RESEARCH PAPER

The Antifungal Efficacy of Heat-Cured Acrylic-Based Soft Denture Liners Infused with Magnesium Oxide Nanoparticles

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ABSTRACT

Candida albicans (*C. albicans*) colonization of denture soft lining materials can cause clinical issues and material deterioration. Given their antibacterial qualities, of magnesium oxide nanoparticles (MgONPs) have generated interest for usage in biomedical applications. This research intended to determine how adding MgONPs to a soft heat-cured acrylic resin denture liner influenced its water sorption, solubility, antifungal activity, and color modification. Moreover, assessing the quantity of MgO released. Soft denture liner was infused with 0.1%, 0.2%, and 0.3% of MgONPs. A total of 200 specimens have been prepped and separated into four groups based on the test that will be run. The antifungal effects of a composite material comprised of a soft liner and MgONPs were determined using a viable count of *C. albicans* in combination with a disk-diffusion method (DDM). Using atomic absorption spectroscopy (AAS), researchers could determine the amount of MgO released by synthetic saliva. The findings of the measurements of the color change, shear bond strength, solubility, and water sorption have been statistically examined. When compared to the control group, all experimental groups displayed a highly significant decline in *C. albicans* colony forming units. No inhibition zone surrounded any test specimens utilized in any of the tested groups. The incorporation of MgONPs aids in producing a soft denture liner with antifungal properties. In addition to decreasing water sorption, this addition also raised the material's opacity while having no effect on the shear bond strength.

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INTRODUCTION

In order to equally disperse the stresses placed on soft tissues during function, soft liners designed for relining removable dentures and other oral and maxillofacial prosthesis are employed [1,2]. They are polymeric materials that can be adhered to the tissue surface of a hard denture base. Denture stomatitis is a word used to characterize specific pathologic alterations in the oral mucosa of

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denture-bearing tissues below partial or complete dentures in both jaws, and somewhat often in the maxilla of denture wearers, mostly elderly people [3–5]. Wearers of full dentures have a reported prevalence of 11-67% [6]. According to reports in the literature, *Candida* species are frequently linked to denture stomatitis [7–11] (Fig. 1).

Clinical issues and material degradation may emerge from *Candida albicans* (*C. albicans*) colonization of denture soft lining materials. This tendency can be exacerbated by increased humidity and high temperature beneath the dentures, as well as by the material's surface characteristics. *C. albicans*, which is isolated from the surface of a soft denture liner, was identified as one of the etiological contributors to denture stomatitis [12]. In addition, several studies have demonstrated that *C. albicans* can penetrate soft lining materials at varying depths [13–16]. This may diminish the effectiveness of the various chemical cleaning agents available. Plaque control techniques, both mechanical and chemical, are frequently employed to prevent the development of denture stomatitis [17]. Some elderly and hospitalized patients with cognitive impairment, reduced motor dexterity, or memory loss may find it challenging to clean their dentures [18]. In addition, the use of these techniques can cause severe damage to the materials that comprise the soft lining [19]. Researchers across the globe are becoming interested in nanoparticles due of their antibacterial capabilities [20]. Due to the advent of infections that are resistant to antibiotics and the subsequent spread of infectious diseases, metal and metal oxide nanoparticles are frequently employed as an antibacterial agent [21]. Magnesium oxide (MgO) is well-known for its antimicrobial properties against numerous bacteria, fungi, and viruses [22,23]. The nanoparticle has demonstrated some exceptional, encouraging outcomes concerning its use as an antibacterial agent due to its small size, large surface to volume ratio, and other primary unique properties [24]. The discovery of new antibacterial compounds may be facilitated by the capacity to manufacture metal and metal oxide nanoparticles of a certain size and form. Among all metal oxide nanoparticles, MgO nanoparticles have received the most attention for their antibacterial properties [25]. In this research, in order to inhibit *C. albicans*-induced microbial growth, magnesium oxide nanoparticles (MgONPs) are incorporated into an acrylic-based soft denture liner. In addition to determining the amount of MgO released, the study examined whether adding MgONPs would significantly alter the mechanical and physical properties of the soft lining material.

