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RESEARCH PAPER

The Antibacterial Characteristics of Fluorescent Carbon Nanoparticles Modified Silicone Denture Soft Liner

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ABSTRACT

Opportunistic oral fungal infections have become more common despite advancements in treatment, particularly in people who wear dentures. The most logical explanation of this infectious disease's beginning is a confluence of yeast cells becoming stuck in irregularities in denture-relining and denture-base materials, poor oral hygiene, and a number of systemic variables. The growth and colonization of microorganisms are potential downsides of prolonged usage of soft liners. Clinical issues and material damage may emerge from Candida albicans (*C. albicans*) and Staphylococcus aureus (*S. aureus*) colonizing soft lining materials. The purpose of this study was to determine how adding fluorescent carbon nanoparticles (FCNPs) to a denture soft liner affected that liner's capacity to prevent bacteria growth. From a soft denture liner, 48 samples were collected and divided into a test group and a control group, each of which received 5% by weight of FCNPs. Antimicrobial tests were performed on *C. albicans*, *S. aureus*, and a combination of biofilms. Prior to and following six months of storage in distilled water, results on the cell density count and adhesion assay were gathered. The findings demonstrated that the modified group's antibacterial activity was significantly higher than that of the control group. Additionally, both before and after the storage period, the addition of 5% nanofiller considerably decreased the growth of *C. albicans*, *S. aureus*, and the biofilm mixture ($P<0.05$). Adding 5% by weight of FCNPs to the denture soft liner for up to six months significantly prevented the growth of *C. albicans* and *S. aureus*.

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INTRODUCTION

For the treatment of edentulous individuals experiencing pain from damaged oral mucosa, soft denture liners and tissue conditioners are frequently used [1–3]. Through absorbing the chewing pressures of the prosthesis while in use, these materials can lower stresses on denture-bearing surfaces [4,5]. The denture base is the denture component that lies on the foundation tissues and to which teeth are connected [6]. Recently, acrylic resins are used to create the majority of denture bases [7]. Denture bases can

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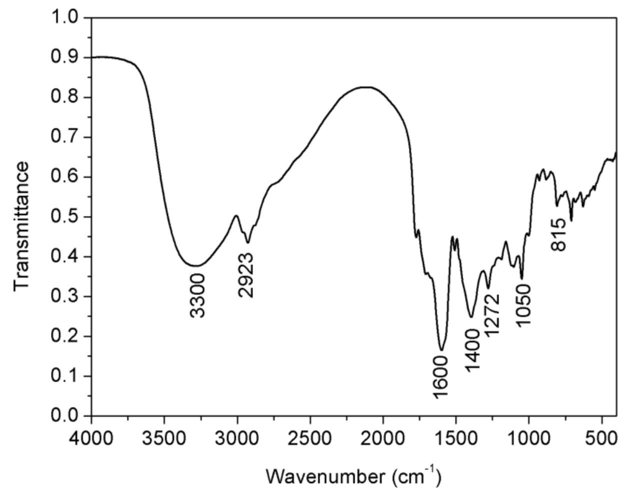
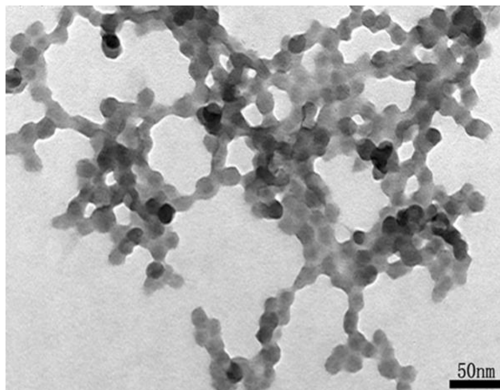
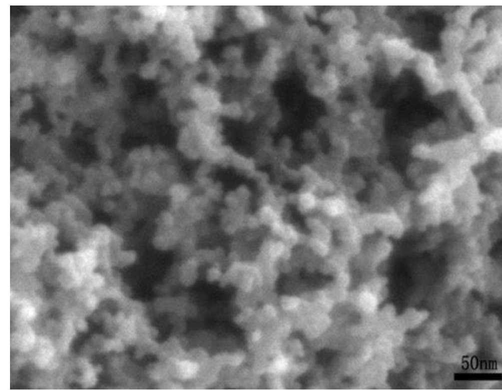
be formed of a variety of materials and serve a variety of practical purposes. The denture base in a tooth-supported prosthesis is basically a space between two abutments that are holding prosthetic teeth. Therefore, through rests, occlusal forces are immediately transmitted to the abutments [8–10]. It is possible for dentures to gradually lose their stability and retention due to alterations in the denture-supporting tissues and occlusal disharmony, both of which contribute to host abuse [11]. Consequently, the goal of relining is to resurface the denture's impression surface in order to better fit and adapt the underlying supporting tissues [12]. According to their consistency, denture lining materials might be hard or soft. They were developed to make wearing dentures more comfortable, particularly for those who have sharp, knife-edged residual ridges and complain of pain and soreness when wearing dentures, making it impossible for them to withstand the masticatory and functional pressures [13]. Typically, the denture soft liners have a porous surface [14–16]; these porosities provide favorable environments for bacterial colonization [17]. The oral cavity is inhabited by numerous microorganisms, including bacteria, viruses, and fungi, all of which can contribute to the production of dental plaque [18–20]. The antibacterial activity of denture soft liners is typically attributable to the incorporation of antimicrobial chemicals; however, this modification frequently results in alterations to



