AJMAS
https://alqalam.utripoli.edu.ly/science/ eISSN 2707-7179

Original article

Effect of Benzalkonium Chloride as Antibacterial Activity on Dental Composites Containing Nano-Titanium dioxide Particles: *in vitro* study.

Atbayga A[1], Abdelaaziz M[2*]

[1] Department of Dental Technology, Faculty of Medical Technology, Bani Walid University, Libya.
[2] Department of Dental Technology, Faculty of Health Sciences, Sirte University, Libya.

ARTICLE INFO

DOI: 10.5281/zenodo.3938687

*__Abdelaaziz M__: Department of Dental Technology, Faculty of Health Sciences, Sirte University, Libya.
Mobile phone: (+218) 917722112.
Email: muftahali@su.edu.ly

Received: 16-5-2020
Accepted: 25-5-2020

Keywords: Titanium oxide (TiO_2), Antimicrobial activity, Benzalkonium Chloride (BzCl).

ABSTRACT

Aim: *The objective of this in vitro study was to evaluate the antimicrobial activity of resin-based dental composites containing Nano-Titanium dioxide Particles that were set with benzalkonium chloride (BzCl).* ***Methods:*** *TiO_2 nanoparticles were treated with (3-mercaptopropyl) trimethoxysilane (MPTMS). Three groups of resin-based dental composites were prepared, namely: Group 1 (an untreated TiO_2 nano-composite), Group 2 (treated TiO_2 nano-composite) and Group 3 (treated TiO_2 nano-composite was combined with three concentrations of benzalkonium chloride (BzCl). Five bacteria were selected and tested against the composite nano-TiO_2 particles, namely, Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Enterococcus faecalis (E. faecalis), and Pseudomonas aeruginosa (P. aeruginosa), were grown on Müller-Hinton Agar (MHA), and Streptococcus mutans (S. mutans) was grown on Brain Heart Infusion (BHI) agar.* ***Results:*** *The results of the present study showed that neither of the groups of TiO_2 nano-composites, (untreated TiO_2 nano-composite and treated TiO_2 nano-composite) exhibited antimicrobial activity against the pathogens. Only preparations of TiO_2 nano-composites at a concentration of 3 %m/m of BzCl showed antimicrobial activity against S. aureus. Antimicrobial activity against S. mutans, E. coli, P. aeruginosa, E. faecalis and S. aureus, were only realized at a concentration of 10 %m/m for BzCl.* ***Conclusions:*** *Dental composites nano-TiO_2 particles prepared and provided with antibacterial substances such as BzCl may prevent the growth of bacteria and may be beneficial as an additive in dental restorations.*

Cite this article: *Atbayga A, Abdelaaziz M. Effect of Benzalkonium Chloride as Antibacterial Activity on Dental Composites Containing Nano-Titanium dioxide Particles: in vitro study. Alq J Med App Sci. 2020;3(2)1-6.*

INTRODUCTION

Resin composites use in the dental field is still more prone to failure compared to amalgam [1,2]. The two most common reasons for resin composite failures are secondary caries and fractures [3,4]. Pereira-Cenci et al, in their review study, indicated that secondary caries might be the cause of more than 55% of resin composite replacements [5]. It occurs at the border of an existing restoration, which defined as positively diagnosed carious lesion [6]. On the other hand, it is usually accepted that it is a primary carious lesion of teeth at the margin of a replacements, but it appears after period of time from placement the restoration [6,7]. In contrast to the remaining carious, that is cause by imperfect removal of infected tissues during the preparation of the teeth [6]. Often associated with the presence of secondary caries leakage microprobe caused by various effects [8,9], which might be the cause of the occurrence of chemicals and liquids, and finally between the restoration and bacteria [10,11]. Irrespective of doubts about the reasons of caries after placing restorative, it recognized that it is a serious and common clinical problem. Additionally, composites accumulate more than plaques and biofilms from another direct dental replacement [12]. For this reason, it is supposed to be that good resin composite materials must not only have proper esthetic and mechanical properties but also should have antibacterial properties to avoid migration of the replacement teeth interface by pathogenic bacteria, such as Streptococcus mutans (*S. mutans*) [13,14].

