Al-Rafidain Dental Journal

rden.mosuljournals.com

Effect of Different Nanoparticles Incorporation in Acrylic Based Soft Liner on Candida albicans Adhesion

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Article information

Received: January 14, 2021
Accepted: March 22, 2021
Available online: March 9,2022

Keywords:
Nanoparticles
Soft liner
Candida albicans
Adhesion

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Abstract

Aims: To evaluate the effect of the addition of MgO, ZrO2, ZnO nanoparticles in different concentration to acrylic based soft liner on candida Albicans adhesion. **Materials and methods**: acrylic-based soft-liner samples were prepared (10 X10 X 2mm dimension) by addition MgO, ZrO2, ZnO nanoparticles in 0.25, 0.5, 1, 2, 4 % by weight concentration to the monomer resin, the samples were immersed in Candida Albicans suspension for 1 hour then washed and dried, the microorganism were fixed and stained by crystal violet and examined by light microscope (at 400 x) to enumerate the stained candida Albicans in two occasions for thirty fields of view. **Result**: soft liner with 1,2,4 % by weight demonstrated that a decreased the surface adhesion of candida Albicans when compared with control group while specimens with lower concentration (0.25, 0.5% by weight) show no significant difference from control. **Conclusion**: addition of nanoparticles in 1,2,4 % concentration of weight to acrylic based soft liner impaired the adhesion properties of Candida Albicans, and reduce fungal induced denture stomatitis.

الخلاصة

الأهداف: تهدف الدراسة الى تقييم تأثير إضافة جسيمات نانوية MgO، ZrO2، ZnO وبتراكيز مختلفة للبطانة الطقم المرن ذات الأساس الاكريلي على التصاق المبيضات البيض عل سطح البطانة المرنة. **المواد وطرائق العمل**: تم إعداد عينات البطانة المرنة وبالأبعاد التالية ٢١٠ X 10 X ملم , ثم تم إضافة الجسيمات النانوية MgO، ZrO2، ZnO وبتركيز ٠,٢٥ ، ٠,٥، ١، ٢، ٤٪ من الوزن إلسائل الراتنج . تم غمر العينات في معلق المبيضات البيض لمدة ساعة ثم غسلها وتجفيفها، بعد ذلك تم تثبيت الكائنات الحية الدقيقة وصبغها بصبغة بالكريستال البنفسجي وفحصها بواسطة المجهر (قوة تكبير ٤٠٠) ليتم تعداد المبيضات البيض الملتصقة عل سطع النماذج بفترتين مختلفة و لثلاثين حقلا . **النتائج** : لوحظ انخفض عدد المبيضات البيض الملتصقة على سطح البطانة المرنة بعد إضافة الجسيمات النانوية وبتركيز ١,٢,٤ ٪ من الوزن عندما يقارن مع النماذج الغير مضاف عليها بالإضافة الى تم ملاحظة ان العينات المضاف عليها الجسيمات النانوية وبتركيز قليل (٠,٢٥, ٠,٥٪ من الوزن) انه لا يوجد اختلاف احصائي عن النماذج الغير معاملة . **الاستنتاجات**: إضافة الجسيمات النانوية بتركيز ١,٢,٤ ٪ من الوزن إلى بطانة المرنة الاكريليك يقلل ألتصق المبيضات البيض والحد من التهاب الفم الناجم عن الفطريات

DOI: 10.33899/rdenj.2022.173032

INTRODUCTION

Denture (fitting surface) used for the resorbed ridge, Sharpe ridge, atrophic mucosa patients are frequently relined by soft lining materials, that assist in better masticatory force distribution when transmitted to the underlining mucosa. The soft liners improve the well-being of denture wearers patient and support prosthetic treatment. [1]

It has a rougher surface due to tissue site topography and plasticizer leakage that enhance the adhesion of microorganisms onto soft lining materials and may permit fungal growth [2]

Dental plaque that formed in the oral cavity consists of different microorganisms which embrace bacteria, viruses, and fungi [3].

Denture tissue surface acts as a source of numerous fungi, usually Candida, that play role in fungal infection [4,5].

