Research Article

Effect of adding titanium dioxide nanoparticles on anti-microbial activity and surface detail reproduction of dental alginate

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Abstract: Most dental works require a diagnostic impression; alginate is contemplated as the most popular material used for this purpose. Titanium dioxide nanoparticles show evidence of antimicrobial activity in the recent era, for this purpose, this study aimed to evaluate the effect of adding Titanium dioxide nanoparticles on antimicrobial activity and surface detail reproduction of alginate impression material. Materials and methods: Titanium dioxide nanoparticles (purity = 99%, size= 20nm) was added to alginate at three different concentrations (2%, 3% and 5%). 84 samples were prepared in total. Samples were tested for antimicrobial activity using a disc diffusion test, and surface detail reproduction was done using (ISO 21563:2021). One-way ANOVA and independent sample t-test were used for data analysis through SPSS software. Results: for the antimicrobial test, inhibition zones for *Streptococcus mutans* and *Candida albicans* showed significant changes concerning the alteration in Titanium dioxide nanoparticle concentrations. The inhibition zone significantly increased with an increase in the percentage of Titanium dioxide nanoparticles. The mean of the inhibition zone for *S. mutans* was superior to *C. albicans* and the difference was statistically significant. Regarding surface detail reproduction, the control group, 2% and 3% groups manifested very similar results, only the group to which 5% of Titanium dioxide nanoparticles were added showed a decline in detail reproduction when compared to the other three groups. Conclusion: Within the limitation of this study, we can conclude that the antimicrobial activity against *S mutans* and *C. albicans* were significantly increased in modified groups, and this escalation was directly linked to the increase in Titanium dioxide nanoparticles concentration. In contrast, the surface detail reproduction was decreased when adding 5% Titanium dioxide nanoparticles to alginate.

Keywords: *alginate, surface detail, nanoparticle, candida albicans.*

Introduction

Dental impression is a negative replication of hard and soft tissues in the mouth from which a positive reproduction (dental cast) can be formed[1].

Alginate is a biomaterial from a family of irreversible hydrocolloid that has served dental practice for almost century[2].They were the first elastic impression material to be used in dentistry that provided high detail even under the presence of undercuts[3]. Being economical, easy to manipulate, and better tolerance

by the patient has put alginate in the utmost utilized material in the field of dentistry in contrast to other impression materials such as silicon[4].

All types of irreversible hydrocolloids have a hydrophilic nature making them susceptible to microbial retention[5]. It is a well-known fact that the human oral cavity is a favourable host to many microbial agents, during the impression-making procedure the oral cavity fluid could adhere to the impression materials[6]. Therefore, they may increase the susceptibility of cross infection[7]. To overcome this point, many disinfection methods have been used such as spraying and immersing. Unfortunately, both methods are time-consuming and may compromise some of the mechanical properties of the alginate[8].

During the last decade, the use of nanoparticles has become prevalent in the design and development of many dental materials since they can provide a unique combination of properties[9]. Due to the small size of the nanoparticles, they can provide a high surface area to volume ratio compared to particles of the same material[10]. This property gives them great attention in the present century as they possess defined mechanical, chemical, and optical properties crafting them into a suitable candidate for various applications[11].

Many studies proved that nanoparticles could control the formation of biofilms as they possess biocidal and anti-adhesive properties. For this purpose, silver, copper, zinc, magnesium, titanium, and their oxides have been used as antimicrobial agents in many dental materials[12].

It was proven that TiO_2 NPs possess good antibacterial activity against *S. mutans*[13], without deteriorating the mechanical and physical properties[13-15].

Due to their imperfect properties, alginate remains an active material for research. The purpose of this study is therefore to evaluate the antimicrobial property of dental alginate incorporated with TiO_2NPs against *S. mutans* and *C. albicans*, in addition to the surface detail reproduction after this modification.

Materials and Methods

To confirm the identity of the planned TiO_2NPs for use in this study, X-ray Diffraction (XRD) analysis was performed before starting sample preparations.

X-ray diffraction is a powerful non-destructive analytical method that is used to determine the structure and composition of unknown nanomaterials[16,17].

