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Antifungal Activity of Magnesium Oxide and Zirconium Oxide Nanoparticles Incorporated into Alginate Impression Material. In Vitro Study

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Abstract

Aims: The aim of this study was to evaluate the antifungal effect of adding nanoparticles with different concentrations into irreversible hydrocolloid impression materials. **Materials and Methods:** This study evaluated the antifungal effect of MgO and ZrO2 nanoparticles by Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) and using the Agar-Well diffusion method after incorporation of different concentrations of nanoparticles into irreversible hydrocolloid impression material. **Results:** The results of this study showed that MIC and MFC of magnesium oxide nanoparticles were 0.2wt% and 0.3wt% respectively and for zirconium oxide nanoparticles were 5wt% and 10wt% respectively. **Conclusions:** The incorporation of MgO and ZrO2 into irreversible hydrocolloid impression materials acts as antifungal agents.

الخلاصة

الاهداف: تهدف الدراسة الى تقييم تأثير إضافة الجسيمات النانوية بتركيزات مختلفة إلى مادة طبعة الالجينيت على نمو الفطريات.**المواد وطرائق العمل**: أظهرت هذه الدراسة في المختبر التأثير المضاد للفطريات لأوكسيد المغنيسيوم وجسيمات أوكسيد الزركونيوم النانوية بالتركيز المثبط الأدنى (MIC) والحد الأدنى من تركيز مبيدات الفطريات (MFC) لهذه الجسيمات النانوية. تم إثبات نشاط مضاد للفطريات باستخدام طريقة الانتشار القرصي بعد دمج تركيزات مختلفة من الجسيمات النانوية في مادة طبعة الالجينيت.**النتائج**: أظهرت نتائج هذه الدراسة أن MIC و MFC لجسيمات أوكسيد المغنيسيوم النانوية كانت ٠,٢٪ و ٠,٣٪ بالوزن على التوالي ولجسيمات أوكسيد الزركونيوم النانوية كانت ٥٪ و ١٠٪ بالوزن على التوالي. **الاستنتاجات**: أوكسيد المغنيسيوم وأوكسيد الزركونيوم المدمجين في مادة طبعة الالجينيت فيه يعملان كعوامل مضادة للفطريات.

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INTRODUCTION

Alginate impression material has been widely used in dentistry; it is an irreversible hydrocolloid impression material. [1]

It has been reported that the dental impressions can become contaminated with blood and saliva of the patient, and therefore these are considered as a potential source of cross‑infection not only to the patient and dentist but also to the dental technician. [2]

Alginate containing disinfectants, popularly known as self-disinfectant alginates, was developed with an aim to avoid conventional disinfection after impression recording. [3]

Numerous researchers have developed self-disinfecting impression materials by incorporating different antimicrobial nanoparticles into impression materials. [4]

Antimicrobial efficacy of Zinc oxide and Copper oxide nanoparticles was also experimented with by numerous researchers and these nanoparticles were also proved to be effective self-disinfecting agents for alginate impression materials with no significant negative effect on physical and mechanical properties. [5] Silver nanoparticles are employed as an antimicrobial agent; and have been used in various medical applications. [6‑8]

MATERIALS AND METHODS

The nanoparticles in this study were provided by Nanoshel company,USA. The Particle size was 20nm, these two nanoparticles are: Magnesium oxide and Zirconium oxide nanoparticles. *Candida albicans* was obtained from Microbiology department, College of Dentistry, Mosul University. The Irreversible hydrocolloid impression material algistar chromatic was provided by, Müller Omicron, Germany.

Minimum Inhibition Concentration and Minimum Fungicidal concentration:

The lowest concentration of nanoparticles that inhibit growth of microorganism is applied in this test. The agar and broth micro-dilution method [9

Various concentrations of nanoparticles with normal saline starting from 0.1wt % till 10wt% concentration. Nanoparticles were suspended in sterile normal saline and ultrasonicated for 5 minutes. [10]

Sterile test tube preparing the dilution of nanoparticles volumetrically in the brain heart infusion broth (BHI), 1 ml for each dilution was needed for each test tube.