MATERIALS AND METHODS

In this investigation, a soft acrylic-based denture liner was used (GC Dental Industrial Corp., Tokyo, Japan). The soft liner contained 0.1%, 0.2%, and 0.3% of MgONPs. These percentages represent the weight of the powder. Their separation followed the preparation of 200 specimens into four distinct groups, each determined by the type of examination to be conducted. A To ascertain whether a chemical interaction occurs between the MgONPs and the soft liner, Fourier-transform infrared spectroscopy (FTIR) was used. Fig. 2

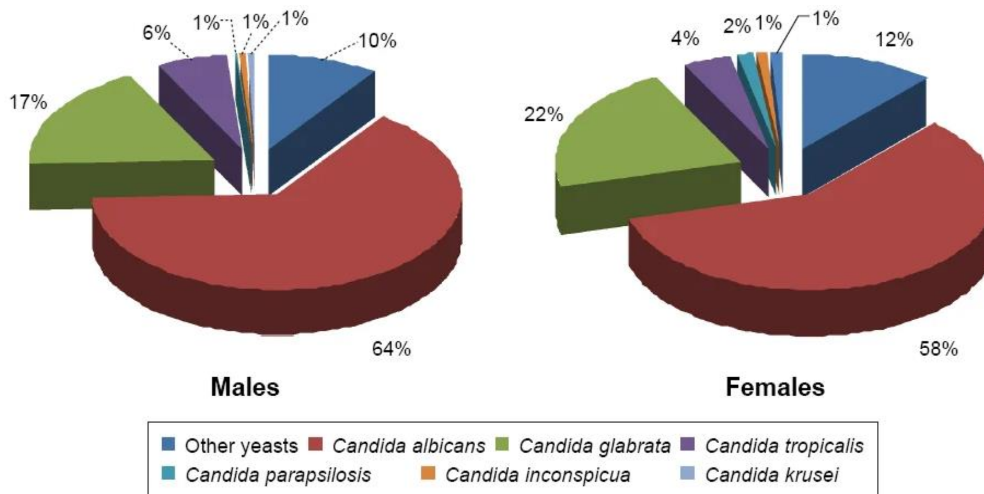

Fig. 1. The most prevalent Candida species found in both males and females.

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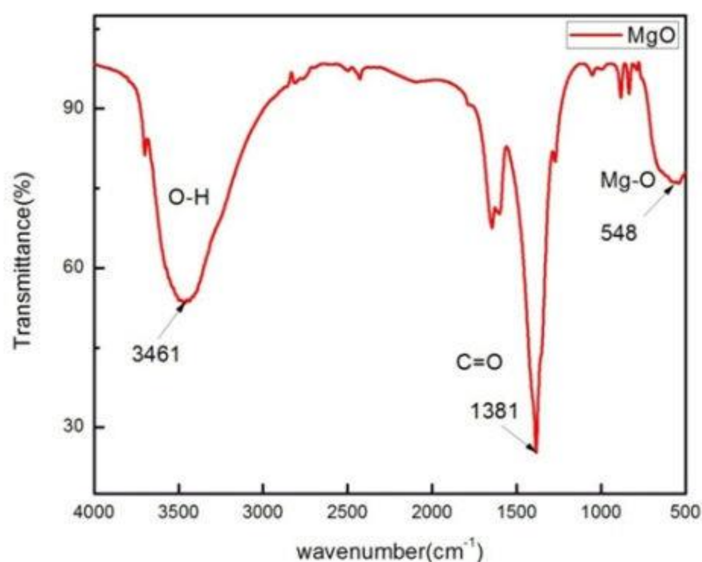