XRD test was performed using PANalytical X'pert powder (Figure 1.a) with Cu-Kα x-ray source, a wavelength of λ=1.54060$\dot{A}$ was used. The TiO_2NPs were deposited on to the specimen holder (Figure 1. b) and packed using a glass slide. The NPs inside the sample holder were loaded into the XRD machine and diffraction data was recorded at 2θ range from 10° to 79.9950° with step size 0.0100° per 0.5s. Low scan speed was elected to provide higher sensitivity for the recognition of impurities[18].

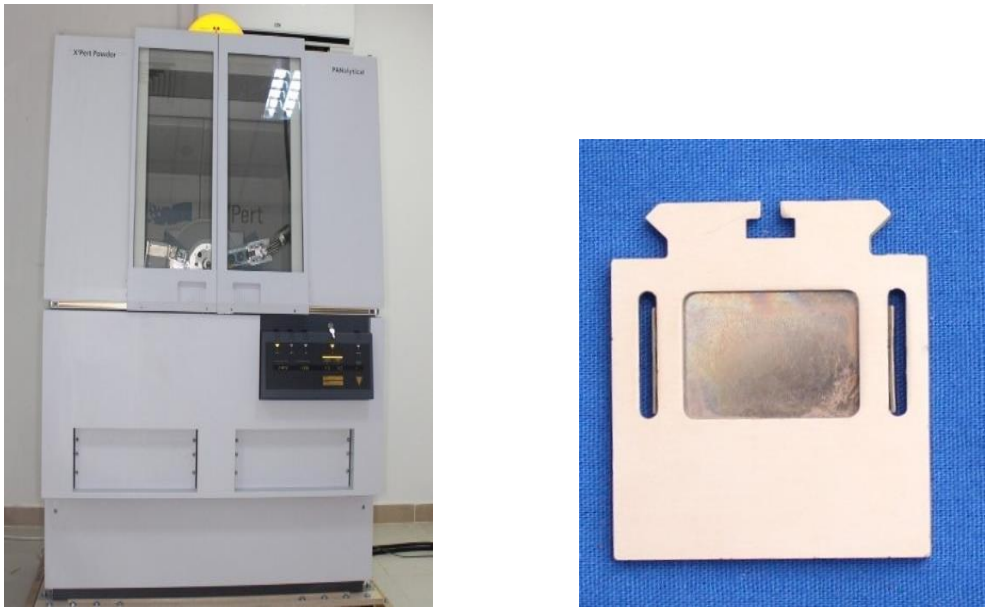

Figure 1: a) PANalytical X'pert powder b) specimen holder

The PANalytical software was used to compare X-ray XRD patterns to identify the NPs. The result of the analysis identified the sample as TiO_2 and its diffraction pattern are shown in figure (2):