An inoculum of Candida albicans equivalent to 0.5 on McFarland scale visualized, 0.1 ml of inoculum of C.albicans was added to each test tube. Positive control tube is included in the test it contains the inoculum and nutrient media without the nanoparticles. The negative

control consists of BHI broth with nanoparticles without the C.albicans.

The inoculated tubes were incubated at 37 C˚ for 24 hours in an incubator to allow the microorganism to grow. The tubes and agar plates were examined for growth and turbidity by the naked eye. (11)

The Agar well diffusion method:

The specimens were divided according to the type of nanoparticles into four groups: Varying concentrations (0.5%, 1%, 2%, and 5 wt%) of nanoparticles were accurately weighed in a container and then they were added to the water of mixing of alginate. (4)

Antimicrobial activity was measured using The Agar well diffusion method. The nanoparticles with the water was mixed with alginate powder according to the manufacturer's instructions. The mixed irreversible hydrocolloid material was inserted into the holes in the Mueller Hinton agar plates that contain the lawn cultures of the Candida albicans corresponding to 0.5 McFarland's standard and later they were incubated at 37C° for 24 hours. At the end of 24 hours, the zone diameter of inhibition was measured in mm (n=3). (12)

Each plate was examined after incubation. The diameter of zones of inhibition was measured with a digital calliper (accuracy 0.01mm), which was placed on the back of an inverted Petri dish that was illuminated with reflected light located few inches above a black, non-reflecting background. The zone margin is the area where there is no visible candida growth. The inhibition zone was measured in millimetres. [13]

RESULTS

the results of determining the lowest concentration (MIC) of nanoparticles that Candida albicans cannot grow are 0.2wt% for 4 magnesium oxide nanoparticles. 5wt% is the MIC for zirconium oxide nanoparticles These results are for 24 h incubation at 37C ˚, which is done visually and recorded.

MFC determination results:

The minimum Fungicidal concentration for magnesium oxide against Candida albicans was 0.3wt% and the MFC for zirconium oxide against Candida albicans was 10wt%.

Zone of inhibition:

Figure 1 shows the zone of inhibition (Agar well Diffusion Test) for the susceptibility of Candida albicans Isolate to alginate impression material incorporated with different concentrations of magnesium oxide nanoparticles. The magnesium oxide incorporated within irreversible hydrocolloids exhibited dose-dependent antimicrobial activity. The Zone of inhibition was observed in table 1, which represents the means and standard deviation of the antifungal effect of different concentrations of MgO nanoparticles incorporated within alginate

impression material, The values are represented in millimetres. One-way analysis of variance (ANOVA)as shown in Table 2 demonstrated that there was a significant difference at $P < 0.05$. The mean diameter and standard deviation of the zone of inhibition against the tested microorganisms observed with the control and irreversible hydrocolloids incorporated with magnesium oxide nanoparticles are presented in Figure 3.

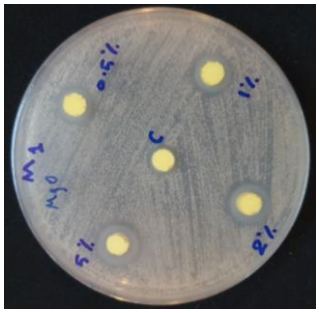

Figure (1): The Agar-Well diffusion test for the susceptibility of *Candida albicans* Isolate to alginate impression material incorporated with different concentrations of magnesium oxide nanoparticles.

Table (1): The zone of inhibition in millimeters of the antifungal effect of irreversible hydrocolloids incorporated with different conc. of magnesium oxide nanoparticles

Concentration	N	Mean	Std. Deviation
Control	3	7.0000	.00000
0.5 wt%	3	10.7067	.34020
1 wt%	3	12.0667	.34312
2 wt %	3	12.8567	.12503
5 wt %	3	13.6700	.09644

Table (2): One way analysis of variance (ANOVA) for comparison of different concentrations of magnesium oxide incorporated with alginate impression material against *Candida Albicans* isolate.

