

Original Article

Antimicrobial Effect of Titanium Oxide (TiO₂) Nano Particles in Completely Edentulous Patients. A Randomized Clinical Trial

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Submitted: 17-8-2021

Accepted: 22-10-2021

Abstract

Objective: to investigate the effect of addition different concentration of TiO₂ nanoparticles on acrylic resin polymethyl methacrylate to improve the anti-microbial properties of denture base material

Subjects and Methods: 24 completely edentulous patients were selected to be enrolled in of the following groups: Group I: 8 patients were rehabilitated with complete dentures had no nanoparticle (Control group), Group II: 8 patients were rehabilitated with complete dentures had a 1% concentration of titanium oxide nanoparticles and Group III: 8 patients were rehabilitated with complete dentures had a 3% concentration of titanium oxide nanoparticles. Microbiological evaluation was done from the fitting surface of dentures after one week, two weeks, and one month of denture use.

Results: Regarding staphylococcus aureus and candida albicans colonization, the result revealed that between one week, two weeks, and one month follow up periods for each group and between different groups for each follow up period, there was a statistically significant difference between all groups. Regarding staphylococcus epidermis colonization, there was negative colonization of staphylococcus epidermis.

Conclusion: Addition of TiO₂ nano particles with 1% and 3% concentrations to PMMA has a great anti-microbial effect with reduction of the colonization of *S. aureus*, *S. epidermis* and *C. albicans* than the conventional dentures

Key words: complete denture, Titanium Oxide (TiO₂), anti-microbial effect

Introduction

Several people have been affected by teeth loss which causes physiological and functional disorders, so rehabilitation treatments with a satisfactory prosthesis are indicated (Rosa et al., 2012)

Acrylic resin polymethyl methacrylate (PMMA) has been the most prevalent material for the construction of dentures for several decades as it has many advantages as good aesthetics, perfect fit, stability in the oral environment, easy

laboratory and clinical management, and cheap equipment's (Nejatian et al., 2006)

Though, this material has some disadvantages, which still show problematic in some clinical and prosthodontic practices, regarding to the surface properties, as surface roughness, obvious porosity that can act as a reservoir for microorganisms (Budtz-Jørgensen, 1981)

Denture stomatitis is a disease associated with *Candida albicans*, and it is a common repetitive problem for complete-denture users.

The early attachment and adhesion of *Candida albicans* to the surface of a denture base is a necessary step in the colonization and pathogenesis that causes Denture stomatitis. (Pattanaik et al., 2010; Zomorodian et al., 2011)

Thus, several attempts have been made to reduce *Candida albicans* adhesion and consequent colonization on the denture base during the use of a variety of antifungal agents, however this treatment method has proven to be a short-term therapy and not always effective. (Izumida et al., 2014)

Lately, much attention has been pointed toward the incorporation of nanoparticles into PMMA to enhance its properties. (Gad et al., 2017)

Nano-sized materials have contributed to progressive applications in the field of nanomedicine and biomedical sciences due to their wonderful physical and chemical properties. Among nanoparticles are metal oxides that are precious not only due to their wide variety of physical and chemical properties but additionally due to their antibacterial effect. The chemical reactivity and surface energy of nanoparticles show a central role in describing the antibacterial and antifungal properties. (Wang et al., 2010)

Between several nanoparticles constituents, TiO₂ nanoparticles are widely used due to their non-toxic, chemically inert, and little cost, high refractive index, antibacterial property under a variety of spectrum, corrosion resistant and high hardness. (Reijnders, 2009)

Literature has also presented that nanoscale TiO₂ reinforcement agents brings recent optical, electrical, physiochemical properties attained at very low TiO₂ concentration, that makes polymer-TiO₂ nanocomposites an encouraging new class of materials. (Chatterjee, 2010b, 2010a)

The integration of TiO₂ Nanoparticles to PMMA decreases the porosity of the denture bases. This finding shown the suitability of metal

oxide Nanoparticles as additives for the improvement of PMMA formulations as high porosities increases the growth of *Candida* hyphae which is accountable for denture stomatitis (Shirkavad & Moslehifard, 2014)