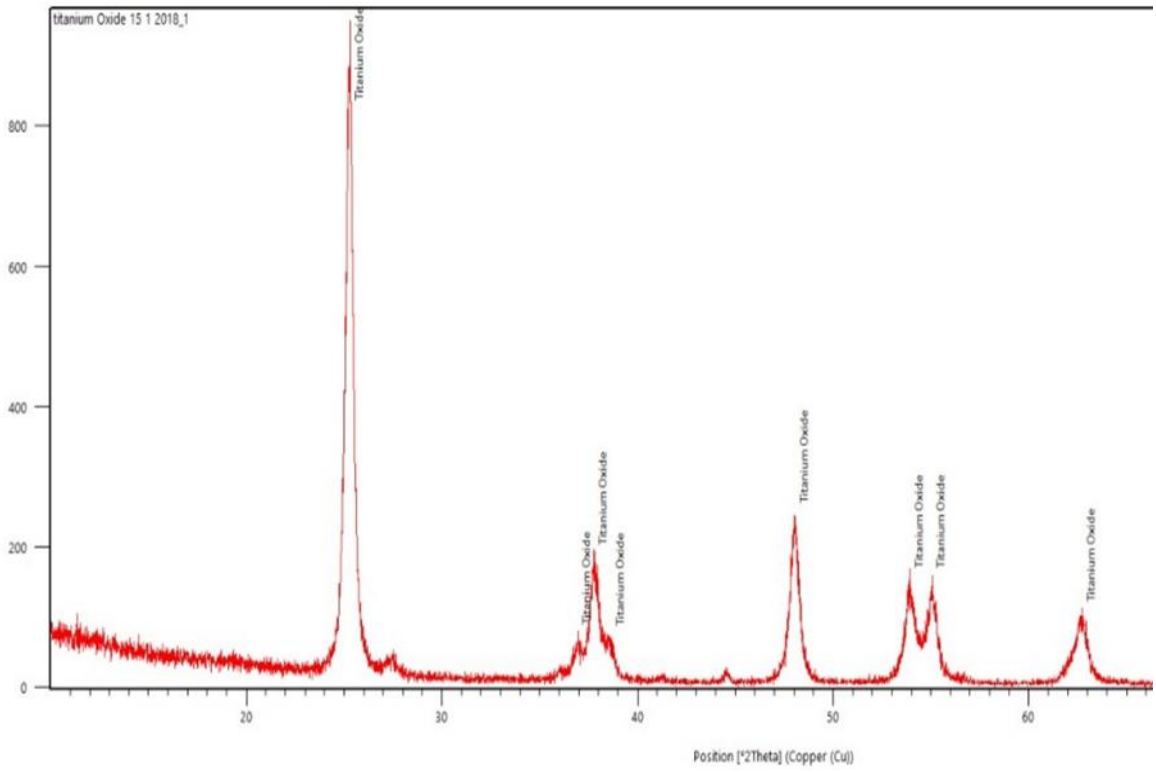

Figure 2: XRD pattern for Titanium oxide nanoparticles

After confirming the identity of the chosen TiO_2NPs, a pilot study was done by FTIR analysis to reveal any possible chemical structure changes (alteration of functional groups) after adding (2%, 3% and 5%) of TiO_2NPs to the alginate, control and modified groups were analysed by the FTIR spectroscopy (Shi-

madzu 8400, Japan). The result (Figure 3) provided a clear clue that the in cooperation of TiO_2NPs at all concentrations doesn't induce any significant change in the main functional groups' structure for the (SI-O-SI group, C-O-C group, O-H group and C-H group) which was present in the unmodified alginate as the stretching and bending of the peaks did not alter after the addition of TiO_2NPs.

The only detected change was the increase in the percentage of IR transmittance which was detected only in the 5% TiO_2NPs group, indicating the weakening of the bonds between the alginate molecules.

Similar to the control group, sharp, strong peaks at 619.21 cm^{-1}, 669.83 cm^{-1} and 793.54 cm^{-1} for SI-O-SI bands, also the peak at 1078.81 cm^{-1} for C-O-C bands were observed in all modified groups indicating that the added TiO_2NPs does not interact with the available structural bonds in the alginate, this finding agrees with Skocaj et al [19] who stated that TiO_2NPs considered as a chemically inert material.

The weak sharp bending peak of the O-H group at 1621.88 cm^{-1} was very similar in control, 2% TiO_2NPs and 3% TiO_2NPs as the IR transmittance located at the same levels, but for the 5% group although the bands located at the same wavelength level but, the IR transmittance increased which is an indication that the higher TiO_2NPs concentration might cause weakening of these bands due to the agglomeration of the TiO_2NPs[20].

The weak stretch peak of the C-H group at 2924.61 cm^{-1}, also the strong stretch peak of the O-H group at 3421.89 cm^{-1} and 3527.51 cm^{-1} for the control, 2% TiO_2NPs and 3% TiO_2NPs groups was almost identical but again there was a difference in the IR transmittance rate in which for the 5% TiO_2NPs group was 80%, while for the former three groups at about 50%, this might be due to formation of small gaps between these molecular groups band which ultimately caused the bonds to become weaker[20] thus the IR easily penetrated the samples contained a higher percentage of 5%TiO_2 NPs.