(a)





(b) (c)

Fig. 1. Analysis results of FCNPs: (a) FTIR spectroscopy, (b) TEM, and (c) SEM image.

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the materials' physical, chemical, and mechanical properties, which limits their application [21–23]. Candida albicans (*C. albicans*) and Staphylococcus aureus (*S. aureus*) are the microbial species that colonizes soft liner materials most frequently [24]. The soft denture liners were given numerous additions that served as antibacterial agents. [25–27]. Antibacterial properties have been reported for fluorescent carbon nanoparticles (FCNPs), also known as carbon dots, CDs, and C-dots [28]. On the other hand, carbon nanoparticles (CNPs) possess a low intrinsic risk of cellular toxicity, in contrast to the vast majority of other metal oxides formulations. This study aims to test and evaluate the antibacterial properties of a silicone-based denture soft liner to which 5% FCNPs have been added.

MATERIALS AND METHODS

In this study, a silicone-based resilient denture liner (Mollosil) was utilized (DETAX GmbH, Germany). It comprised of two pastes, a base and a catalyst, which were blended in an equal ratio per the manufacturer's instructions. The employed FCNPs exhibited a purity of greater than 95% and an average diameter of 2-6 nm. Fig. 1 shows the transform infrared spectroscopy (FTIR) spectra, the scanning electron microscope (SEM) and the transmission electron microscope (TEM) image of the FCNPs acquired.

After determining the weight of the base and the catalyst using an electronic scale, 5% of the catalyst's weight in FCNPs was added to the mixture. An exploratory investigation was used to guide the choice of this concentration. In the investigation, the soft liner was injected with FCNPs at different concentrations, including 4%, 5%, 6%, 7%, and 8% by weight, to determine which concentration offers the most effective antibacterial effect. Compared to the control group and the other modified groups, the modified group containing 5% FCNPs had the greatest impact on inhibiting the growth of *C. albicans* and *S. aureus* (Fig. 2).

FCNPs were sterilized in a hot air oven at 250°C for 30 min prior to the preparation of the samples because other sterilization or disinfection methods for FCNPs are not permitted due to their hygroscopic nature. As a result, autoclaving is not regarded as the best method for sterilization because there is a chance that the FCNPs will change phase due to the presence of the autoclaving process. Also, FCNPs absorb ultraviolet (UV) radiation and interact with oxygen molecules [29–32]. After trace amounts of FCNPs were added to the base of the soft liner and stirred for one 30 seconds in a circular motion (180 cycles/min), then mixed for an additional 5 min with a lentulo spiral file that was attached to a slow-speed handpiece. In line with the manufacturer's instructions the catalyst portion was added to the mixture and stirred for 20 seconds (Table 1).