This aim of this study was to investigate the effect of addition different concentration of TiO₂ nanoparticles on acrylic resin polymethyl methacrylate (PMMA) to improve the antimicrobial properties of denture base material

Subjects and Methods

The present study was conducted in removable Prosthodontics department Faculty of Oral and Dental Medicine, Kafr Elsheikh University, Egypt. The participants of the present clinical study composed of 24 completely edentulous. All patients have been rehabilitated with complete dentures. All patients approved with the trial protocol and signed a voluntary consent agreement. This trial was agreed by the ethical committee of the Faculty of Dentistry, Kafr Elsheikh University with approval number of (KD/20/21)

Sample size calculation

Sample size calculated depending on a previous study (Gomaa et al., 2019) as reference. According to this study, the response within each subject group was normally distributed with standard deviation 0.79. If the true difference in the experimental and control means is 1.41, minimally the study needed 5 subjects in each group to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. Total sample size increased to minimally 8 subjects per group to compensate 20 % drop out. So, Total sample size = 24 patients

The Patients were randomly classified into three groups eight patients for each group

according to the form and concentration of nanoparticles within the denture base, Group I : Denture had no nanoparticle (Control group), Group II : Denture had a 1% concentration of titanium oxide nanoparticles and Group III : Denture had a 3% concentration of titanium oxide nanoparticles. Participants were requested to pick-up their number from opaque, sealed and sequentially numbered envelopes which contained computer generated random number (RANDOM.ORG) to identify the group he/she belonged.

Age of the patients varied between 45 and 66 years with average age of 54 years, patients were free from any systemic disease that may influence the salivary flow or soft tissue of the oral cavity, different infectious disease, or history of antibiotic treatment for the last 3 months. Heavy smoker patients or patients with para-functional habits were rejected.

Construction of complete dentures: Mandibular and maxillary primary impressions were made by using irreversible hydrocolloid impression material (Chromopan-lascod B.A. sestoflorentino Firenze, Italy). Final impressions were made from zinc oxide and eugenol impression material (Cavex outline B.V., Holland). Then, Impressions were boxed and poured in dental stone (Zeus dental stone hard type, Italy) to gain definitive casts on which occlusion blocks were constructed . After adjusting maxillary occlusion rim, a maxillary face bow record (Whip Mix #8645 quick mount. Louisville, K.Y., USA) was adjusted to mount the maxillary cast on a semi-adjustable articulator (Whip Mix #8500 Semi-adjustable articulator, Louisville, K.Y., USA). Centric occluding relations were recorded by using wax wafer technique to mount the mandibular cast. Artificial teeth were set by using cross-linked acrylic resin teeth (Acrylic teeth, Cosmo MEA, Dentsply-USA). Before , flasking and packing of heat cure acrylic resin , it was modified according to the group of each patient was ,as they were randomly

classified into three groups eight patients for each according to the form and concentration of nanoparticles within the denture base, Group I : Denture had no nanoparticle (Control group), Group II : Denture had a 1% concentration of titanium oxide nanoparticles and Group III : Denture had a 3% concentration of titanium oxide nanoparticles.

The nanoparticles were made in a private laboratory (Nanogate Laboratory, Cairo, Egypt). The common size of the nanoparticles was < 20 nm with a spherical form. The structure of the nanoparticle was established using a high-resolution transmission electron microscope (JEM-2100, Jeol, Akishima, Japan) and X-ray diffraction examination by using a powder diffractometer system (X'pertPro- Panalytical, Malvern, United Kingdom). Furthermore, The nanoparticles of titanium oxide were added to the monomer of the acrylic resin by using volume concentration (v/v%).

All patients were given similar post-insertion instructions and a regular oral and denture hygiene protocol through the study.