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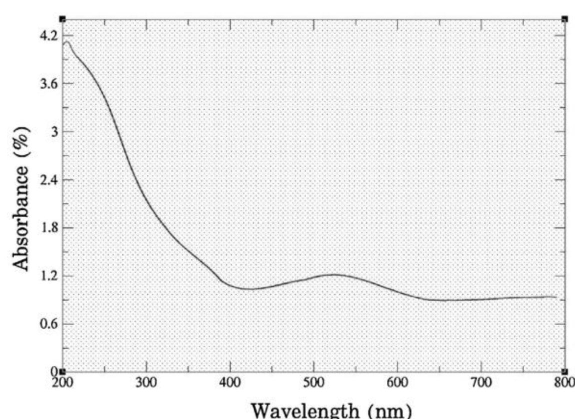

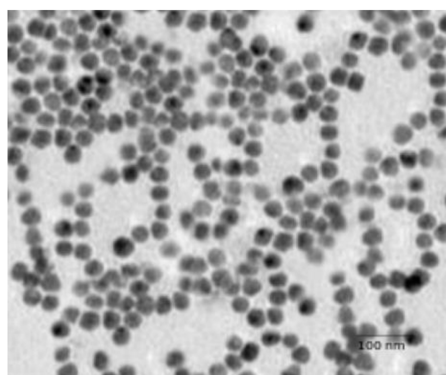

Fig. 2. Analysis results of MgONPs: (a) FTIR spectroscopy, (b) absorption spectra, and (c) TEM image.

shows the FTIR spectra, the absorption spectra and the transmission electron microscope (TEM) image of the MgONPs acquired.

A broad band at around 433-769 cm^-1 is assigned to the metal-oxygen bending vibration. A broad band at around 3461 cm^{-1} is attributed to stretching frequency of H-O-H. The broad band near 1381 $cm^{-1}$is due to C=O stretching frequency shows the presence of aromatic ring. Specimens of 10×10×2mm dimensions were prepared using plastic patterns to create a silicon-stone mould [26]. According to the manufacturer's specifications, each specimen was prepared, packaged, and cured. After adding the MgO NPs to the liner monomer, a probe sonication device was used for three minutes to disperse the MgO NPs and create individual nanoparticles (Soniprep-150, MSE, Sussex, UK). The container was submerged in a cooling bath, sometimes known as an ice-water bath, to prevent excessive liquid evaporation or material degradation caused by the mixture heating up during sonication [27]. After the specimens had fully cured, a final polish was applied, and they were autoclaved to ensure sterility. By rubbing the lesional tissue with a sterile cotton swab, 20 people with denture stomatitis and oral thrush had C. albicans isolated from their mouths. After that, the organism was grown on Sabouraud Dextrose Agar (SDA) for 24 to 48 hours and kept at 37°C. When it forms pasty convex, smooth, creamy colonies on SDA [28], the colony morphology and gram stain were used to identify it. Additionally, a Germ Tube Test (GTT) approach was performed [29–31], and API Candida system

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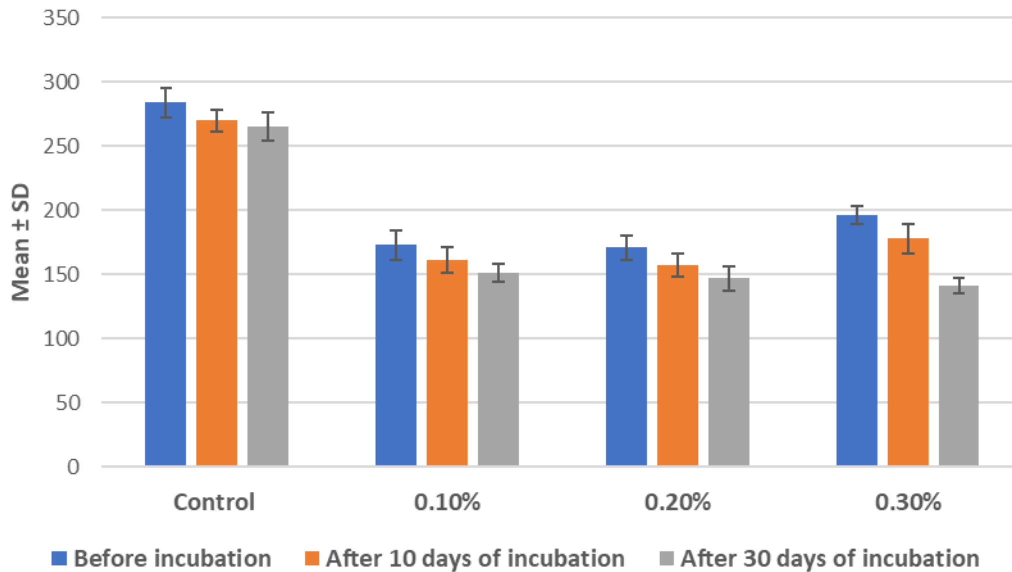

