTIR) spectra, the absorption spectra and the transmission electron microscope (TEM) image of the ZrO_2NPs acquired.

Different percentages of ZrO_2NPs were applied to the soft liner (1.5% and 2% by weight). The specimens were made using plastic patterns with dimensions of 8×8×2mm [32]. According to the manufacturer's specifications, each specimen was prepared, packaged, and cured. ZrO_2NPs were introduced to the liner monomer for the experimental specimens, then dispersed for three minutes by a probe sonication equipment (Soniprep-150, MSE, Sussex, UK) to separate them into separate nanoparticles [33]. To avoid the liquid heating up in large amounts during sonication, that would lead to significant liquid evaporation or material damage, the combination was cooled down by submerging the container in a cooling bath [34]. The specimens were polished

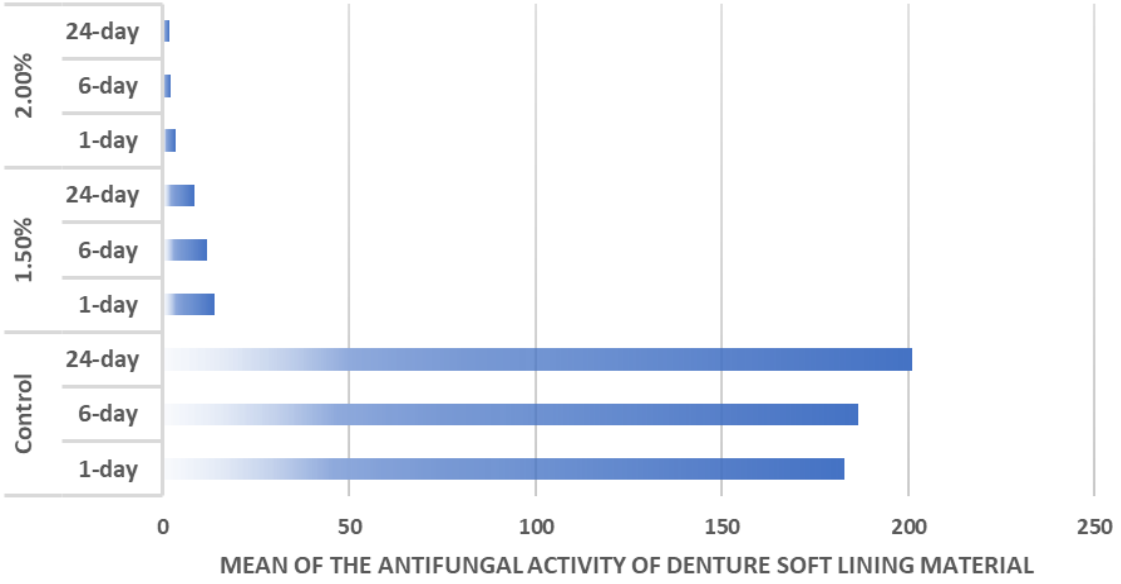

Fig. 2. Mean CFU/ml values for each experimental group over the course of the investigation at various points.

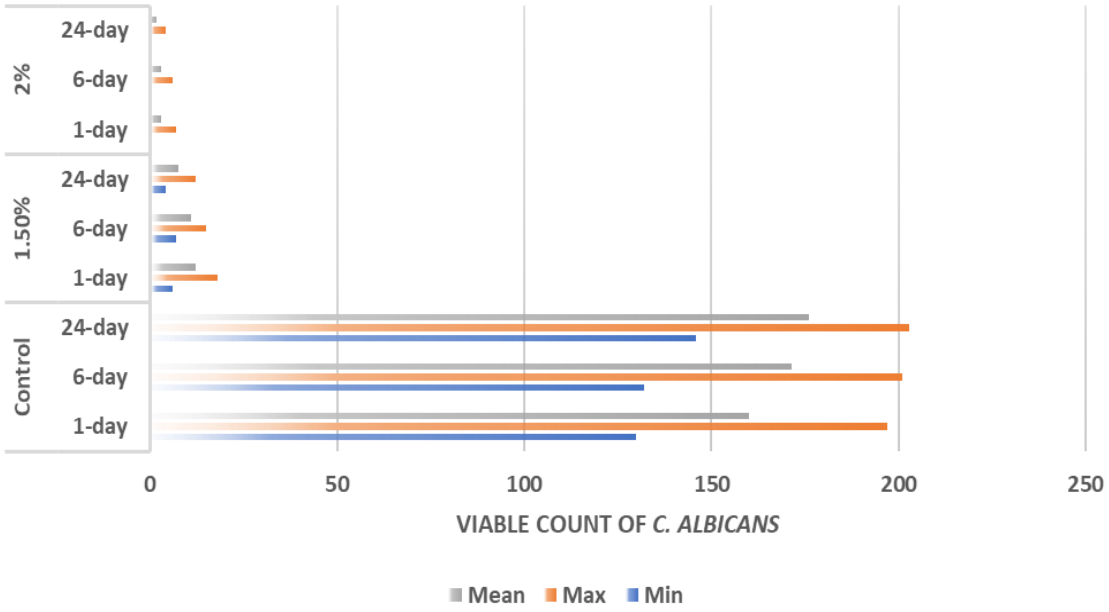

Fig. 3. For all study groups and time periods, descriptive statistics on the viable count of *C. albicans* are compared.