Throughout the entire process, every specimen was prepared at room temperature. Table 2 presents the sample numbers in addition to the grouping that was performed.

The certification of *C. albicans* obtained from a reference laboratory was given by the American Type and Culture Collection (ATCC) 10231. After developing the medium of Sabouraud dextrose agar (SDA), the medium was incubated for 12 hours at 50°C. The *C. albicans* suspension was then standardized by dilution with sterile phosphate buffer saline (PBS), and the yeast culture concentration was maintained at 10^{7} CFU/mL. After inoculating 8 mL of SDA broth with one *C. albicans* colony, the combination was incubated at 40°C for 20 hours. Once the *C. albicans* sample was placed in autoclaved test cups, the microbiological test

Table 1. Sample component for an antibacterial activity test

Sample label	FCNPs (g)	Catalyst (g)	Base (g)	Overall (g)
Control with 0% FCNPs	0	0.5	0.5	1
5% FCNPs	0.015	0.5	0.485	1

Table 2. Sample numbers and grouping

Pathogenic yeast	Control group (number) Before and after storage	Modified (test) group Before and after storage
C. albicans	10	10
S. aureus	10	10
Mixed *C. albicans and S. aureus*	10	10

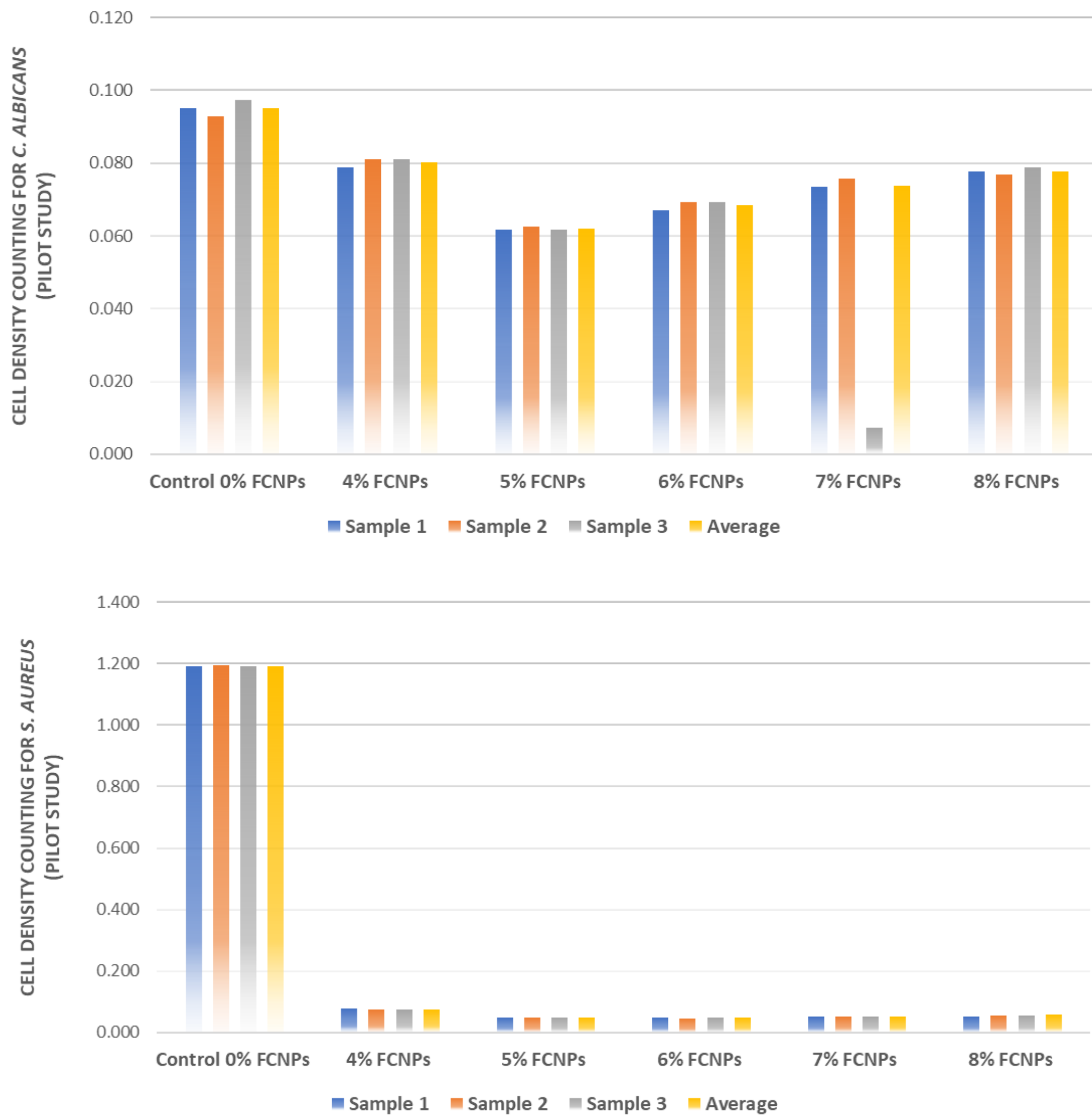

Fig. 2. Results of *C. albicans* and *S. aureus* cell density counting (pilot study).