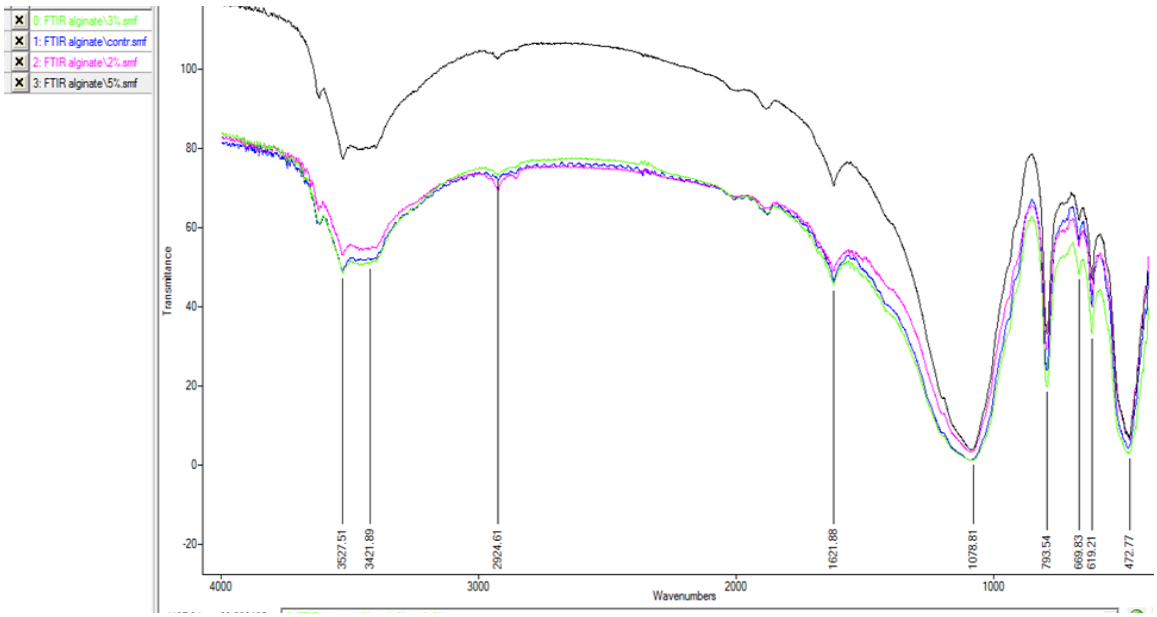

Figure 3: FTIR analysis of Alginate (Blue =Control, Pink=2%, Green =3%, Black= 5%)

Study design and sample preparation

In the present study, 84 samples were prepared from Alginplus (Major- ISO 21563. Italy) extra high precision alginate impression material. Antimicrobial activity against *S. mutans* (n=28) and *C. albicans* (n=28) in addition to surface detail reproduction (n=28) were tested. One control group and three modified groups to which (2%, 3% and 5%) spheric shaped TiO_2NPs were added respectively to the alginate have been studied, each group consisting of seven samples.

Digital electronic balance (OHAUS GmbH- Switzerland) with precise accuracy of 0.0001 mg was used to weigh the alginate powder and the amount of TiO_2NPs powder.

The samples were prepared by mixing the blend of both powders (Alginate and TiO_2NPs) with a premeasured volume of distilled water as recommended by the manufacturer by using an automatic alginate mixer (Cavex- Netherlands) for 10 seconds.

Antimicrobial test

A disc diffusion test was used to investigate the antimicrobial activity released from the tested alginate specimens. For this purpose, two main oral pathogens namely *S. mutans* ATCC 25175 and *C. albicans* ATCC 10231 yeasts were chosen.

The *S. mutans* bacteria were cultivated on blood agar media. The culture media was prepared according to the recommended protocol for *S. mutans*. Seven Petri dishes were used, and in each petri dish, four specimens were placed at equal distances from each other, marked with numbers 1, 2, 3 and 4 representing the control, 2%, 3% and 5% groups respectively and incubated aerobically at 37°C for 24 hours.

For the *C. albicans* ATCC 10231, Sabouraud Dextrose Agar was used for growing and cultivation[21]. The protocols from Antifungal susceptibility testing of the National Committee for Clinical Laboratory Standards (NCCLS) and Manual of Antimicrobial Susceptibility Testing were followed[22,23]. The seven Petri dishes containing samples were incubated for 24 hours.

The measurement of inhibition zones for both pathogens was performed using scientific image analysis known as Image J software[24].

After completion of 24 hours incubation, the Petri dishes were taken out from the incubator, and images were taken at 90º with a reference of a ruler for calibration of image J software. Inhibition zones were measured around the ingots at six different positions.

For the entire procedure, the working environment was conditioned under Bio air Top safe with continuous air ventilation and a Bunsen burner that was turned on near the working field to prevent contamination of the testing components by airborne pollutants[25.26].