Fig. 3. Viable *C. albicans* count for all study groups and for various time periods (P<0.05).

(bioMérieux, France) was used for the biochemical method's final verification [32,33]. To evaluate the antimicrobial activity of the soft liner/MgONPs composites, *C. albicans* was diluted in 1% NaCl. A yeast suspension containing approximately 10^7 CFU/ml was prepared using the McFarland density method (0.5 McFarland standard) [34]. After placing each sample in a tube containing 10 mL of SDA broth, 100 µL of yeast suspension was added to each tube. A final cell density of 10^5CFU/ml was achieved. After 24 hours of incubation at 37°C, 100 µL of each mixture were diluted tenfold by adding them to 10 mL of NaCl solution. 100 µL of the second dilution was spread out on SDA before being incubated aerobically at 37°C for 24 hours. After storing the specimens in synthetic saliva at 37°C for 10 days and 30 days, this procedure was repeated. In this examination, specimens with a diameter of 8 mm and a thickness of 0.5 mm were utilized. The testing substrate for this experiment was Mueller-Hinton agar containing 2% glucose and 5 µg/ml of methylene blue (prepared in

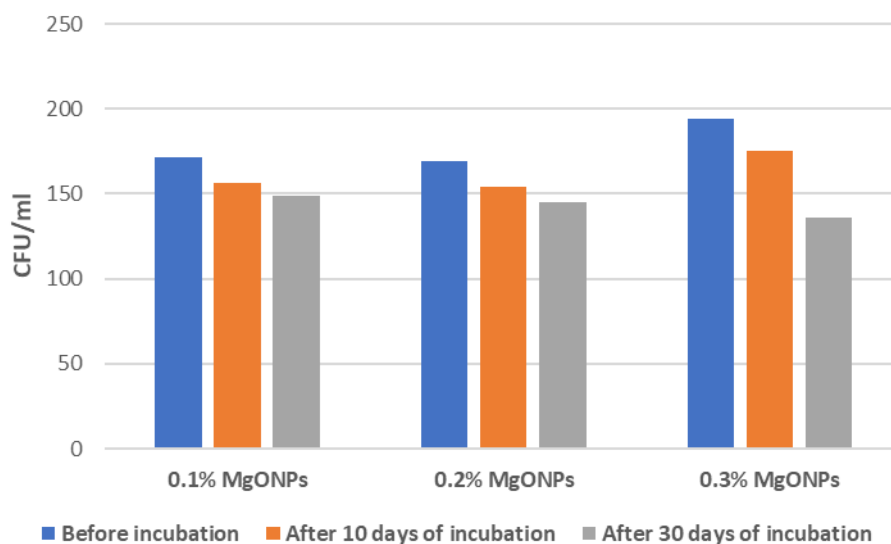

Fig. 4. Mean CFU/ml values for each experimental group at various research intervals.