Candida is one of the main causes of implicated for denture stomatitis of denture wearer [6]. Candida albicans is recoded as most infectious among other Candida species. The virulency of different species is due to variance in the ability of the microorganisms to adhere to epithelial cells. In which the *C. albicans* stuck to Buccal epithelial cells higher than other Candida species. The adherence of candida to denture surface material considers the initial step for colonization [7,8]

Candia adherer to both hard and soft denture materials, this adherence may be due to surface-free energies of the microorganisms and the surfaces of the materials or maybe due to specific adhesion–receptor interactions [9].

The material used, surface roughness, microporosity, presence of saliva, strain variability, and concentration. May also considered additional factor of candida adhesion [10,11]

Different study supported those nanoparticles can offer preventive and therapeutic approaches, due to their action in controlling and reducing dental plaque biofilms and enhancing the antibacterial activity of dental materials [12].

Incorporation of metal oxide nanoparticles in acrylic resin denture may improve the hypogenic performance of it [13].

Colony-forming unit of bacteria was significantly reduced after acrylic resin after immersion MgO NPs solution, due to the antibacterial properties of MgO particles. MgO nanoparticles provide antimicrobial activity against both Gram-positive and Gram-negative bacteria, spores, and viruses [14,15].

Zirconium oxide nanoparticles showed remarkable antimicrobial activity against bacterial pathogens and *Candida albicans* [16].

Fungal strains were significantly reduced by the growth inhibition activity of ZrO_2 nanoparticles, in which fungal hyphae were distorted and cell function interfered [16].

Zinc oxide NPs produce reactive oxygen species (that may cause damage to DNA, RNA, and proteins) that lead to an excellent anti-microbial activity. [18]

ZnO nanoparticles cover polymethyl methacrylate showed substantial antifungal activity against Candida albicans biofilms [19].

Water absorption and solubility properties were increased as nanoparticles concentration increased while the hardness and tensile bond strength of the soft liner was greatly reduced. [20].

The aim of this study to evaluate the effect of the addition of MgO, ZrO2 and ZnO nanoparticles to the acrylic-based soft liner on *C. albicans* adherence.

MATERIALS AND METHODS

Candida preparation:

Isolation of *Candida albicans* was carried out from non-antifungal treated denture wearer (for 2 years at least) patients with denture stomatitis attended to the prosthodontic clinic at the College of Dentistry / University of Mosul. The *candida SPP* was cultured on Sabouraud dextrose agar (with chloramphenicol 50mg/L). *Candida albicans* was identified gram stain reaction, germ tube formation under a microscope, and Chromogenic agar medium that performed by a specialized microbiologist in the college of dentistry/ university of Mosul [21].

Minimum inhibitory concentration (MIC):

The minimum inhibitory concentration of MgO, ZnO_2 and ZnO nanoparticles concentrations at 0.125, 0.25,0.5, 1, 2, 4, 8 % by weight were evaluated by serial two-fold dilutions.

A stock solution was prepared by the addition of 160 µgm of nanoparticles to the test tube for each 1ml of sterile normal saline and sonicated for 5 min. Two-fold serial dilution was performed by transferred 1ml of stock solution to test tube containing 1ml of sterile brain heart infusion broth (representing 8% wt/wt), then mixed and 1 ml of 2nd test tube was transferred to 3rd one. This process was repeated till 0.125%wt/wt nanoparticles solution was obtained.[22]

Candida albicans suspension was prepared at 0.5 McFarland's standard (10^8 CFU/ml) concentration, 0.1 ml of candida suspension was added to the prepared nanoparticles test tube. The test tubes were agitated and incubated at 370C for 24 h. the MIC was evaluated visually to the determination antifungal efficacy of Nanoparticles by assessing the fungal growth in brain heart infusion broth. [19]

Sample preparation

Eighty square-shaped samples of the acrylic-based soft liner (vertex soft, Vertex-Dental, Netherlands) were prepared and nanoparticles incorporated with it (5 samples for each group) table (1). A stone mold was prepared by

investing a square-shaped plastic foil (10 mm X 10mm X 2 mm) in class IV dental stone (Elite stone, Zhermack dental, Italy) poured in the metal flask [20].

After setting of stone, the plastic foil was removed. A separating medium was painted overset the stone mold in two layers.