Surface detail reproduction

A stainless-steel die with three scribed parallel lines ISO 21563: 2021[27] is used for surface detail reproduction. The widths of these lines were 20-µm, 50-µm and 75-µm respectively. A stainless-steel ring was

placed on top of the steel die; the mixed alginate was poured inside the ring over the testing mould. A glass slab was then placed on top of the ring and a one-kilogram weight was positioned upon the slab for 10 minutes. Then, the samples were carefully removed from the mould and immediately examined with a digital microscope UM012C (5M 300X with 8 LEDs- China). Prior to the measurement procedure, the microscope eyepieces lens was calibrated for precise measurements of the samples.

Specimens were reported to either pass (1) or fail (0) the test based on their ability to capture the entire length of the scribed 20-um line over the full length of 25mm distance between the cross line[27,28]. The surfaces were assessed according to the ranking system established by Owen[29] which are:

Score 1: Line reproduced clearly and sharply over the entire length between the marks.

Score 2: Line clear over more than 50% of length, or line indistinct over less than 50% of length, the line appears to be reproduced well over the entire length, but not sharply.

Score 3: Line clear over less than 50% of length, line indistinct over more than 50% of length, or line visible over entire length but blemished not sharp.

Score 4: Line is not reproduced over the entire length; rough, blemished, pitted.

Statistical analysis

Statistical Package for Social Sciences (SPSS, version 23.0) and Microsoft Office Excel were used for statistical analysis. Descriptive statistics for frequency, mean, and standard deviation. Student T-test was used for comparisons between two independent groups, in addition to One-way ANOVA and post hoc test for multiple comparisons. The $p<0.05$ value was considered statistically significant.

Results

The obtained results from the antimicrobial test showed that the control group exhibited the least antimicrobial activity; furthermore, it was observed that the inhibitory effect against both pathogens was directly linked to the increase in the concentration of TiO_2NPs as shown in Figure (4)

The inhibitory effect of the modified alginate against *S. mutans* was more dominant compared to *C. albicans* which revealed higher resistance to the added TiO_2NPs at the same concentrations.

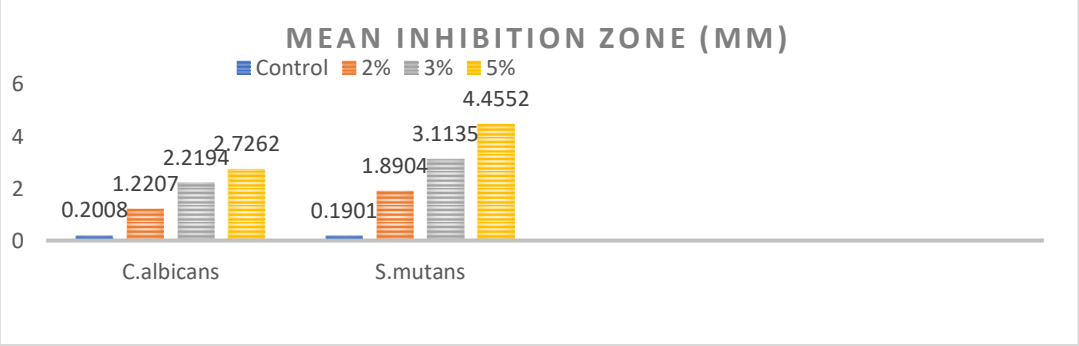

Figure 4: Mean inhibition zone for *S. mutans* and *C.albicans*

For *S. mutans*, the results of One-way ANOVA revealed a statistically significant difference ($p < 0.05$) in the inhibition zone measurements as shown in Table (1).

Table 1: One-way ANOVA test for *S.mutans*

Group	N	Mean+SD	95% CI for mean		F	Sig
			Lower band	Upper band		P-Value
Control	7	0.19014 (0.00661)	0.18402	0.19626	534.957	0.000
2%TiO_2NPs	7	1.89043 (0.12906)	1.77106	2.00979		
3%TiO_2NPs	7	3.11357 (0.37468)	2.76704	3.46010		
5%TiO_2NPs	7	4.45529 (0.12333)	4.34122	4.56935		
Total	28	2.41236 (1.61205)	1.78727	3.03745		

Post hoc (LSD) test for multiple comparisons depicted a statistically significantly different between the groups in such a way that the added TiO_2NPs improved the antibacterial activity of the used alginate in all used concentrations as shown in Table (2).

