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Investigation of Antibacterial Activity of Chlorhexidine Gluconate (CHxG) on Dental Composites Containing Nano-Titanium Dioxide Particles: in vitro study

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Abstract: this in vitro study was conducted for evaluating the antibacterial activity of resin-based dental composites containing Nano-Titanium dioxide Particles that were prepared with chlorhexidine gluconate (CHxG). Methods: TiO2 nano-composite was prepared and divided into three groups: (1) untreated TiO2 nano-composite. (2) Treated TiO2 nano-composite. (3) Treated TiO2 nano-composite which was incorporated with CHxG. TiO2 nano-composite was prepared in three groups, untreated TiO2 nano-composite and treated TiO2 nano-composite. Five of selected bacteria to be tested against the TiO2 nano-composite named, S. mutans was grown in Brain Heart Infusion (BHI) and E. faecalis, S. aureus, E. coli, and P. aeruginosa were grown in Müller-Hinton Agar (MHA). Results: Neither of the groups of untreated TiO2 nano-composite and treated TiO2 nano-composite and treated TiO2 nano-composites containing 3% (m/m) of CHxG showed no microbial inhibition when tested against previously mentioned facultative bacteria and showed an inability to inhibit the growth and the effectiveness of these bacteria. The other groups containing treated TiO2 nano-composite prepared with CHxG concentrated differently as 3%-5%-7% and 10% (m/m) which tested against the same facultative bacteria were antibacterial restoratives. They inhibited the bacterial growth and clear inhibition zones were demonstrated around the nano-composite materials. CHxG exhibit bacterial resistance at concentrations of 10% (m/m). At concentrations of 5 and 7% (m/m) for CHxG the nano-composites could not resist the bacterial growth except for S. aureus, where there was small inhibition zones observed around the nano-composites. At concentration of 3% of CHxG, the TiO2 nano-composites show no resistance of all bacteria.

Keywords: Antibacterial Activity, Carbon dioxide (CO2), Chlorhexidine Gluconate (CHxG), Dental resin, nano-Composites, Titanium oxide (TiO2), Urethane dimethacrylate (UDMA), 2-(Dimethylamino)ethyl methacrylate (DMAEMA), 3- methacryloyloxy-propyl (MPTMS).

Introduction

Bowen in 1962 was the first scientist who introduced resin composite in dentistry field, since then they became widely used. Resin Composites had many desirable properties, such as bonding to tooth body, Removal of minimal tooth structure, as well as high and desirable aesthetics, therefore, making it one of the nowadays wide used dental composite materials [1].

After treatment of primary caries by restorative materials secondary caries could occur again. Notice should be taken in mind that in cases of secondary caries occurred the tooth has already lost some tissue structure because of the primary lesion [2]. The tooth structure Demineralization happens after acid invasion which often produce certain types of bacteria, such as *S. Mutans* in the presence of fermentable carbohydrates.

Bacteria are main factor that basically responsible for caries development and bacterial colonization could not be prevented by composite restorations. In order to overcome this frequent problem and inhibit the growth of bacteria, directed efforts for development of resin composite materials used in dental applications which prevent bacterial accumulations at the interface of tooth composites [4].

Streptococcus mutans is an Acidogenic bacterium which considered as the most important micro-organism in the initiation of tooth lesion.

Although many other micro-bacteria play a role in the pathogenesis of the tooth decay *s. mutans* is the most bacteria which contributed in the transition process from non-pathogenic to cariogenic biofilms [5].

S. mutans is the bacteria which contribute in the transition from non-pathogenic to cariogenic biofilm, although many other microorganisms also play a role in the pathogenesis of the dental caries [5]. Therefore, dental restorative material which is effective against *S. mutans* when incorporated with certain antibacterial substances could be considered as an anti-cariogenic agent [6].

Recently, highly increased attentions have been paid to obtain dental resin materials with antimicrobial properties [7]. For example, chlorhexidine gluconate (CHxG) used as antimicrobial agent could incorporate with the resin matrix which has been approved to be active against many micro-organisms [8], [9].

Because of the well-known excellent performance, TiO_2 composite is widely used in dental applications such as teeth restoratives and implantology but it could be affected by bacteria that perhaps accumulate on the restored tooth [10], [11]. With all that, association of bacterial infection with TiO_2 still needs investigations in order to be inhibited and eliminated. The purpose of current *in vitro* study was to evaluate the effect of incorporating different loading of CHxG substances on TiO_2 nano-composites that used in dental applications

Materials and methods

TiO_2 particles sized 80 nm was purchased from Evonik Company, Krefeld, Germany. MPTMS, UDMA, DMAEMA, CQ, CHxG as a 20% (m/v) solution was purchased via Sigma Aldrich Company, Johannesburg, South Africa as well as the bacterial strains were purchased from Davies Diagnostics laboratories, Johannesburg, South Africa.