and made sterile by autoclaving them following complete cure. Using a sterile cotton swab, *C. albicans* was isolated from the oral cavities of 25 patients treated by the college of dentistry with symptoms of oral thrush and denture stomatitis [35]. All swab samples were initially cultured on Sabouraud Dextrose Agar (SDA), and brooded aerobically for 12 to 24 hours at 40°C, and then left at 5°C for additional analysis. When it forms pasty convex, smooth, creamy colonies on SDA, the colony morphology and gram stain were used to identify it. Additionally, a Germ Tube Test (GTT) approach was performed, and API Candida system (bioMérieux, France) was used for the biochemical method's final verification [36]. A yeast culture containing about 10^7 CFU/ml has been created employing a McFarland densitometer and *C. albicans* to explore the antifungal efficacy of the Poly (Ethyl Methacrylate) (PEMA) matrix/ZrO_2NPs composites (0.5 McFarland standard) [37]. Each sample was placed into a tube that held 95 µL of the yeast suspension and 10 mL of SDA. 98 CFU/mL of cells were the total cell density. After being incubated for 24 hours at 40°C, the samples were collected and washed five times in sterile deionized water in accordance with standard procedure to remove loosely connected cells. Then they were placed on SDA plates to determine the number of vital organisms. This process was repeated after storing the samples in synthetic saliva for 6 and 24 days, respectively, at 40°C. The Disk Diffusion Method (DDM) required tiny discs measuring 6mm to be carved from a rectangular metal pattern measuring approximately 30×60×0.5mm [38]. The extra fluid was squeezed out after dipping a sterile swab into the inoculum mixture. To ensure that all of the Mueller-Hinton agar supplemented with 2% glucose and methylene blue (MH-GM)'s surface

Table 1. The means of the *C. albicans* count in various groups during each incubation time were compared using a one-way ANOVA.

Incubation period	Effects	SS	Df	MS	F	P
1-day	Between groups	147987.84	2	73993.92	393.71	0.001
	Within groups	5431.59	28	201.17		
6-day	Between groups	172744.39	2	86372.19	620.74	0.001
	Within groups	3948.91	28	146.26		
24-day	Between groups	186832.87	2	93416.43	799.75	0.001
	Within groups	3338.08	28	124.33		

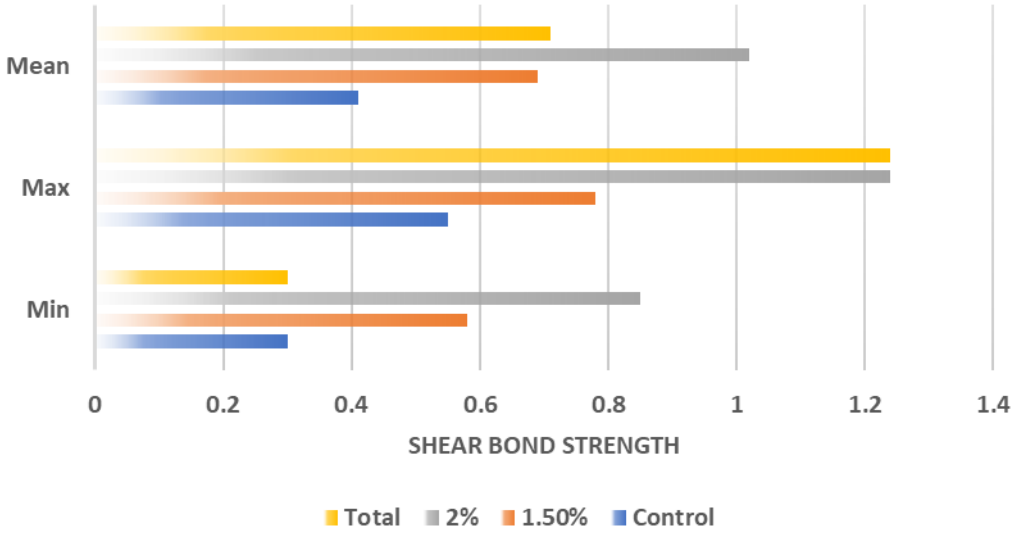

Fig. 4. Shear bond strength assessments with descriptive statistics.