Łukomska-Szyma et al, concluded that resin composites containing calcium fluoride as filler reinforced had presented a significant reduction of L. acidophilus and *S. mutans*, which were perhaps relate with creating hydrofluoric acid, which may inhibit enzymes and generate acidification of cytoplasm after penetrating the bacteria membrane [10]. Nowadays the application of nanomaterials to resin composites is consider as one of the most important advances in dental materials field. TiO_2 nanoparticles and their application has been widely used in many medical fields and other sciences because of its antimicrobial properties.

Haghi et al, evaluated the antimicrobial effect of TiO_2 NPs on pathogenic strain of E. coli and indicated that the TiO_2 NPs cause little pores in bacterial cell walls, thereby increasing the permeability and cell death [15]. The objective of this in vitro study is to investigate the antimicrobial activity of composites resin containing nano-TiO_2 particles prepared with benzalkonium chloride.

MATERIALS AND METHODS

Materials

TiO_2 of 80 nm particles size purchased from Evonik, Krefeld, Germany. UDMA, CQ, DMAEMA, MPTMS, BzCl as a solid. The bacteria obtained from Davies Diagnostics, Johannesburg, South Africa.

Preparation of resin composite

The treatment of TiO_2 nano-composite was performed by mixing MPTMS in xylene in the presence of 2% (wt/v) n-propylamine used as catalyst and then nano-TiO_2 was mixed with MPTMS by adding 2.5% m/v of MPTMS to 1 g of nano-TiO_2. The composite mixture was prepared by adding 0.5 gm of each CQ as an initiator and DMAEMA as an accelerator to UDMA. Antimicrobial agent BzCl added during the mixing of the composite. The mixture was then casted into a plastic template, light-cured until solidification reached and then sterilized by alcohol. BzCl incorporated into the mixture of the composite in different concentrations, and left until well molded. TiO_2 nano-composites then divided into four groups, as each concentration was added separately, for instance, 3% (m/m), 5% (m/m), 7% (m/m) and 10% (m/m) of BzCl respectively. In this study, the TiO_2 nano-composite prepared in different ways, introduced in this *in vitro* study for evaluating their antimicrobial activities, were prepared following manufacturers' instructions and mention as follows:

- Untreated TiO_2 nano-composite.
- Treated TiO_2 nano-composite.
- Treated TiO_2 nano-composite mixed with three concentrations of BzCl, 3% (m/m), 5% (m/m), 7% (m/m) and 10% (m/m) separately.

Table: 1. *Summary of the synthesis of the TiO_2 nano-composite materials*

	Nano-TiO_2	MPTMS	Benzalkonium chloride (BzCl)
1	10%	None	None
2	10%	Treated	None
3	10%	Treated	3%
4	10%	Treated	None
5	10%	Treated	5%
6	10%	Treated	None
7	10%	Treated	7%
8	10%	Treated	None
9	10%	Treated	10%
10	10%	Treated	None

Selection of the medium and bacteria

Facultative bacteria used in this *in vitro* study were *Streptococcus mutans* ATCC 25175, *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 12600. These selected bacteria were chosen because they considered as standard for antibacterial tests. A standard Gram- stain performed on all cultures and these found to be pure. Two types of commonly used media used with the selected bacteria to evaluate the antimicrobial activity of the TiO_2 nano-composites. BHI only used with *S. mutans* and MHA used with the other bacteria, *E. coli*, *S. aureus*, *E. faecalis* and *P. aeruginosa*. *S. mutans* is a difficult bacterium to grow, therefore, it had to be grown in a different medium such as BHI Agar, and because *S. mutans* needs to grow in a CO_2. CO_2 only used with *S. mutans* and not with the other bacteria, because incubation in a CO_2 enriched atmosphere is not recommend because of its pH effect on the medium.

Methods

All samples performed in triplicate and equidistantly in each plate and then the experiments divided into four groups corresponding to the nano-composite materials used in this study. Each group contained one of the TiO_2 nano-composites and tested *in vitro* for its antimicrobial activity separately against each one of the five strains respectively. For instance, untreated TiO_2 nano-composite was cultivated with each bacterium separately and incubated for 24 hours (overnight) at 37°C. Treated TiO_2 nano- composite was cultivated with each bacterium for 24 hours at 37°C. Treated TiO_2 nano-composite incorporated with concentration of 3% (m/m) of BzCl was cultivated with each one of the bacteria separately for 24 hours at 37°C, and repeated using treated TiO_2 nano-composite with concentration of 5% (m/m), 7% (m/m) and then with 10% (m/m) of BzCl respectively. All the nano-composite specimens tested against all the bacteria independently by following the same procedures except *S. mutans* exposed to 5% CO_2 inside a CO_2 water-jacketed incubator for 48 hours at 37°C.