Preparation of resin composite

Treatment of TiO_2 nano-composite was obtained by mixing MPTMS in xylene in the presence of 2% (wt/v) n-propylamine used as catalyst and by adding 2.5% m/v of MPTMS to 1 g of nano-TiO_2, nano-TiO_2 was incorporated with MPTMS. 0.5 gm of each CQ was added as an initiator to the prepared composite mixture and UDMA as an accelerator was added to DMAEMA. Antimicrobial substance CHxG added during the composite mixing process. The mixture was then casted up into a plastic template and then light-cured until reaching to the final hardening degree and then alcohol used to sterilize Samples obtained from this process. In different concentrations CHxG incorporated into the mixture of the composite, and left until well molded. TiO_2 nano-composites then divided into four equal groups separately according to each concentration was added, for example, 3% (m/m), 5% (m/m), 7% (m/m) and 10% (m/m) of CHxG respectively and individually.

The TiO_2 nano-composite prepared in different methods, showed in this *in vitro* study to investigate their antimicrobial activities, was conducted following manufacturers' instructions as follows:

1) Treated TiO_2 nano-composite.
2) Untreated TiO_2 nano-composite.
3) Treated TiO_2 nano-composite incorporated separately with different concentrations of CHxG, 3% (m/m), 5% (m/m), 7% (m/m) and 10% (m/m).

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

	Nano-TiO_2	UDMA	MPTMS	Chlorhexidine gluconate (CHxG)
1	10%	90%	None	None
2	10%	90%	Treated	None
3	10%	87%	Treated	None
4	10%	87%	Treated	3%
5	10%	85%	Treated	None
6	10%	85%	Treated	5%
7	10%	83%	Treated	None
8	10%	83%	Treated	7%
9	10%	80%	Treated	None
10	10%	80%	Treated	10%

Selection of the medium and bacteria

In this *in vitro* investigation the five used facultative micro-bacteria were *Staphylococcus aureus* ATCC 12600, *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212 and *Streptococcus mutans* ATCC 25175. These chosen strains were carefully selected because they considered as standard for antibacterial examinations. All cultures which have been prepared in this study performed on standard Gram-stain and these found to be pure. Two types of commonly used media were applied with the above mentioned bacteria to investigate the microbial activity of the TiO_2 nano-composites which widely used in dental applications. *S. mutans* was evaluated with BHI Agar and the rest of the strains were evaluated with MHA, *S. mutans* used in this *in vitro* study considered as difficult strains to grow, therefore, it had to be grown in a different medium, and furthermore a CO_2 was needed for growing *S. mutans*. CO_2 only used with *S. mutans* and not with the other bacteria, because incubation in a CO_2 enriched atmosphere is not recommend due to its pH effect on the medium.

Methods

All specimens conducted were triplicate in each plate equidistantly and according to the nano-composite materials used in this *in vitro* investigation all the tests were divided individually into four groups. Only one specimen of TiO_2 nano-composites was included in each prepared group and then separately investigated *in vitro* for its antibacterial activity against each one of the five strains respectively as following.

- Untreated TiO_2 nano-composite was separately cultivated with each one of the five bacteria and incubated at 37°C for 24 hours.
- Treated TiO_2 nano-composite was separately cultivated with each one of the five bacteria and incubated at 37°C for 24 hours.
- Treated TiO_2 nano-composite incorporated with CHxG at concentration of 3% (m/m) was independently cultivated with each one of the five bacteria at 37°C for 24 hours. And then, repeated respectively using treated TiO_2 nano-composites containing CHxG at different concentrations of 5% (m/m), 7% (m/m) and 10% (m/m).

By following the same procedures all TiO_2 nano-composite samples were respectively and separately investigated against all the five bacteria except *S. mutans* which was exposed to 5% CO_2 inside a CO_2 water-jacketed incubator for 48 hours at 37°C. In this performed *in vitro* study, all experiments conducted in triplicate, including the preparation procedures of the nano-composite materials in order to confirm the obtained findings. This was qualitative descriptive study so the statistical analysis was therefore not applicable.

Results and discussion

The results that were obtained in this study confirmed that treated and untreated TiO_2 nano-composite had clearly no antibacterial activity (Fig. 1, 2).

As well as the results obtained in this study are concluded in Table 2. The results evident that treated and untreated TiO_2 nano-composites possess no antimicrobial activity at all (Fig: 1 and 2).