Nanoparticles were added to the monomer of the soft liner at 0.25, 0.5, 1, 2, 4% wt concentration and sonicated to get homogenously dispersed nanoparticles/ monomer solution).

Acrylic base soft liner powder was added to monomer suspension at 1.25:1 powder / liquid ration (according to manufacturer instruction). The dough packed in the stone mold flask and pressed under a hydraulic press.

The dough was cured in the water bath for 90 min at 70c^{o} and 30 min at 100C (according to manufacturer instruction), and then the flask was removed from the water bath and left to bench cooling for 30 min. The specimens were stored in distilled water without finishing to represent tissue site of the denture (only the boundary excess was removed by the sharp blade) for 24hours at 37 c^{o}.

Table (1): nanoparticles used in the study

nanoparticles	Size	manufacture
Magnesium oxide MgO	40 nm	Nano shel USA
Zirconium oxide ZrO_2	20 nm	Nano shel USA
Zinc oxide ZnO	35-45 nm	Nano materials USA

Adhesion assay

McFarland standards (0.5) of Candida albicans suspension was arranged from 48-hour cultures in sterile normal saline to attain 1×10^8 Cfu/ml [23].

A sterile acrylic-based soft-liner specimen [23] (sterilized by UV light sterilizer for 20 minutes) was immersed in a sterile petri dish containing 20 ml candida suspension and incubated for 1 hour at room temperature. The specimens were washed by phosphate-buffered saline solution two times for 1 minute, and then the samples were dried at room condition to remove poorly adherent cells.

Fixation of adherent candida cells was performed by 1ml of 99% methanol for 1 min. and stained for 30 seconds with crystal violet.

The soft-liner samples were washed by a phosphate-buffered saline solution for 30 seconds and inspected by light microscopy (optika, Italy) under 100 and 400X magnification.

Thirty fields of view were examined (0.25 mm^2 per field) and Adherent *Candida albicans* were counted, and the

results were expressed as candida cells/mm^2 of material. Assays were repeated two times [24,25]. (Figure 1)

One way ANOVA with Duncan's multiple range test using the SPSS statistical analysis program at a significant level of 5%.