Table 2: Post hoc test (LSD- multiple comparisons) for *S. mutans*

Group I	**Group J**	**Mean Difference (I-J)**	**95% CI for mean**		**Sig**
			Lower band	**Upper band**	**P-value**
Control	**2% Tio_2NPs**	-1.700286*	-1.92925	-1.47132	0.000
	3% Tio_2NPs	-2.923429*	-3.15239	-2.69446	0.000
	5% Tio_2NPs	-4.265143*	-4.49411	-4.03618	0.000
2% Tio_2NPs	**3% Tio_2NPs**	-1.223143*	-1.45211	-0.99418	0.000
	5% Tio_2NPs	-2.564857*	-2.79382	-2.33589	0.000
3% Tio_2NPs	**5% Tio_2NPs**	-1.341714*	-1.57068	-1.11275	0.000

Regarding *C. albicans*, the obtained results were identical to the results of *S. mutans* as there was a statistically highly significant increase in the antifungal activity of the alginate in the modified groups. The inhibition zone was more dominant at the highest percentage (5%) of used TiO_2NPs compared to the 2% and 3% groups. The control group possessed a minimum inhibition zone as shown in Table (3).

Table 3: One-way ANOVA test for *C. albicans*

Group	**N**	**Mean+SD**	**95% CI for mean**		**F**	**Sig**
			Lower band	**upper band**		**P-value**
Control	7	0.20086 (0.00470)	0.19651	0.20521	926.760	0.000
2% Tio_2NPs	7	1.22071 (0.06380)	1.16170	1.27973		
3% Tio_2NPs	7	2.21943(0.14397)	2.08627	2.35258		
5% Tio_2NPs	7	2.72629 (0.11391)	2.62093	2.83164		
Total	28	1.59182 (0.99070)	1.20766	1.97598		

Post hoc test for multiple comparisons between the groups showed the presence of highly significant difference ($p < 0.001$) between the control group and the other groups as well as between the groups themselves as shown in table (4).

Table 4: Post hoc test (LSD- multiple comparisons) for *S.mutans C.albicans*

Group I	Group J	Mean Difference (I-J)	95% CI for mean		Sig
			Lower band	Upper band	P-value
Control	**2% Tio_2NPs**	-1.019857*	-1.12710	-0.91261	0.000
	3% Tio_2NPs	-2.018571*	-2.12582	-1.91133	0.000
	5% Tio_2NPs	-2.525429*	-2.63267	-2.41818	0.000
2% Tio_2NPs	**3% Tio_2NPs**	-0.998714*	-1.10596	-0.89147	0.000
	5% Tio_2NPs	-1.505571*	-1.61282	-1.39833	0.000
3% Tio_2NPs	**5% Tio_2NPs**	-0.506857*	-0.61410	-0.39961	0.000

An Independent sample t-test was used to evaluate whether *S. mutans* and *C. albicans* differ significantly in their inhibition zone. The result specified that the mean of the inhibition zone for *S. mutans* and *C. albicans* was statistically significant as shown in Table (5). The means indicated that *S. mutans* (M = 2.41236, SD = 1.61205) showed significantly more inhibition zone than *C. albicans* (M=1.5917, SD=0.99070).

Table 5: Independed Student T-test for *S. mutans* and *C. albicans*.

	Levene's Test for Equality of Variances		t-test for Equality of Means				
	F	Sig.	t	df	Sig.(2 tailed)	Mean Difference	Std. Error Difference
Equal variances assumed.	8.204	0.006	2.295	54	0.026	0.820536	0.357582
Equal variances not assumed			2.295	44.849	0.026	0.820536	0.357582

Surface detail reproduction

The alginate used in this study is branded as an irreversible hydrocolloid material that satisfies ISO 21563. All the tested groups efficiently and sharply recorded the 75-μm line in the entire length thus satisfying Owen's score 1. Regarding 50-μm line, the control group, 2% and 3% groups reproduced that line on the alginate samples surface with Owens score 2 except for 5% TiO_2NPs group that fall into Owens score 3.

As mentioned previously, due to extra high quality of the used alginate in this study, the reproduction of the 20-um line was selected and considered as the base line for comparison between the groups. The group that was altered by addition of 5% of TiO_2NPs failed to record the 20-μm line (Figure 5), while the remaining three groups reproduced 20-μm line and this ability is considered as an equivalent to the detail reproduction of the addition silicones according to ISO specifications 4823[30].