Microbiological evaluation was done by using a sterile cotton swabs to collect saliva from the fitting surface of maxillary dentures and palatal tissues , and the mandibular dentures and the buccal vestibule figure(1) . Samples were obtained in the fore noon hours between 10 and 12 O'clock letting patients to use their dentures for a couple of hours preceding the collection of swabs.

Procedure of sample collection(Green & Goldman, 2021): every patient had instructed to use Betadine mouth wash 1% concentration with 20 ml. rinsing for 30 sec.(Shin & Nam, 2018), an area of about 1cm. square dimension was swabbed for 30 seconds for the vestibular and palatal tissues and denture fitting surfaces of each patient figure (2) . A rotating movement with the swab held securely and laterally against the denture for three times with the same swab to confirm proper adherence of the plaque. The

similar process was applied for the vestibular and palatal tissues.

This step repeated for each patient 3 times after one week, after two weeks and after one month of delivery of complete dentures. The denture and tissues were swabbed as stated before. Swabs were directly transferred to commercially obtainable sterile swab holder and preserved in ice box for less than 2 hours before plating.

Procedures of samples preparation and bacterial counting (ISO 4833, 2003) : Quantification of the bacterial colonies was made by the number of Colony Forming Units per ml. (CFUs) which was assessed by the following technique: the swab tip of every sample was cut by sterile scissor and sited in 9 ml. of sterile 1% buffered peptone water and completely mixed for 30 sec. by using vortex to prepare culture homogenate (initial dilutions). This dilution was consecutively diluted into additional decimal (tenth fold) dilutions till fourth dilution. For calculating the total bacterial count, plate count agar petri dishes were inoculated in duplicate by dispersion with 0.1ml. from every dilution. Incubation was made for 72 hours at 30°C. Plates with fewer than 3 colonies were counted negative for enumeration (plates that had 3 to 30 colonies only are to be stated). Entire bacterial count (TBC) was assessed as follows according to the ISO protocol (ISO.18593, 2004):

TBC = Colonies count at last positive plate X 10 X dilution (10n).

Procedures of bacterial identification (AOAC, 2000): Various colonies shapes from positive plates were isolated and transferred to distinct sterile buffered peptone water 1% test tube and incubated at 30°C. For 12 hours, for productivity. Loopful is removed from the peptone test tubes and distributed by streaking over specific media surface (EMB. Eosin Methylene Blue, Rose Bengal Agar and blood agar media) to recognize the isolated micro-

organisms figure (3) . Therefore, the isolated colonies were assessed and recognized according to their morphology (ISO.4832, 2006a, 2006b; ISO.16649-2, 2001). The present study was a double blinded study for patients and investigator.

All complete dentures were constructed in the same prosthodontic laboratory, by the same technician making standardized finishing and polishing methods.

For each patient, the samples were collected from both maxillary and mandibular denture and mucosa

Microbiological count data Collected data were obtained through laboratory tests staphylococcus aureus, staphylococcus epidermis and candida albicans colonization. Data were statistically analyzed by Microsoft Excel[®] 2016¹, Statistical Package for Social Science (SPSS)[®] Ver. 24². and Minitab³ statistical software Ver. 16. CFU data were revealed as mean and standard deviation which were logarithmically transformed into parametric data, with a significance level (p<0.05).

Results:

Regarding staphylococcus aureus colonization after one week, mean ± standard deviation of group (I), group (II) and group (III) were (2.29±0.01114), (2.551±0.1467) and (2.505±0.1123) respectively, as listed in table (1) and showed in figure (4).

While after two weeks, mean ± standard deviation of group (I), group (II) and group (III) were (2.161±0.01498), (2.353±0.1794) and (2.676±0.04098) respectively, as listed in table (1) and showed in figure (4).

Finally after one month, mean ± standard deviation of group (I), group (II) and group (III) were (1.984±0.01436), (2.277±0.03765) and (2.828±0.05448) respectively, as listed in table (1) and showed in figure (4).

¹ Microsoft Cooperation, USA.

² IBM Product, USA.

³ Minitab LLC, USA.