Table 2. Shear bond strength test results using one-way ANOVA

Groups	SS	Df	MS	F	P
Between groups	1.77	2	0.88	113.36	0.001
Within groups	0.22	28	0.01		

area developed equally, swabbing was done in three different directions [39]. The soft-liner discs (containing and lacking ZrO_2NPs) were placed on the agar surface once it was preserved for about 5 min. The antimicrobial compounds were then allowed to diffuse over a period of 120 min while the plates remained at room temperature. For 24 hours at 40°C, these agar plates underwent aerobic incubation. A computerized electronic caliper was used to assess the inhibitory zone that might develop around the disks. Making acrylic blocks with the required measurements of 80×20×5mm and a stopper with a depth of approximately 3.5 mm was necessary in order to test the strength of shear bond of the soft lining material to the acrylic denture base [40]. Next, acrylic resin that has been heat-cured was employed (Lucitone 550, (LU)). The manufacturer's recommendations for mixing packaging and curing were followed. Afterward, one acrylic block was placed on top of the other, providing a 20×20×2.5mm wax-filled area between them. To create a mould for curing the very last specimen, the entire specimen (two wax-coated blocks) was therefore submerged in silicon material. After the wax was removed, soft lining material was placed inside the 20×20×2.5mm chamber that had been created, and curing was then completed. In order to determine the shear bond strength value for each test sample, the highest load necessary for failure was divided by the cross-section area. To create silicone rubber molds for evaluating Zr release, a 9.5 mm in diameter and 2.5 mm in thickness plastic circular pattern was used. The proportions, mixing, and curing of experimental sample (1.5%, 2% ZrO_2NPs) were done. All samples were stored at 40°C in plastic plane tubes with 25 mL of synthetic saliva for two time points (6 and 24 days). The synthetic saliva's volume was regenerated on a regular basis to account for evaporation. Every tube's collected solution was tested for Zr dosage using Atomic Absorption Spectroscopy (AAS), and the Zr released dosage from standard solutions at different levels was counted using a linear calibration curve included into the apparatus (VarianAA-800). A one-way ANOVA (analysis of variance) was used to compare the means of the *C. albicans* count and shear bond strength test results in various groups ($P<0.05$).

RESULTS AND DISCUSSION

According to FTIR research, ZrO_2NPs and the

soft lining material did not interact chemically. The 2% group displayed the lowest mean value (3.36 CFU/ml), which increased as the incubation duration in synthetic saliva increased (182.88 CFU/ml) (Fig. 2).

All experimental groups (1.5% and 2% ZrO_2NPs) displayed lower mean values than the control group (Fig. 3 and Table 1).

DDM demonstrated that, even after 6-days and 24-days periods of incubation in artificial saliva, there is no inhibitory zone around any of the PEMA/ZrO_2NPs discs employed in the Kirby-Bauer disk diffusion susceptibility test of any ratios of ZrO_2NPs. At any point throughout the incubation period, no Zr leakage into synthetic saliva was observed. The findings of the shear bond strength test in the current study also indicated that the 2% group had the largest mean value (1.02 N/mm^2) amongst study groups, followed by the 1.5% group (0.69 N/mm^2) in terms of mean value. In contrast, the control group's mean value was 0.41 N/mm^2 (Fig. 4 and Table 2).

By mixing ZrO_2NPs into the acrylic-based soft denture lining material, it will be possible to increase the antibacterial activity of the material against *C. albicans*, the primary culprit in denture-induced stomatitis. Due to the incorporation of ZrO_2NPs to the soft denture liner, the findings of the current investigation showed a statistically highly significant decline in CFU/ml of Candida albicans, demonstrating the development of a polymer with antifungal capabilities. This research supports [41] which studied the antibacterial properties of ZrO_2NPs and Zr mixed ligand complexes on bacterial strains of *S. aureus*, *E. coli*, and fungus strains of *A. niger*. The results of [42], which showed that Zr(IV) and ZrO_2 complexes with active facet play a major part in defining the activity against microorganisms, are consistent with the findings of current research. Additionally, research showed that the studied soft liner ZrO_2NPs composite's antifungal efficacy enhanced with longer incubation times in synthetic saliva.

CONCLUSION

This investigation indicated that the Zr release result was similar to the DDM, which had no inhibition zone around any specimens during Zr release. Also, no prior research has been done to show how ZrO_2NPs affect the strength of shear bond of soft lining materials. The current study's findings showed that the 2% group had the highest mean value among the experimental groups, indicating a significant increase in the mean value of shear bond strength with increasing percentages of ZrO_2NPs added to the soft liner. Another element that enhances shear bond strength is the high flow capability of soft lining materials utilized in this research, which allows the material to adapt easily to the bonding surface and create good contact.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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