	Sum of squares	df	Mean Square	F	Sig.
Between Groups	82.386	4	20.596	398.538	.000*
Within Groups	.517	10	.052		
Total	82.903	14			

(*) significant difference ($p \leq 0.05$)

Figure (2): The Agar-Well diffusion test for the susceptibility of *Candida albicans* Isolate to alginate impression material incorporated with different concentrations of zirconium oxide nanoparticles.

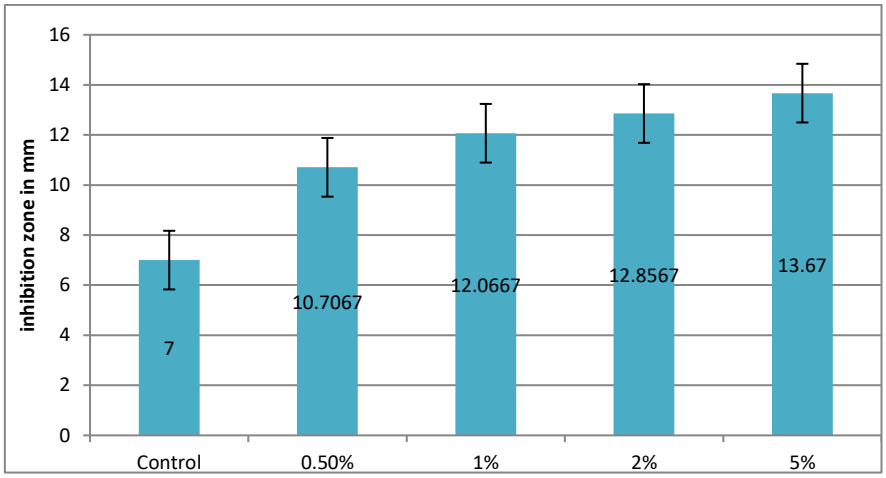

Figure (3): Duncan test for Antifungal Activity (Zone of Inhibition in Millimeters) of Different Concentrations of Magnesium Oxide Nanoparticles on *Candida albicans* Cells

Figure 2 shows the (Agar well Diffusion Test) for the susceptibility of Candida albicans Isolate to alginate impression material incorporated with different concentrations of zirconium oxide nanoparticles. The Zone of inhibition was observed in Table 3, which represent the means and standard deviation of the antifungal effect of different concentrations of zirconium oxide nanoparticles incorporated within alginate impression material. The values are represented in millimetres.The one-way analysis of variance ANOVA was shown in Table 4. for different concentrations of zirconium oxide incorporated with alginate impression material against Candida albicans isolate which was a significant difference at $P < 0.05$. The mean diameter and standard deviation of the zone of inhibition against the tested microorganisms observed with the control and 5 irreversible hydrocolloids incorporated with zirconium oxide nanoparticles are presented in Figure 4

Table (3): The zone of inhibition in millimetres of the antifungal effect of irreversible hydrocolloids incorporated with different concentrations of zirconium oxide nanoparticles

Concentration	N	Mean	Std. Deviation
Control	3	7.0000	.00000
0.5 wt %	3	7.0000	.00000
1 wt %	3	7.0000	.00000
2 wt %	3	7.0000	.00000
5 wt %	3	9.5633	.58244

Table (4): ANOVA for comparison of the zone of inhibition in millimetres of different concentrations of Zirconium oxide incorporated within impression material against *Candida albicans* isolate.