began. This occurred quickly after the preparation of *C. albicans*. The test cups were incubated in an incubator with an aerobic atmosphere at 40°C for 2 hours. After a while, the samples were removed from the test cups and washed with PBS. Each sample was subsequently transferred to a new test cup containing 18 mL of fresh brain heart infusion (BHI) and yeast nitrogen base (YNB) media. The final step involved placing test samples in an incubator at 40°C under aerobic conditions for 18 hours. This was done for the biofilm to form and grow on the test samples. Thirty samples in all were prepared for the anti-*Candida* test. Fifteen of these samples were placed in autoclaved test cups containing distilled water (DW) and stored at 40°C at a constant temperature for six months. After 24 hours, the remaining 15 samples were utilized to establish whether or not the anti-*Candida* treatment was effective. Regarding the technique for bacterial cultivation, the ATCC 25923 certified that *S. aureus* was obtained from a reference laboratory. Following the growth of the bacteria on blood agar, one colony of the strain was placed in 8 mL of 30% BHI with 70% YNB at 40°C for 20 hours. At 10^7 CFU/mL, the bacterial concentration remained maintained. Following this, the sample

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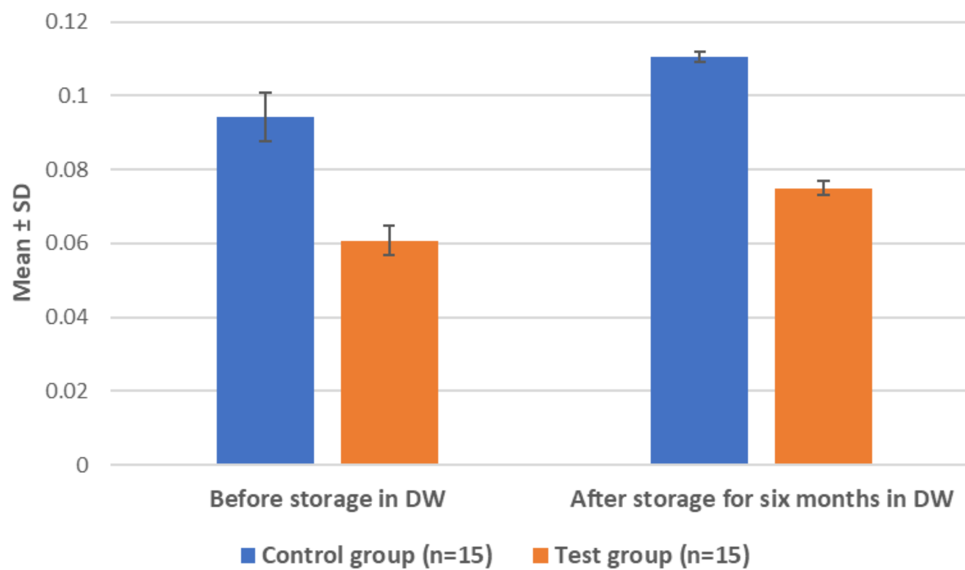