In this study, all tests performed in triplicate, including the preparation of the nano-composite materials in order to confirm the obtained findings. This was qualitative descriptive study and statistical analysis was therefore not applicable.

RESULTS AND DISCUSSION

The obtained results showed that untreated TiO_2 nano-composite and treated TiO_2 nano-composite had no antibacterial activity (Fig. 1, 2).

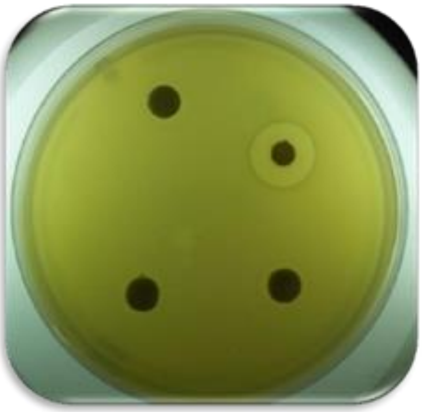

Figure: 1. *Antimicrobial activity of untreated TiO_2 nano-composite tested against S. mutans. It is evident that the untreated TiO_2 does not possess antimicrobial activity.*

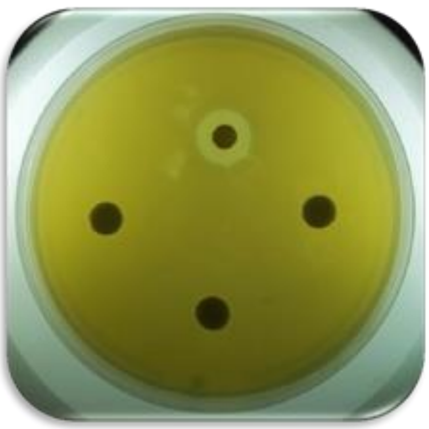

Figure: 2. *Antimicrobial activity of treated TiO_2 nano-composite tested against S. mutans. It is evident that the treated TiO_2 does not possess antimicrobial activity.*

Treated TiO_2 nano-composite contained 10% (m/m) BzCl gave antibacterial inhibition zones (Fig. 3-7) produced significant degrees of antibacterial effectiveness against *S. mutans*, *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus*. This confirmed that BzCl effective antimicrobial substances when they are added to the nano-composites. Furthermore, the inhibition zones clearly observed around all the treated nano- composite specimens, except with *P. aeruginosa* (Fig. 5).

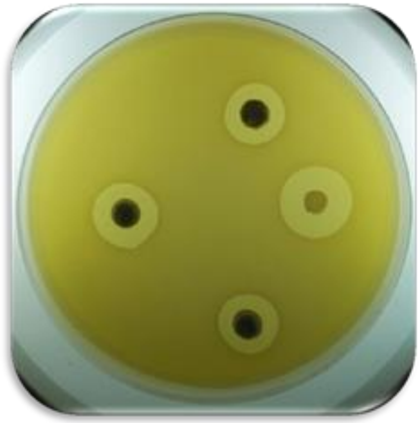

Figure: 3. *Antimicrobial activity of a preparation of treated TiO_2 nano-composite composite with 10% (m/m) BzCl against E. coli. It is evident that the preparation possesses antimicrobial activity.*

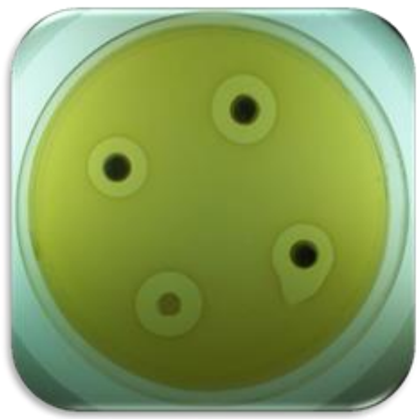

Figure: 4. *Antimicrobial activity of a preparation of treated TiO_2 nano-composite composite with 10% (m/m) BzCl tested against E. faecalis. It is evident that the preparation possesses antimicrobial activity.*