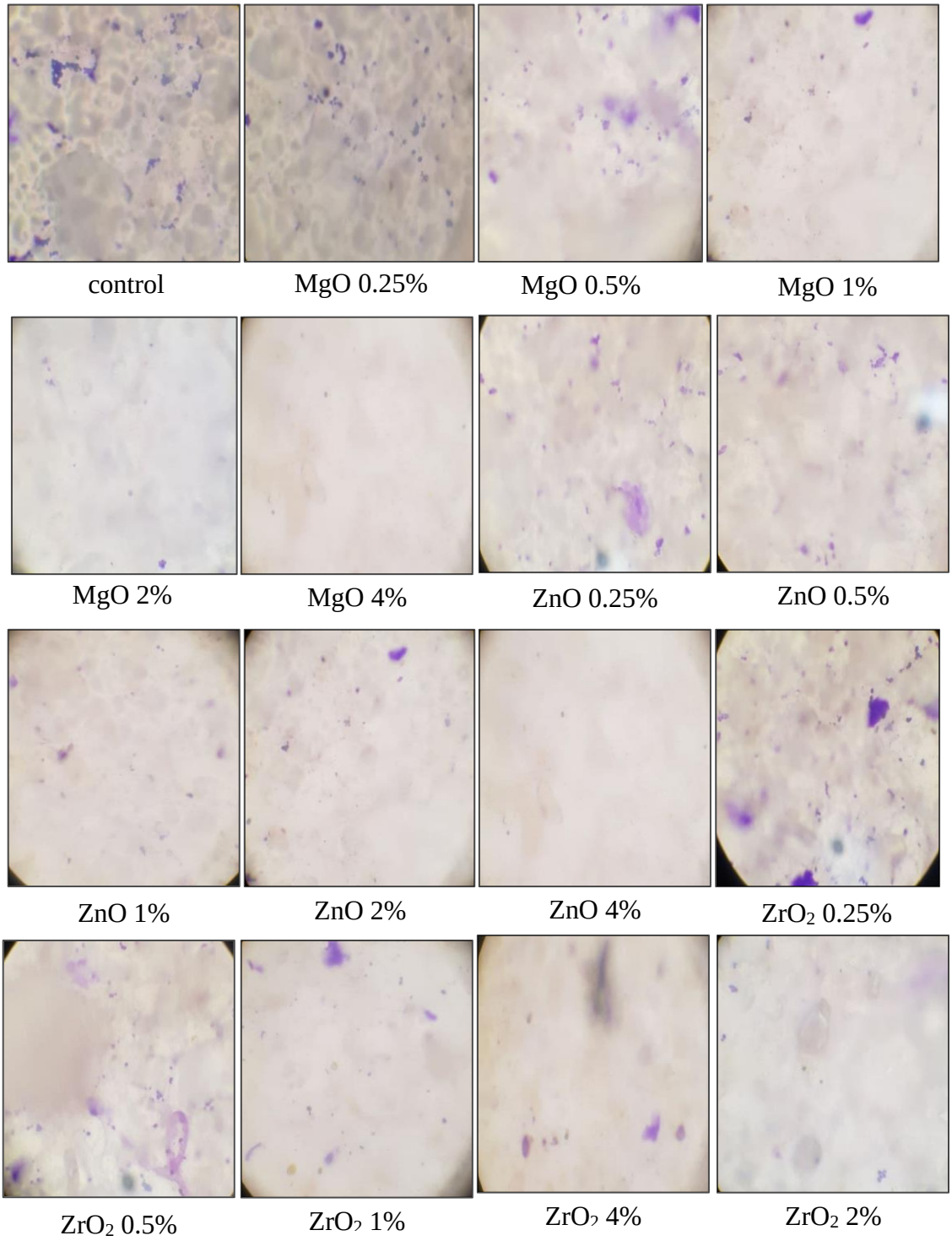

Figure (1): microscopical view of candida albicans adhesion on soft liner with nanoparticles (under 400X).

RESULTS

Mean of *Candida albicans* /mm^2 was demonstrated in the table (2) that shows there is a lower value of *Candida albicans* adherence unite on acrylic based soft liner surface with all nanoparticles additives used in this study at higher concentration (1,2,4% Wt) than the control group.

Table (2): Mean of Candida adhesion on modified acrylic soft-liner surface and control groups (candida/mm2).

Variables	N	Mean*	Std. Deviation
Control	5	204.6000	9.37017
MgO 0.25%	5	207.0000	22.60531
MgO 0.5%	5	198.8000	8.10555
MgO 1%	5	52.0000	8.15475
MgO 2%	5	31.8000	3.03315
MgO 4%	5	30.8000	6.90652
ZnO 0.25%	5	208.8000	21.19434
ZnO 0.5%	5	193.8000	5.80517
ZnO 1%	5	96.8000	5.80517
ZnO 2%	5	31.8000	4.02492
ZnO 4%	5	23.4000	4.15933
ZrO_2 0.25%	5	203.6000	13.86723
ZrO_2 0.5%	5	191.0000	4.58258
ZrO_2 1%	5	56.6000	10.16366
ZrO_2 2%	5	24.2000	4.65833
ZrO_2 4%	5	21.8000	2.58844

*Adhesion unite cell/mm^2

Table (3) illustrated the analysis of variance (ANOVA) of study groups and demonstrated that there are highly significant differences ($p \leq 0.01$) between study groups and control group at a significant level 0.01%.

Table (3): analysis of variance of candida adherence on the acrylic-based soft liner with MgO, ZrO_2, ZnO nanoparticles with different concentration and control group.

	SS	df	MS	F	Sig.
Between Groups	529325.800	15	35288.387	335.481	0.000
Within Groups	6732.000	64	105.188		
Total	536057.800	79			

Duncan's multiple range test of study groups displayed in figure (2), which confirm that there is a significant reduction in *Candida albicans* adherence

on acrylic based soft liner incorporated with ZrO_2, ZnO, MgO at 1,2, 4% Wt/Wt concentration nanoparticles in contrast to control group.

Soft liner with 0.25 and 0.5 %Wt/Wt concentration nanoparticles (ZrO_2, ZnO, MgO) have no significant differences when compared with the control group.

There are no significant differences between Nanoparticles (ZrO_2, ZnO, MgO) at 2% and 4% concentration incorporated with soft liner, while there are differences between 1% nanoparticles concentration and 2%,4% concentrations.