Figure (1): **A:** swabbing of the fitting surface of maxillary denture, **B:** swabbing of the fitting surface of mandibular denture



Figure (2): **A:** swabbing of the palatal tissues, **B:** swabbing of the mandibular vestibular tissues

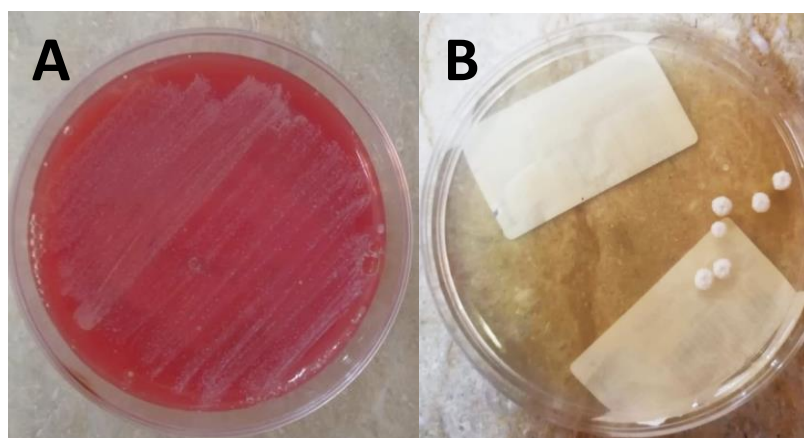


Figure (3): **A:** bacterial growth of staphylococcus aureus for 3% TiO₂ NPs group , **B:** growth of candida albicans for 3% TiO₂ NPs group

Using One Way Analysis of Variance (One Way ANOVA) followed by Tukey`s post hoc test for

multiple comparisons among different follow up period for each group and between different

groups for each follow up period, there was significant difference between all groups as P-value < 0.05, as listed in table (1).

Regarding staphylococcus epidermis colonization

After one week, mean \pm standard deviation group (III) were (2.501 \pm 0.1004) respectively.

While after two weeks, mean \pm standard deviation of group (III) were (2.666 \pm 0.04325) respectively.

Finally after one month, mean \pm standard deviation of group (III) were (2.821 \pm 0.05218) respectively .

For group (I) and group (II), it was revealed that there was negative colonization of staphylococcus epidermis.

Using One Way Analysis of Variance (One Way ANOVA) followed by Tukey`s post hoc test for multiple comparisons among different follow up period for group (III), there was significant difference between all groups as P-value < 0.05.

Regarding candida albicans colonization

After one week, mean \pm standard deviation of group (I), group (II) and group (III) were (1.236 \pm 0.1191), (1.78 \pm 0.07748) and (2.149 \pm 0.2625) respectively, as listed in table (2) and showed in figure (5). While after two weeks, mean \pm standard deviation of group (I), group (II) and group (III) were (1.05 \pm 0.0844), (1.617 \pm 0.1187) and (2.292 \pm 0.1845) respectively, as listed in table (2) and showed in figure (5).

Finally after one month, mean \pm standard deviation of group (I), group (II) and group (III) were (0.7757 \pm 0.05177), (1.344 \pm 0.07344) and (2.413 \pm 0.1515) respectively, as listed in table (2) and showed in figure (5).

Using One Way Analysis of Variance (One Way ANOVA) followed by Tukey`s post hoc test for multiple comparisons among different follow up period for each group and between different

groups for each follow up period, there was significant difference between all groups as P-value < 0.05, as listed in table (2).