Fig. 3. *C. albicans* yeast cell concentration (P<0.001).

pattern, size, and numbers were determined in a manner comparable to the approach described before for *C. albicans*. After the incubation phase was complete, each sample was carefully removed from the cup using sterilized tweezers. Each sample was then rinsed by dipping it into the sterile cup containing 45 mL of PBS solution for 80 seconds three times. To ensure that all cells were completely freed from the surface of the specimen, each sample was then suspended once more in 2 mL of PBS solution and vortexed at maximum speed. The specimen was then withdrawn from the solution. The specimens were used to assess the results of the adherence assay test, while the solution was used to count the number of cells. The resulting solution was sonicated for 5 min to remove the microbiological aggregates. Subsequently, to evaluate the rate of absorbance, the suspended biofilms were transferred into a 100-well plate that had been sanitized and then placed within a spectrophotometric apparatus operating at a wavelength of 500 nm. The specimens were carefully washed by dipping them into the sterile PBS solution for roughly 1 min three times to eliminate the unattached pathogen cells from the sample surfaces. This was done to detect whether or not a biofilm had established on the surface of the soft liner samples. After fixing the specimens by exposing them to ethanol at one 100% concentration for five seconds, they were allowed to dry in sterile plates at room temperature. In the final phase, the samples were dyed for 2 min with a 0.1% concentration of sterilized methylene blue dye. In the final step, samples were examined with an optical light/dissecting microscope to see if biofilm had grown on the surface of the soft liner samples.

RESULTS AND DISCUSSION

The modified group performed substantially better than the control group when it came to suppressing *Candida* growth, as determined by the results of an anti-*Candida* test conducted on the soft liner using the optical density method. As shown in Fig. 3, this resulted in a statistically significant decrease in the mean value of *Candida* growth.

There was a decline in anti-*Candida* activity relative to the value prior to the conditioning method after six months conditioning (incubation) of both groups in DW. The mean value of *C. albicans* growth after six months of storage in DW increased significantly for the control group. The test group outperformed the control group once more and showed superior anti-*Candida* growth resistance, although the difference in *C. albicans* growth grew dramatically after six months of storage in DW. The results for the antibacterial growth indicated that before conditioning the samples in DW, adding 5% of FCNPs to the soft liner greatly reduced the development of *S. aureus* (Fig. 4).

Since there was an increase in bacterial growth

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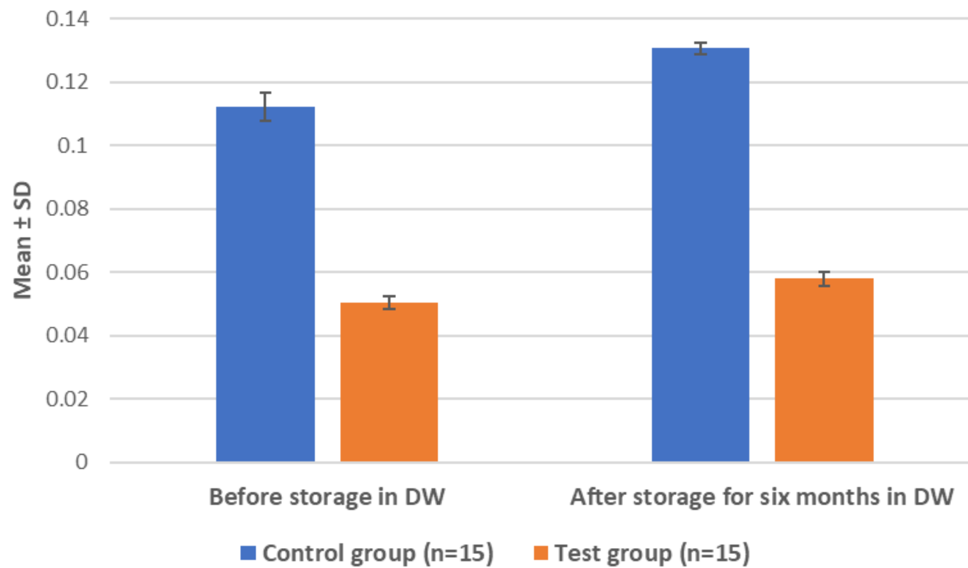

