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The Antibacterial Activity of Titanium Dioxide Nanoparticles Incorporated into Resin Composite Restoration (In Vivo Study)

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ABSTRACT

Objectives: - This study was conducted to evaluate the antibacterial activity of titanium dioxide nanoparticles that incorporated into; resin composite, adhesive, and both. **Materials and methods:** 10 wt% titanium dioxide nanoparticles (TiO_2NPs) were incorporated into resin composite and adhesive to obtain modified resin composite and adhesive. Forty patients have Class V caries were selected to receive composite restorations. Class V cavities were prepared and divided into 4 groups according to the incorporation of titanium dioxide nanoparticles into the restoration; **group A1** (Resin composite incorporating TiO_2NPs), **group A2** (adhesive incorporating TiO_2NPs), **group A3** (Both resin composite and adhesive incorporating TiO_2NPs), and **group A4** (Composite restoration free of TiO_2NPs). Plaque samples were collected from the gingival margin of the restoration immediately, 1 week, and 1 month after the restoration, then colony forming units (CFUs) were counted to evaluate the count of streptococcus mutans in plaque samples**. Results:** There was a statistically significant reduction in CFUs count with all restorations incorporating TiO_2NPs as compared to the control group. After 1 week of composite restoration, there was no statistically significant reduction in CFUs count as compared to immediately after the restoration, whereas, there was a statistically significant reduction in CFUs count after 1 month. **Conclusions:** Composite restorations incorporating TiO_2NPs enhanced the antibacterial activity especially that blended TiO_2NPs in both resin composite and adhesive. There was time dependent improvement in the antibacterial activity of composite restorations incorporating titanium dioxide nanoparticles.

KEYWORDS

Titanium dioxide nanoparticles resin composite, adhesive, plaque samples, CFUs counting.

INTRODUCTION

Resin composites are widely used in dentistry due to their excellent esthetic, strength, and the ability to micromechanically bond with the adequate tooth structure. Despite the improvements in dental adhesive

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and resin composite, the bonded interface is still the weakest area of composite restoration [1]. There are significant problems such as secondary caries and the resulting need for restoration replacement. Part of this problem may be attributed to that resin composite accumulate more biofilm/plaque than other restoratives[2]. Plaque contribute to secondary caries, which is a main reason for restoration failure. Replacing failed restorations accounts for 50–70% of all restorations performed [3].

Bacteria are responsible for caries development and composite restorations cannot hinder bacterial colonization. To overcome this problem, efforts are directed for development of dental adhesive and resin composite materials that suppress the bacterial activity at the tooth composite interface [4].

One of the most important advances of the last few years is the application of nanotechnology to improve the performance of composite restorations[5]. Multiple studies investigated the effect of incorporation of different nanoparticles into resin composite or adhesive on the bacterial viability. [6]

Titanium dioxide nanoparticles have excellent optical properties consisting of a high reflective index, chemical stability, bioactivity, and antibacterial activity. Extensive efforts have been undertaken to improve the performance of resin composite and adhesive using titanium dioxide nanoparticles to overcome the problem of secondary caries [7,8].

However, literature is sparse in evaluating the combined effect of incorporating titanium dioxide nanoparticles into both resin composite and adhesive. Since time may affect the treatment outcome and due to lack of definitive approach for secondary caries prevention under composite restorations, thus the purpose of the present study was to evaluate the antibacterial activity (in vivo) of composite restorations incorporating TiO_2NPs into resin composite only, adhesive only, and in-combination at different time intervals.

MATERIALS AND METHODS

Preparation of resin composite containing TiO_2NPs:

10% of TiO_2NPs were calculated by mass fraction of composite paste weight, they were added into dark glass bottle which sealed with aluminum foil. Composite paste was added into the bottle containing the calculated powder, and then sonicated together within the glass bottle. The incorporation was performed for 10 min [9] using the autoclaveble ultrasonic tip of high speed ultrasonicator in a lightproof environment at room temperature. Immediately after the incorporation, the mixture was aspirated again into the original composite syringe, and then the syringe was recapped securely again until used.

Preparation of adhesive containing TiO_2NPs:

10% of TiO_2NPs were calculated by mass fraction of the adhesive volume, and then added into the adhesive bottle. The adhesive solution and TiO_2 nanoparticles were sonicated together [10] within the adhesive bottle for 10 min using an autoclaveble ultrasonic tip of the high speed ultrasonicator (100B-HB ultrasonic processor, 25000 revolutions per minute), to obtain a homogenous mix in a lightproof environment at room temperature. Immediately after the incorporation, the adhesive bottle was recapped securely again until used.