	Sum of square	df	Mean Squar	F	Sig.
Between Groups	15.770	4	3.942	58.108	.000*
Within Groups	.678	10	.068		
Total	16.448	14			

(*) significant difference (p ≤0.05)

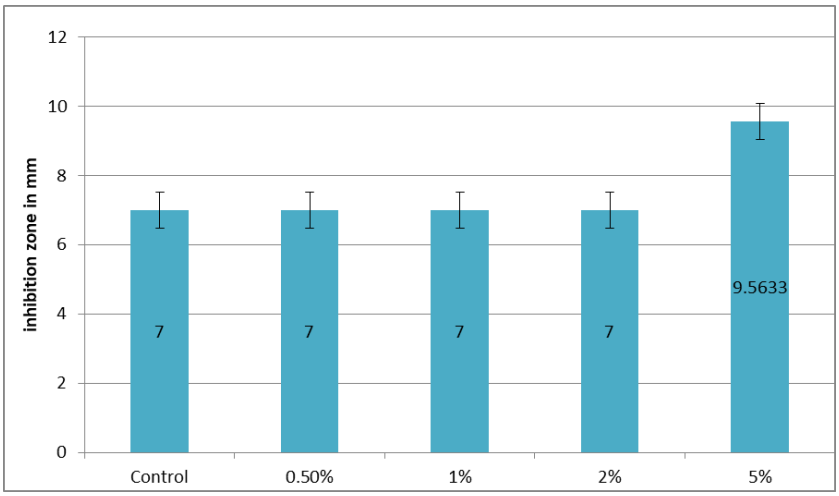

Figure (4): Duncan test for Antifungal Activity (Zone of Inhibition in Millimeters) of Different Concentrations of zirconium Oxide Nanoparticles on *Candida albicans* Cells

DISCUSSION

Various antimicrobial agents have been incorporated into alginate composition to self-disinfect the alginate without the need for spray or immersion disinfection. Among the antimicrobial agents, increased attention is being directed at nanoparticles. There are different studies about the effect of nanoparticles on Candida albicans, but the information about the antifungal effects of these nanoparticles is limited. The results from the microbiological tests demonstrated that the magnesium oxide was not only fungistatic but also fungicidal for Candida albicans. this study agrees with Kim et al. study that investigated the effects of silver nanoparticles on various Candida species. [14.]

Our study showed that magnesium oxide had better antifungal effect than zirconium oxide nanoparticles, this may be related to the apoptotic cell death in Candida. albicans by increasing of free radicals as had been induced by Some nanoparticles as silver.15 this result does not agree with results of Najafzadeh et al. 16 that concluded nano-MgO did not have antifungal effect against Candida albicans at in vitro condition. This could be attributed to different shape and size of nanoparticle which had been used in this study that influence the antimicrobial effects.[17]

In this study, the antimicrobial activity was measured using the Agar-Well diffusion method. For dental applications, Candida albicans is well known to be associated with fungal infections. This study has found that nanoparticles with different concentrations inhibit the growth of Candida albicans. As the concentration of nanoparticles increased the size of The inhibition zone also increased significantly. this agrees with a study done by Khaza'l 2020.18 Nanoparticles exhibit very high surface area and hence possess superior antimicrobial activity than macro-sized particles, Zirconium and magnesium oxide nanoparticles were found to exhibit fungicidal properties. In the present study, the addition of ZrO2-NP and MgO-NP to alginates was found to increase the antimicrobial activity against Candida 6 albicans. inhibition of fungal growth for ZrO2 was recorded at a concentration of 5wt%. Antifungal ability for the ZrO2 NPs may due to their lower size. The antimicrobial activity of ZrO2-NP and MgO-NP, like most metal-based nanoparticles, can be attributed to their ability for the disruption of microbial cell membrane along with damaging the DNA or inhibiting its replication and causes, cell death. [19, 20]

CONCLUSION

It was concluded that the incorporation of Magnesium oxide and Zirconium oxide nanoparticles into alginate impression material has significant antifungal activity against Candida albicans.

Ethical approval and consent to participate:

REC reference no. UoM.Dent /A.L.22/21

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