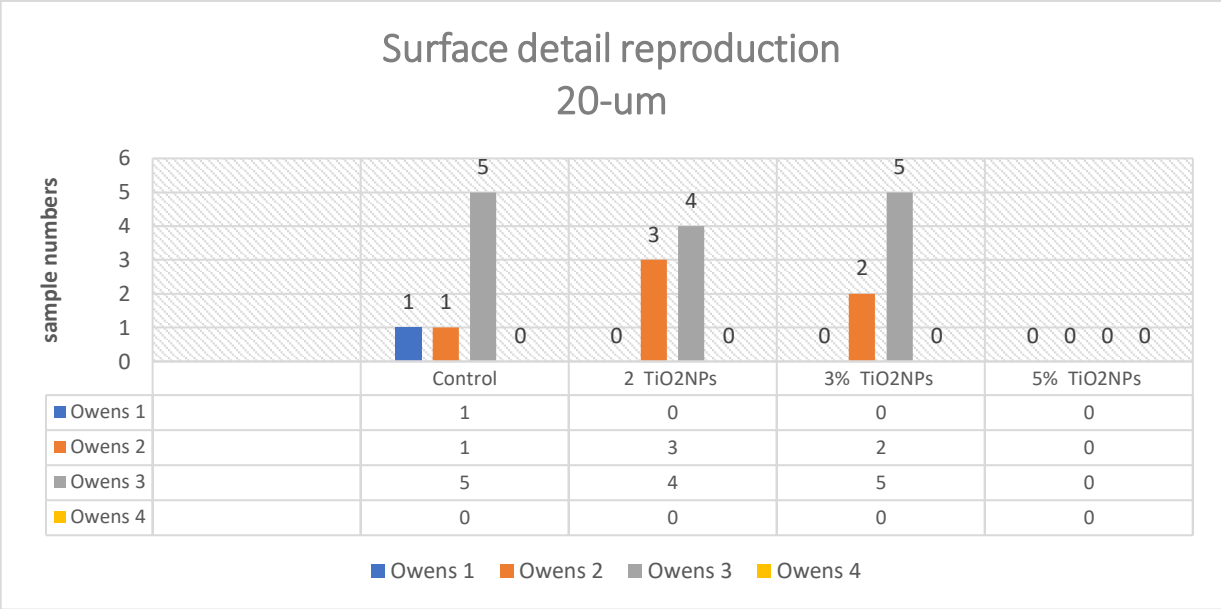

Figure 5: Surface detail reproduction

Discussion

Infection control is a fundamental procedure in dental practice. It is documented that there are about 750 million microorganisms in only 1 mL of the saliva of a healthy person[31].

According to many researchers, spherical-shaped nanoparticles with sizes 15-50nm exhibit maximum antimicrobial properties[32]. Due to this, spheric-shaped 20 nm TiO_2NPs were chosen for this study. The result of the inhibition zone for *S. mutans* showed a significant increase when the percentage of TiO_2NPs increased, a similar finding was obtained in a study by Al-Hawezi[13] when TiO2NPs were in cooperated into a flowable dental composite resin and agreed with the result obtained in studies done for testing the effect of silver nanoparticles on *S. mutans*[33,34].

The antibacterial activity of TiO_2NPs is practically due to a reaction of the high surface energy TiO_2NPs with water. TiO_2NPs release free radicals which are considered a potent oxidizing agent (Reactive oxygen species) that ultimately destroy the cell membrane[35] or alternatively, in the absence of light, direct contact and adsorption of cells onto TiO2NPs may cause a loss of bacterial cell membrane[36].

Additionally, reports in the literature have shown that electrostatic attraction plays a great role in the bactericidal effect of the material[37]. This attraction probably overcomes other factors, such as the size and shape of NPs which can influence bacterial cell death[38].

The antifungal effect of TiO2NPs against *C. albicnas* was obvious in the modified groups when compared to control group, this finding agrees with results of a study[39] who found that up to 65% of the *C. albicans* were killed after exposure to 100 µg/mL of TiO2NPs. A similar results was concluded with of Kermani et al[40] who found that higher percentage of the titanium and zinc oxide nanoparticles increased their toxicity.

It was documented that TiO2NPs cause *C. albicans* yeast cell death by producing intracellular reactive oxygen species (ROS), this in turn causes oxidation of the Coenzyme-A and peroxidation of lipids which subsequently decreases respiratory activity and ultimately causes cell death[41]. Another explanation for the antifungal activity of TiO2NPs, is the tear of the fungi cell membrane that disturb its integrity, causing loss of intracellular substances [42].