Discussion

The oral cavity of healthful people with or without teeth may be inhabited by yeast and bacteria coexisting in a relationship of commensalism. Many factors may govern a rise in the number of microorganisms in the mouth, which can improve colonization and allow them to become pathogenic. Among the predisposing factors are denture-wearing and poor denture hygiene (Dàistan et al., 2009). So, The aim of this study was to investigate the effect of addition different concentration of TiO₂ nanoparticles on acrylic resin polymethyl methacrylate (PMMA) to improve the anti-microbial properties of denture base material

In the present study, the tiO₂ was used only in the form of two concentrations 1% and 3% according to Venkatesh Anehosurand Kulkarni (Venkatesh Anehosur & D. Kulkarni, 2012)who reported that TiO₂ nanoparticles had inhibitory activity against *S. aureus*. But more effective inhibitory activity against *S. aureus* detected at 3 w% concentration of nanoparticles proved anatase form of TiO₂ to be a better material. Both 3 w% and 5 w% concentrations of nanoparticles permitted similar results, 3 w% concentration of TiO₂ was preferred (AKITA. et al., 2005)

The samples of the present study was collected after one week, two weeks, and one month follow up periods for each group as (Sharma et al , 2016) were reported that the frequency of isolation of gram negative bacilli was little at 24hrs, it increased considerably at 1-week from both the denture surface and tissues surface

Table (1): Descriptive and Comparative Study of Staphylococcus Aureus Colonization among One Month Follow Up

	N	3 % TiO2 NPs		1 % TiO2 NPs		Conventional Heat Cured Acrylic Resin		P-value
		M (log10)	SD	M (log10)	SD	M (log10)	SD	
After One Week	8	2.290 ^{Aa}	0.01114	2.551 ^{Ab}	0.1467	2.505 ^{Ab}	0.1123	<0.0001*
After Two Weeks	8	2.161 ^{Ba}	0.01498	2.353 ^{Bb}	0.1794	2.676 ^{Bc}	0.04098	<0.0001*
After One Month	8	1.984 ^{Ca}	0.01436	2.277 ^{Bb}	0.03765	2.828 ^{Cc}	0.05448	<0.0001*
P-value		<0.0001*		0.0209*		<0.0001*		

N; Number, M; Mean, SD; Standard Deviation, P; Probability Level

Means with same (UPPERCASE) superscript letter in the same column were insignificant different using Tukey's post hoc test

Means with different (UPPERCASE) superscript letter in the same column were significant different using Tukey's post hoc test

Means with same (lowercase) superscript letter in the same row were insignificant different using Tukey's post hoc test

Means with different (lowercase) superscript letter in the same row were significant different using Tukey's post hoc test

*Significant Different

Table (2): Descriptive and Comparative Study of Candida Albicans Colonization among One Month Follow Up

	N	3 % TiO2 NPs		1 % TiO2 NPs		Conventional Heat Cured Acrylic Resin		P-value
		M (log10)	SD	M (log10)	SD	M (log10)	SD	
After One Week	8	1.236 ^{Aa}	0.1191	1.780 ^{Ab}	0.07748	2.149 ^{Ac}	0.2625	<0.0001*
After Two Weeks	8	1.050 ^{Ba}	0.08440	1.617 ^{Bb}	0.1187	2.292 ^{Ac}	0.1845	<0.0001*
After One Month	8	0.7757 ^{Ca}	0.05177	1.344 ^{Cb}	0.07344	2.413 ^{Bc}	0.1515	<0.0001*
P-value		<0.0001*		<0.0001*		0.0029*		

N; Number, M; Mean, SD; Standard Deviation, P; Probability Level

Means with same (UPPERCASE) superscript letter in the same column were insignificant different using Tukey's post hoc test

Means with different (UPPERCASE) superscript letter in the same column were significant different using Tukey's post hoc test

Means with same (lowercase) superscript letter in the same row were insignificant different using Tukey's post hoc test

Means with different (lowercase) superscript letter in the same row were significant different using Tukey's post hoc test

*Significant Different

Regarding staphylococcus aureus and candida albicans colonization, the result of the present study revealed that between one week, two weeks, and one month follow up periods for each group and between different groups for each follow up period, there was a statistically

significant difference between all groups as P-value < 0.05, as the . These results of this study agreed with Ribeiro et al (Ribeiro et al., 2012) who Documented that Candida spp., Strep. mutants and Staph. aureus on complete dentures belongings to 90 patients.