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accordance with the manufacturer's guidelines) [35]. The decision was made to perform a Kirby-Bauer disk diffusion susceptibility test. Five-well colonies of *C. albicans* were suspended in 0.85% sterile normal saline containing 5 mL to accomplish a turbidity of 0.5 McFarland in order to produce a yeast stock suspension. After dipping a clean swab into the inoculum suspension, the sterile swab was used to squeeze out any excess fluid. The agar plate was swabbed three times to ensure uniform growth [36]. After leaving the agar surface alone for approximately 5 min, MgO NPs-containing and MgO NPs-free soft liner disks are placed on the agar. The plates are then stored at room temperature for 120 min to permit the antimicrobial agents to diffuse throughout the agar [37]. Finally, the agar plates undergo an aerobic incubation at 37°C for 24 hours. A digital caliper is used to measure the potential inhibition zone that may form around the disks. Specimens measuring 3 mm in thickness, 10 mm in diameter and two atomic absorption spectrophotometers (AAS), with detection limits of 0.025ppm and 0.015ppb, respectively, were used to determine the amount of MgO that was released (Model: ELICO SL194) [38]. Every ten days, the volume of synthetic saliva was reconstituted to account for natural evaporation. In each period (T1=10 days; T2=30 days), the solution from each tube was collected, and the amount of MgO released was determined using AAS. For the experimental and control specimens, 0.5±0.05 mm thickness and 50±1 mm diameter metal disks were manufactured using American National Standards Institute/American Dental Association Specification No.12 compliant metal patterns. All disk-shaped specimens were dried at 37°C±2°C for 24 hours in a desiccator containing dry silica gel, cooled to room temperature for 1 hour, and then weighed using a digital electronic balance with an accuracy of 0.0001g. This cycle was repeated until a constant weight (±0.5 mg) was reached. This was taken into account as the initial weight (W1). The samples were then heated for 7 days at 37°C±2°C in distilled water. After this time, each specimen was removed from the water, wiped with a clean, dry hand towel until all visible moisture was removed, waved in the air for 15 seconds, and weighed 1 min later (W2). The specimens were then dried in a desiccator and weighed every 24 hours until they reached a constant weight (±0.5 mg). This weight reflects the quantity of the substance present (W3). The following formulas were used to determine the water sorption and solubility of each specimen:

$$\text{Sorption} = \frac{W2 - W1}{\text{surface area}}\ (mg/cm^2)\,,$$

$$\text{Solubility} = \frac{W1 - W3}{\text{surface area}}\ (mg/cm^2)$$

Soft lining material was evaluated for shear bond strength to an acrylic denture base using acrylic blocks with specific dimensions 80×20×5mm and a stopper with an anticipated depth of 3 mm. Next, acrylic resin that has been heat-cured was employed (Lucitone 550, (LU)). All processes followed the manufacturer's instructions, including mixing, packaging, and curing. Following this, one acrylic block was placed on top of the other, and a space measuring 20×20×3.5mm was filled with wax. In order to create a mold for the final stage of the specimen's curing process, the entire specimen was cast in silicon material. After removing the wax, the formed space was filled with soft lining material and cured. The specimens were evaluated with the aid of an Instron Universal Testing Machine (PTC/O83/ME, INSTRON, USA). 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 (ASTM D 638-03 (2005)). Disk-shaped specimens with dimensions of 0.5±0.05 mm in thickness and 50±1 mm in diameter were prepared for use in Ultraviolet-Visible spectrophotometer (Shimadzu UV-1700) measurements of color change. This instrument measures the thickness of specimens and evaluates color change by measuring the percentage of absorbed light. A one-way ANOVA (analysis of variance) was used to compare the means in various groups. Values of $P<0.05$ were considered to be significant and values of $P<0.01$ highly significant.

RESULTS AND DISCUSSION

According to the FTIR analysis results, the material used for the soft lining had no chemical interaction with the MgO NPs. All experimental groups using different concentrations of MgO NPs (0.1%, 0.2%, and 0.3%) demonstrated a highly significant decrease in the number of *C. albicans* colony forming units relative to the control group ($P<0.01$). This decrease became more pronounced as the incubation time in synthetic saliva increased (Figs. 3 and 4).

ion test results did not reveal the existence of an inhibition zone around any test specimens. At no point during the incubation process did the synthetic saliva exhibit any signs of MgO release. The incorporation of MgO NPs resulted in a highly significant decrease in the mean value of water sorption ($P<0.01$) (Fig. 5). At the same time, only the 0.3% group exhibited a highly significant decrease in solubility ($P<0.01$) (Fig. 5). None of the test groups demonstrated statistically significant differences in shear bond strength ($P>0.05$) (Fig.