Impression-making is a routine in the dental practice, for this purpose, a variety of impression materials are available to capture oral cavity structures, the final decision for the selection of these products is usually based on the required type of dental treatment and clinician's preference[43].

Surface detail reproduction is considered fundamental criteria for any irreversible hydrocolloid material, the latest ISO 21563 and ADA specification 18 is used as a standard protocol for measuring this property.

The results of the present study were similar to another study [44] when they found no adverse effect of incorporating up to 1000 ppm of silver nanoparticle on the surface detail reproduction of alginate. This could be the result that the TiO_2NPs were small (20nm) thus the particles were evenly distributed within the alginate matrix and did not influence the intermolecular bond. In addition, these nanoparticles are considered an inert material and do not induce any chemical structure alteration, this fact was supported by the FTIR analysis results.

At 5% TiO_2NPs, caused deterioration of the surface detail reproduction and it was impossible to record the 20-μm line, this may be due to agglomeration of the used TiO_2NPs inside the alginate matrix because of their high surface energy [45], this in turn triggered a poor intermolecular bond.

According to obtained data, the requirements were met for irreversible hydrocolloid material as the tested groups reproduced the 75-μm and 50-μm groove which is considered satisfactory for alginate impression materials[,46.]

Conclusion

Within the limitation of this study, we can conclude that the addition of TiO2NPs to alginate improved the antimicrobial activity significantly. TiO2NPs are more powerful against *S. mutans* at the same used concentration. The addition of TiO_2NPs doesn't compromise the ISO 21563 requirement for surface detail reproduction.

Conflict of interest: None**.**

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المستخلص:

الخلفية: معظم أعمال طب الأسنان تتطلب انطباعًا تشخيصيًا ،الجينات هي المادة المستخدمة الأكثر شيوعًا لهذا الغرض. تظهر الجسيمات النانوية لثاني أكسيد التيتانيوم دليلاً على نشاط مضادات الميكروبات في العصر الحديث ، ولهذا الغرض تهدف هذه الدراسة إلى تقييم تأثير إضافة **TiO2NPs** على النشاط المضاد للميكروبات ونسخ تفاصيل السطح لمادة الانطباع الجينات.

تمت إضافة **TiO2NPs** (نقاء = 99٪ ، الحجم = 20 نانومتر) إلى الجينات بثلاث تركيزات مختلفة (2٪ ، 3٪ و 5٪). تم تحضير 84 عينة في المجموع. تم اختبار عينات النشاط المضاد للميكروبات باستخدام اختبار انتشار القرص ، وتم اختباراستنساخ تفاصيل السطح باستخدام **ISO 21563: 2021**.

لتحليل البيانات تم استخدام **ANOVA** أحادي الاتجاه واختبار **t** المستقل من خلال برنامج **SPSS**.

بالنسبة لاختبار مضادات الميكروبات ،أظهرت مناطق التثبيط للمكورات العقدية الطافرة والمبيضات تغيرات معنوية ذا علاقة بالتغير في تركيزات **TiO2NPs** ،وزادت منطقة التثبيط معنويا مع زيادة نسبة **TiO2NPs**. كان متوسط منطقة التثبيط لـ **S. mutans** أعلى من **C. albicans** وكان الاختلاف ذا دلالة إحصائية. فيما يتعلق باستنساخ تفاصيل السطح ، أظهرت المجموعة الضابطة، 2٪ و 3٪ نتائج متشابهة جدًا ، فقط المجموعة التي تمت إضافة 5٪ من **TiO2NPs** إليها أظهرت انخفاضًا في استنساخ التفاصيل عند مقارنتها بالمجموعات الثلاث الأخرى.

الخلاصة: ضمن حدود هذه الدراسة ، يمكننا أن نستنتج أن نشاط مضادات الميكروبات ضد **S mutans** و **C. albicans** قد زاد بشكل كبير في المجموعات المعدلة ، وكانت هذه الزيادة مرتبطة بشكل مباشر بزيادة تركيز **TiO2NPs**. في المقابل ، تم تقليل استنساخ تفاصيل السطح عند إضافة 5 ٪ **TiO2NPs** إلى الجينات.