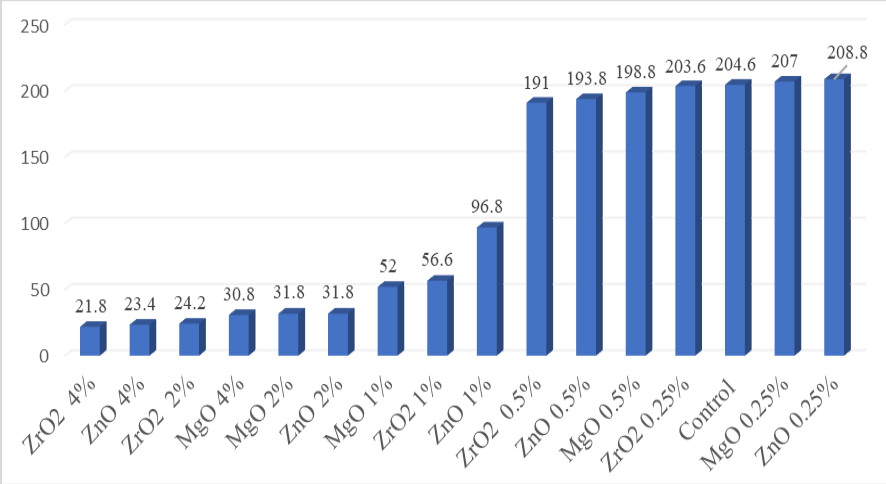

Figure (2): Duncan's multiple range test of *Candida albicans* adherence on different study groups.

DISCUSSION

Candida albicans adhesion to the soft liner is a multifaceted process, liner denture surface roughness, poor oral and denture hygiene, salivary pH, systemically diseased patient enhanced yeast colonization [26].

Candid albicans adherence is greatly related to the free surface energy of soft liner and candida, as the free surface energy of candida closes to that of attached surface, the candida albicans adherence increased. In addition, the *Candida albicans* hydrophobicity property and surfaces electrostatic interaction considered for high adherence of yeast.

The second cause may be blamed for the tight binding of candida is the presence of adhesin-receptor interactions, in which the candida had adhesins that bind stereochemically to complementary receptors on the surface [27].

The result of the present study revealed that *Candida albicans* adhesion to soft liner was significantly decreased when high nanoparticles concentration (1,2,4% wt/wt) incorporated with it in contrast to the control group, which is may due to an electromagnetic attraction between negative charged fungal Mannosylphosphate cell wall and positive charge of metal oxide nanoparticle (present in high amount), that lead to yeast death due to its oxidation. [28,29].

Also, ZrO2 nanoparticles deformed fungal hyphae by cell wall interfering that causes fungal growth inhibition. While ZnO nanoparticles destructed hyphae by interrupt conidia and conidiaphores growth. [30,31]

The lower nanoparticles concentration (0.25, 0.5%wt/wt) in soft liner showed no significant effect on *Candida albicans* adhesion which may due to the lower nanoparticles contained soft liner to be in direct contact with Fungai on its surface, in addition to minimum superoxide anions released from the nanoparticles [32].

Soft liner samples with high nanoparticles concentration may have lower surface roughness when compared with control samples [33] that lead to lower candida adhesion on specimens surface[9]

This study agrees with Yassir and Abdul Fatah [34] who exposed that ZrO2 nanoparticles have significant antifungal activity when added to the soft liner.

The finding of this study also agrees with Cierech et al[35] which demonstrated that the addition of ZnO nanoparticles to PMMA in high concentration provides antifungal properties for denture base. While disagrees with Homsiang *et al.* [36] who mentioned that ZnO nanoparticles at 5% concentration show no significant effect on *Candida albicans* which is maybe due to differences in nanoparticles size.

Conclusion:

The study concluded that (with the limitation of this study) the addition of MgO, ZnO, ZrO^2 (metallic oxide) nanoparticles to acrylic based soft liner at 1,2,4 % by weight concentration decreases *Candida albicans* adhesion ability to liner surface, and improve soft liner resistance to fungal induced denture stomatitis. While low concentration nanoparticles do not affect candid adherence on the liner surface.

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