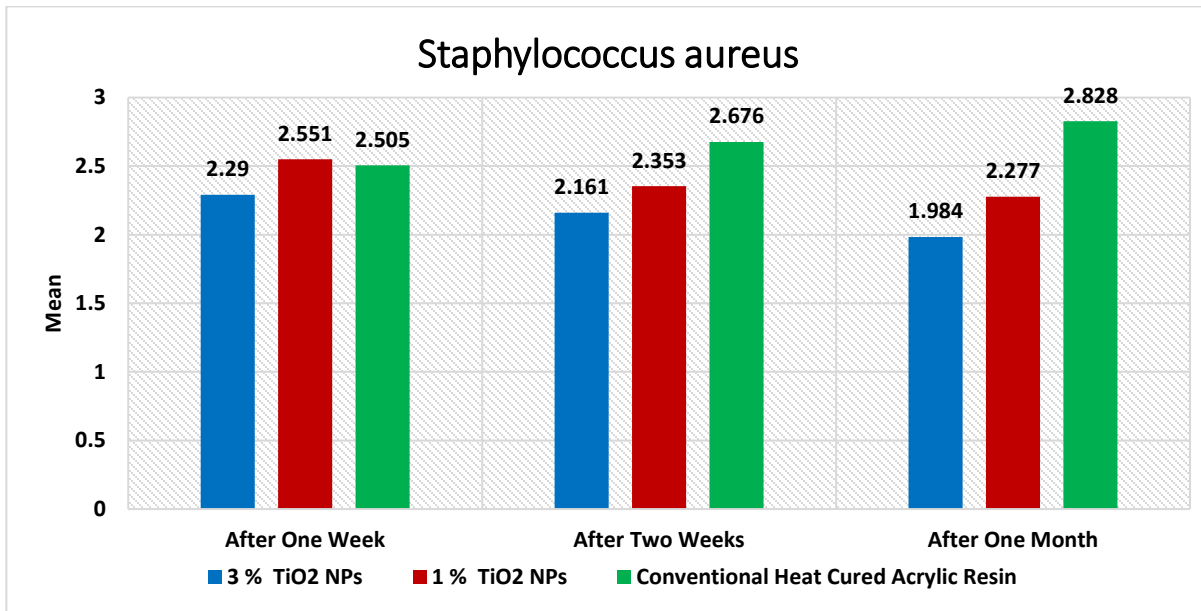


Figure (4): Bar Chart revealing Staphylococcus Aureus Colonization among One Month Follow Up