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Table 1: Incorporated specimens of treated TiO_2 nano-composite with different percentages of CHxG against tested bacteria

(+ indicates that bacteria were killed and the antibacterial substances effect were positive)

(-Indicates that bacteria were grown and the antibacterial substances effect were negative)

CHxG Concentrations	*E. coli*	*E. faecalis*	*S. aureus*	*P. aeruginosa*	*S. mutans*
3 wt%	–	–	–	–	–
5 wt%	–	–	+	–	–
7 wt%	–	–	+	–	–
10 wt%	+	+	+	+	+

Figure: 1. untreated TiO_2 nano-composite tested for its antibacterial activity against *S. mutans.* It is clear that the untreated TiO_2 does not show antibacterial activity

Figure: 2. treated TiO_2 nano-composite tested for its antibacterial activity against *S. mutans.* It is clear that the untreated TiO_2 does not show antibacterial activity

Inhibition antibacterial zones produced significant degrees by the preparation of Treated TiO_2 nano-composite with 10% (m/m) CHxG (Fig. 3-7) which tested against *S.*

mutans, *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus*.

This confirmed that the effective of CHxG as antibacterial substances when they are prepared with the nano-composites. Moreover, except with *P. aeruginosa,* the inhibition zones could be observed clearly around the whole treated nano-composite specimens (Fig. 5).

Figure: 3. Antibacterial activity of treated TiO_2 nano-composite prepared with 10% (m/m) CHxG against *E. coli.* It is clear that the test showed antibacterial activity.

Figure: 4. Antibacterial activity of treated TiO_2 nano-composite prepared with 10% (m/m) CHxG tested against *E. faecalis.* It is clear that the preparation showed antibacterial activity.

Figure: 5. Antibacterial activity of treated TiO_2 nano-composite prepared with 10% (m/m) CHxG tested against *P. aeruginosa.* It is clear that the test showed antibacterial activity.

Figure: 6. Antibacterial activity of treated TiO_2 nano-composite prepared with 10% (m/m) CHxG tested against *S. aureus.* It is clear that the test showed antibacterial activity.

Figure: 7. Antibacterial activity of treated TiO_2 nano-composite prepared with 10% (m/m) CHxG tested against *S. mutans*. It is clear that the preparation showed antibacterial activity.

Figure: 8. Antibacterial activity of treated TiO_2 nano-composite prepared with 5% (m/m) CHxG tested against *S. aureus*. It is clear that the preparation showed antibacterial activity.

Due to the resistance effect, the latter exhibited small and little inhibition zones of the antibacterial substances. However, CHxG as antibacterial substances at concentrations of 10% (m/m) clearly demonstrated an antibacterial effectiveness. *P. aeruginosa* is the only bacteria at concentration of 10% (m/m) displayed a resistance which confirms that this kind of microorganisms could easily be accumulated on the composite materials on teeth in the oral environment. However, no microbial inhibition was appeared for treated TiO_2 nano-composites containing 3% (m/m) of CHxG at all. Furthermore, no bacterial resistance observed with treated TiO2 nano-composites contained CHxG at concentration of 5% (m/m) and the microbial growth which means the bacteria could easily be grown except with S. aureus, which showed small inhibition zones around the nano-composites (Fig. 8). Moreover, no different results could be showed with CHxG at concentration of 7% (m/m) and nearly the same results were carried out with the previous concentration of 5% (m/m).

Although incorporation of treated TiO_2 nano-composite with CHxG at concentration of 10% (m/m) was antimicrobial composition, moreover, although treated TiO_2 nano-composites incorporated with CHxG at concentration of 5% (m/m) of the same antimicrobial agent showed resistance to the bacterial growth with one of tested bacteria. The treated and untreated TiO_2 nano-composites with no addition of antimicrobial could not prevent the growth of any tested bacteria and never showed significant antimicrobial activities. Furthermore, these nano-composite materials showed no resistance capability to the bacterial effectiveness. This study achieved the confirmation of using CHxG as antimicrobial substances when added to TiO_2 in order to eliminate the reoccurrence of caries. This is, in agreement with literature which carried out that, the microbicidal capability of TiO_2 nano-composites could depend on the improvement of antimicrobial activity, which should increase and enhance the elimination of micro-bacteria and viruses in the oral environment [12].

Conclusion

The incorporations and additions of certain and different concentration of CHxG as antibacterial agent increased and supported the antimicrobial activity without influencing or changing the chemical or physical handling characteristics and properties of used nano-composites. CHxG incorporated into the nano-composite materials with Lower concentrations for example, 3% (m/m), showed results negatively with all tests that have been done and the bacteria had not inhibited and the growth was easily. Moreover, except with *S. aureus*, the other incorporated concentrations of 5% and 7% (m/m) of CHxG as an antimicrobial substance with TiO_2 nano-composites also showed no positive results as well. For *S. aureus*, clear inhibition zones were positively observed around the TiO_2 nano-composites samples. With incorporated concentration of 10% (m/m) of CHxG, observed inhibition zones positively appeared around the TiO_2 nano-composites specimens and completely eliminated the bacterial growth.

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