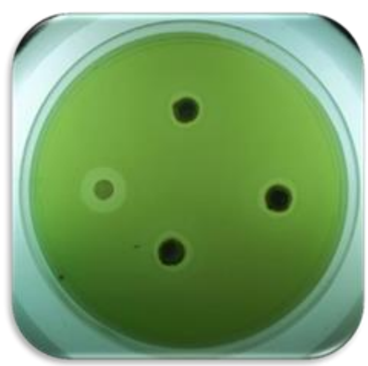

Figure: 5. *Antimicrobial activity of a preparation of treated TiO_2 nano-composite composite with 10% (m/m) BzCl tested against P. aeruginosa. It is evident that the preparation possesses antimicrobial activity.*

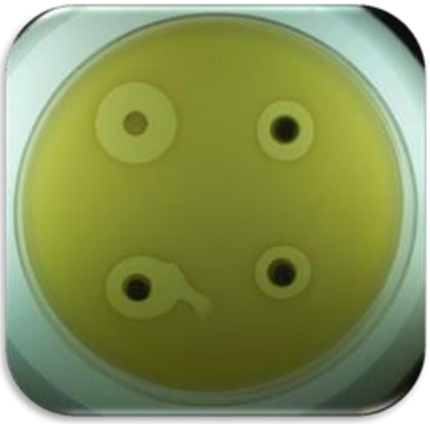

Figure: 6. *Antimicrobial activity of a preparation of treated TiO_2 nano-composite composite with 10% (m/m) BzCl tested against S. aureus. It is evident that the preparation possesses antimicrobial activity.*

The latter showed a small inhibition zone because it resists the influence of the antibacterial substances However, antibacterial substances, BzCl clearly appeared an antibacterial effectiveness at concentrations of 10% (m/m). The only bacteria that showed a resistance at 10% (m/m) is *P. aeruginosa*, which confirms that this type of bacteria could easily accumulate on the restorative materials on teeth inside the oral cavity. However, treated TiO_2 nano-composites containing 3% (m/m) of BzCl showed no microbial inhibition at all. Moreover, treated TiO_2 nano-composites that contained 5% (m/m) concentration of BzCl could not resist the bacterial growth and the bacteria easily grown except with *S. aureus*, which produced small inhibition halos around the nano-composites (Fig. 8). Also, with a concentration of 7% (m/m) of BzCl, no difference could be observed and the results were almost the same as for the concentration of 5% (m/m).

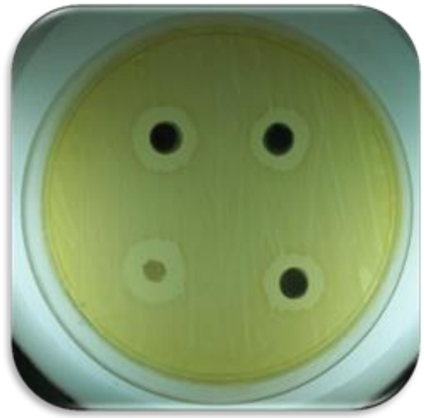

Figure: 7. *Antimicrobial activity of a preparation of treated TiO_2 nano-composite composite with 10% (m/m) BzCl tested against S. mutans. It is evident that the preparation possesses antimicrobial activity.*

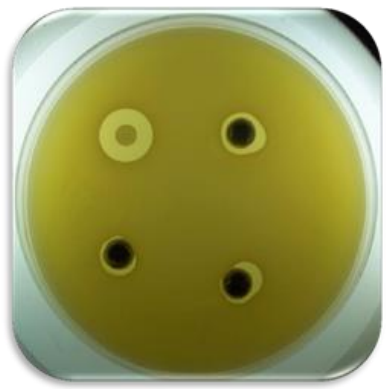

Figure: 8. *Antimicrobial activity of a preparation of treated TiO_2 nano-composite composite with 5% (m/m) BzCl tested against S. aureus. It is evident that the preparation possesses antimicrobial activity.*

In contrast, although treated TiO_2 nano-composites contained 10% (m/m) BzCl concentration were antibacterial resins. Moreover, although treated TiO_2 nano-composites contained 5% (m/m) of the same antibacterial agent inhibited the bacterial growth with one of the bacteria. The untreated TiO_2 nano-composites and treated TiO_2 nano-composites without the antimicrobial additives did not inhibit the bacterial growth of any of the bacteria tested and had no significant antimicrobial activities at all. Moreover, these dental nano- composites had no ability to resist the bacterial impacts. This study carried out the confirmation of using BzCl as antimicrobial substances when added to TiO_2 in order to eliminate the occurrence of secondary caries. This is, in agreement with literature, which carried out that, the microbicidal ability of TiO_2 nano particles depends on the improvement of antimicrobial activity, which should enhance the killing of bacteria, viruses and fungi [16].