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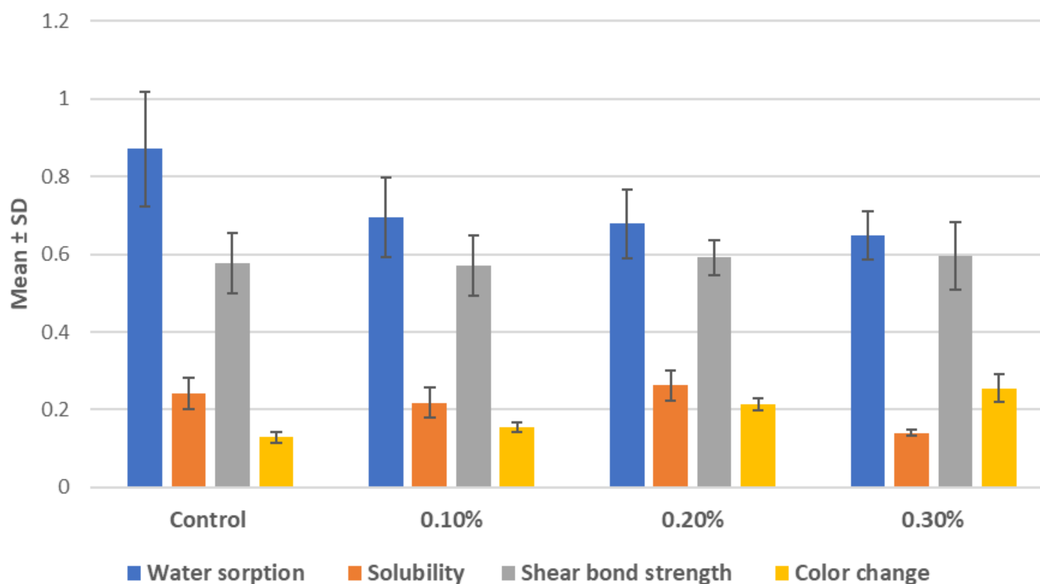

Fig. 5. Descriptive statistics of water sorption, solubility, shear bond strength and color change test results.

5). The percentage of light absorbed increased in all experimental groups and was highly significant (P<0.01) (Fig. 5).

In the present study, MgO NPs were incorporated into a soft denture liner to improve the liner's antimicrobial properties against *C. albicans* yeast, one of the primary contributors to denture-induced stomatitis. After incorporating MgO NPs into the soft denture liner, the results of this study revealed a statistically highly significant reduction in *C. albicans* colony forming units per milliliter. Antimicrobial effectiveness appeared to be proportional to concentration. The length of time the composite of the tested soft liner and MgO NPs was allowed to incubate in synthetic saliva appeared to affect the composite's antifungal activity. This phenomenon may be caused by the longer duration of specimens' immersion in an aqueous environment. No MgO were discovered to be released during this process [39]. These phenomena and a reduction in water sorption may explain the substance's decreased antifungal activity and subsequent improvement (0.3%). The disk-diffusion test revealed no evidence of an inhibition zone surrounding the specimens, regardless of the percentage of MgO NPs used, even after incubating the samples in artificial saliva. This phenomenon may be explained by the absence of magnesium oxide ion release from the soft liner/MgO NPs composite, as confirmed by the results of this study. Using AAS, the researchers did not detect any MgO in the synthetic saliva at any time during incubation. This was another finding that lent credibility to the study's findings. Previous research evaluated the release of MgO from a variety of polymeric materials [40–42]. Some of that research agreed with the present study's findings [41], while other research reached a different conclusion due to a different magnesium oxide concentration in the environment [40,42]. This could be accounted for by variations in MgO NPs incorporation techniques, polymeric material types, and polymerization processes.

CONCLUSION

In the current work, MgO NPs were added to a soft denture liner to enhance its antibacterial capabilities against the yeast *C. albicans*. The outcomes of this investigation showed a statistically highly significant decrease in *C. albicans* colony formation after adding MgO NPs to the soft denture liner.

CONFLICT OF INTEREST

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

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