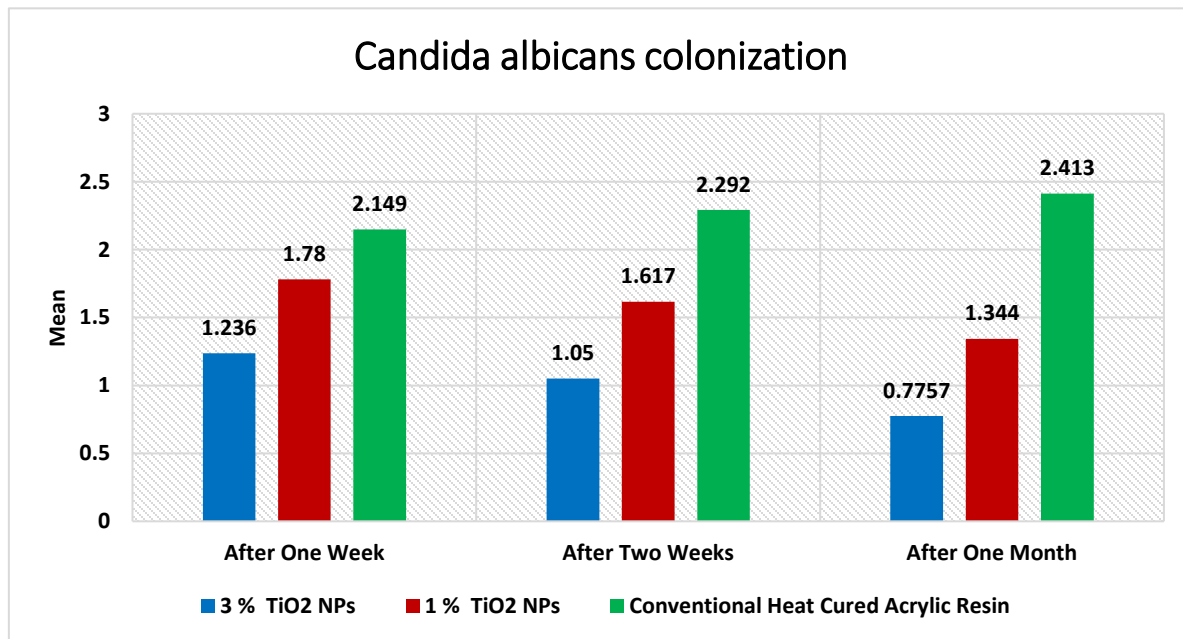


Figure (5): Bar Chart revealing Candida Albicans Colonization among One Month Follow

Candida species were found to be the most frequent microorganisms on the dentures (65.5%), while *Strep. mutans* and *Staph. aureus* was discovered on 53.3 and 34.4% of them.

In addition, the results of the present agree with those reported by Baena- Monroy et al.(Baena-Monroy et al., 2005), who verified the presence of *C. albicans*, *Staph. aureus* and *Streptococcus mutans* on the inner surface of complete dentures. In their study, *C. albicans* was isolated in 66.7% of the dentures, while *Staph. aureus* and *Strep. mutans* were separated from 49.5% of the dentures.

In the present study, The results showed that the addition of TiO₂ NPs 1% and 3% with good proportion in the PMMA had a wide effect on the reduction the colonization of *S. aureus*, *S. epidermis* and *C. albicans* than the conventional dentures this result compatible with Alrahlah et al (Alrahlah et al., 2018) who resulted that the addition of TiO₂ NPs also improved the antimicrobial behavior of PMMA denture bases by reducing their bacterial adherence ability, confirming the practical significance of the addition of TiO₂ NPs. And also the addition of TiO₂ NPs with an adequate distribution in the PMMA had a wide effect on the reduction and removal of toxic or hazardous pollutants and can be used as an antibacterial in dentistry.

Moreover , the results of present study were comparable to Natarajan et al who discovered that TiO₂ acts as a photo-catalyst has been used extensively for destroying different groups of microorganisms including bacteria, fungi and viruses(Natarajan et al., 2016) Also The synthesised TiO₂ nanotubes used in this study indicated powerful antibacterial and antifungal properties against all required species, generation of reactive oxygen species (ROS) such as •OH, •O⁻, •HO and H₂O due to the photocatalytic properties of titanium oversees the disintegration of microorganisms.(Naji et al., 2018) .Furthermore, the attachment of the TiO₂ nanotubes to the cell membrane of species may

affect and upset the permeability of the cells, induce oxidative stresses, and inhibit cell growth(Gong et al., 2012)

Conclusion

Addition of TiO₂ nano particles with 1% and 3% concentrations to PMMA has a great anti-microbial effect with reduction of the colonization of *S. aureus*, *S. epidermis* and *C. albicans* than the conventional dentures .With increasing the concentration of TiO₂ nano particles , the anti-microbial effect will increase

Recommendations

Further clinical studies with increasing sample size are recommended. in addition, further clinical studies on immunocompromises patients are recommended, as those patients are suffering from reducing immunity which accompanied by increasing colonization of bacteria and fungi.

According to the results of the present study, in cases with immunocompromised patients , the use of TiO₂ nano particles with different concentrations may reduce mortality rate in post covid 19 immunocompromised completely edentulous patients who wearing complete denture as result of Mucormycosis which may occurs due to colonization of bacteria and fungi on different surfaces of denture

Conflict of Interest:

This work was self-funded and the authors declare no conflict of interest.

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