Fig. 4. *S. aureus* yeast cell concentration (P<0.001).

for both groups compared to their respective values prior to storage in DW, and since this difference was statistically significant for both groups, storing the soft liner samples in DW for six months reduced the antibacterial effect. Prior to storage in DW, cell density counts for the mixed *C. albicans* and *S. aureus* biofilm revealed that the controlled group's growth of this combination was greater than that of the test group (Fig. 5). The findings demonstrated that the distinction was statistically very significant.

The results showed the same pattern as before storage in DW, and once more the test group demonstrated a better resistance than the control group, the difference being highly significant. Cell density counting for the control and test groups was significantly increased after six months of storage in DW (P<0.001). The results of the adherence assay test revealed that, compared to the control group, the antimicrobial effect on the

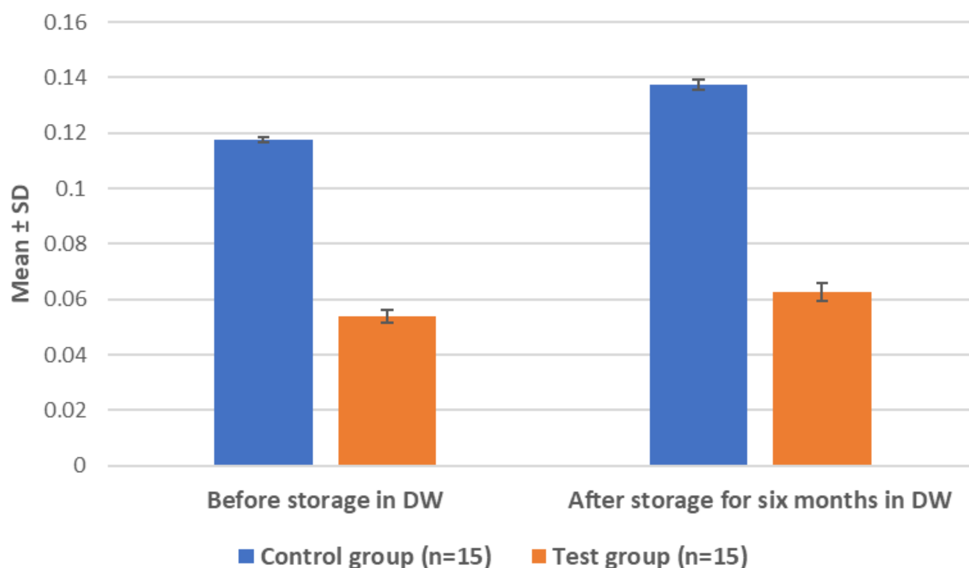

Fig. 5. *C. albicans* mixed with *S. aureus* yeast cell concentration (P<0.001).

surface of the soft denture liner modified with 5% of FCNPs was more effective and performed higher resistance for *C. albicans* development. For both the *S. aureus* group and the mixed group, the same formula was found. Following six months storage in DW, the samples matching showed the same pattern as was seen as that before storage. This shows that the additive (5% FCNPs) continued to exhibit antimicrobial activity after storage in DW, albeit with a less potent impact than before storage. The outcomes of this study were comparable to [33] when they investigated the efficacy of various FCNPs when paired with tissue conditioners. It was discovered that the anti-*candida* activity rose as the CNPs concentration increased. This finding was also observed by [34].

CONCLUSION

In this study, a silicone-based denture soft liner with 5% FCNPs added was tested and evaluated for its antibacterial capabilities. The antibacterial efficacy against *C. albicans*, *S. aureus*, and a biofilm combination of both species was greatly enhanced by the addition of 5% of FCNPs to the denture soft liner for up to six months.

CONFLICT OF INTEREST

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

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