CONCLUSION

The addition of certain concentrations of BzCl antibacterial substances increased the antibacterial activity without changing the physical handling properties of these nano-composites. Lower concentrations of BzCl incorporated into the nano-materials, for instance, 3% (m/m), and they all gave negative results in all the experiments and the bacteria grown easily. Furthermore, the incorporation of 5% and 7% (m/m) concentration of antibacterial substance BzCl also gave negative results except with S. aureus, for which it was positive and there were clear inhibition zones around the nano-composites. With addition of 10% (m/m) it showed positive results as clear inhibition zones appeared around the TiO_2 nano-composites and killed the bacteria.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

REFERENCES

[1] Rasines Alcaraz MG, Veitz-Keenan A, Sahrmann P, Schmidlin PR, Davis D, Iheozor-Ejiofor Z. Direct composite resin fillings versus amalgam fillings for permanent or adult posterior teeth. Cochrane Database Syst Rev. 2014;3.

[2] Hurst D. Amalgam or composite fillings--which material lasts longer?. Evid Based Dent. 2014;15(2):50 - 51.

[3] Demarco FF, Corrêa MB, Cenci MS, Moraes RR, Opdam NJ. Longevity of posterior composite restorations: not only a matter of materials. Dent Mater. 2012;28(1):87-101.

[4] Opdam NJ, van de Sande FH, Bronkhorst E, et al. Longevity of posterior composite restorations: a systematic review and meta-analysis. J Dent Res. 2014;93(10):943-949.

[5] Pereira-Cenci T, Cenci MS, Fedorowicz Z, Azevedo M. Antibacterial agents in composite restorations for the prevention of dental caries. Cochrane Database Syst Rev. 2013.

[6] Lai, G.; Li, M. Secondary Caries. In Contemporary Approach to Dental Caries; InTech. 2012, 403–422. ISBN 978-9-53-510305-9.

[7] Mjör IA, Toffenetti F. Secondary caries: a literature review with case reports. Quintessence Int. 2000;31(3):165-179.

[8] Shih WY. Microleakage in different primary tooth restorations. J Chin Med Assoc. 2016;79(4):228-234.

[9] Nedeljkovic I, Teughels W, De Munck J, Van Meerbeek B, Van Landuyt KL. Is secondary caries with composites a material-based problem?. Dent Mater. 2015;31(11):e247-e277.

[10] Łukomska-Szymańska M, Zarzycka B, Grzegorczyk J, et al. Antibacterial Properties of Calcium Fluoride-Based Composite Materials: In Vitro Study. Biomed Res Int. 2016;2016:1048320.

[11] Chatzistavrou X, Lefkelidou A, Papadopoulou L, et al. Bactericidal and Bioactive Dental Composites. Front Physiol. 2018;9:103.

[12] Imazato S. Antibacterial properties of resin composites and dentin bonding systems. Dent Mater. 2003;19(6):449-457.

[13] Chen L, Shen H, Suh BI. Antibacterial dental restorative materials: a state-of-the-art review. Am J Dent. 2012;25(6):337-346.

[14] Maas MS, Alania Y, Natale LC, Rodrigues MC, Watts DC, Braga RR. Trends in restorative composites research: what is in the future?. Braz Oral Res. 2017;31.

[15] Haghi M, Hekmatafshar M, Janipour MB, Gholizadeh SS, Faraz MK, Sayyadifar F, Ghaedi M. Antibacterial effect of TiO2 nanoparticles on pathogenic strain of E. coli. Int. J. Adv Biotec. Res. 2012, 3, 621-4.

[16] Arora, H., Doty, C., Yuan, Y., Boyle, J., Petras, K., Rabatic, B., Paunesku, T. & Woloschak, G. Titanium dioxide nanocomposites. Nanotech Life Sci. 2012, 25, 206.