Selection of patients:

Forty patients were selected randomly from the restorative dentistry clinic at the Faculty of Oral and Dental Medicine, Girl's branch, Al-Azhar University; the objectives of this study were explained to all participants who signed an informed consent according to the guide of ethics committee of human research before starting the study. Each volunteer was selected according to the inclusion and exclusion criteria followed by Al-Duliamy, in 2014. [11]

Patients grouping:

Sixty Class V cavities were prepared and divided into 4 groups according to the incorporation of titanium dioxide nanoparticles into the restoration. **Group A1** (Resin composite incorporating TiO_2NPs), **group A2** (adhesive incorporating TiO_2NPs), **group A3** (Both resin composite and adhesive incorporating TiO_2NPs), and **group A4** (Composite restoration free of TiO_2NPs).

Cavity preparation:

All operative procedures were performed under local anesthesia (2% lidocaine). A class V cavity was prepared on the buccal surface of each tooth using carbide round bur at high speed with water coolant. A suitable diameter of the round bur (Komet, Lemgo, Germany) was selected corresponding to the size of caries lesion. Initial caries removal was done using the round bur mounted in a high speed handpiece under copious of water. Caries indicator dye was used to discriminate between the caries infected dentin and the affected one, after highlighting the infected dentin, a suitable size spoon excavator was used to remove caries beginning from the outer lateral walls followed by the center.

A forty five degree (45º) bevel of approximately 0.5-1mm thickness was placed on the incisal enamel cavosurface margins using diamond flame shaped bur (KG Sorensen, Sao Paulo, Brazile) [12].

Restoration of teeth.

Upon completion of the cavity preparation, isolation was performed using rubber dam and high volume suction. The cavity was rinsed and gently air dried. In all groups, the etch-rinse technique was performed prior to the application of resin composite.

Etchant application

Phosphoric acid gel (37% conc) was injected initially to the enamel margins and then extended from the cavo-surface margins to the floor of the cavity, rubbed against the cavity walls for 15 sec, and rinsed away with copious of water/air blasts for 10 sec according to the manufacture instructions. Excess water was blotted using absorbent paper until the surface appeared glistening without pooling of water.

Adhesive application

Two groups were treated with Adper single bond 2 adhesive, which were free of TiO_2NPs (Groups A1 and A4). The other two groups were treated using the modified one incorporating TiO_2NPs (Groups A2 and A3). Immediately after blotting, the adhesive was applied according to the manufacture instructions.

Utilizing a disposable applicator, a thin uniform layer of the tested adhesive corresponding to each group as described before was rubbed to the etched cavity walls for 15 sec, gently air thinned with compressed air for 5 sec, and cured for 10 sec using LED curing unit. Another layer of adhesive was applied and cured for 10 sec as described before.

Composite application

Immediately after adhesive application, two groups were restored with Filtek™ 350 XT flowable composite, shade A2, which was free of TiO_2NPs (Groups A2 and A4). The other two groups were treated using the modified one incorporating TiO_2NPs (Groups A1 and A3).

Resin composite corresponding to each group was dispensed incrementally into the axial wall and the gingival margin, adapted using composite instrument (636, 3 MESPE products, USA), and then light cured for 20 sec according to the manufacture instructions. Subsequent layer was applied until filling the cavity following the contour of each tooth.

Plaque samples collection.

Plaque samples were collected immediately after composite restoration as baseline. The tested teeth were isolated using cotton roll, dried, then plaque

samples were collected supragingivally using a sterile microbrush (Disposable fine microapplicator, JHY) to scrub in a one way direction along the border between the enamel and composite restoration [13].

The head of each microbrush was cut using a sterile scissor in a sterile plastic container, then transported in icepack containers, and processed in the laboratory immediately. Patients were advised to get outdoor daily at the time of afternoon to get benefit from the sun light exposure. [14] They also were instructed not to use mouth washes containing fluoride during the period of the research. Patients were recalled again at morning after 1 week and 1 month of composite restoration. They were previously instructed not to eat or brush their teeth two hours before the sampling appointments [15]. Plaque samples were collected as described before.

RESULTS

(Table 1 and 2) and (Figure 1)

Concerning the effect of time on bacterial count, results revealed that, after 1 week, there was no statistically significant reduction in the mean value of Log_{10} CFUs of mean bacterial count with all restorations incorporating TiO_2NPs; whereas, there was a statistically significant reduction in the mean value of Log_{10} CFUs of mean bacterial count after 1 month.

Concerning the effect of incorporation of TiO_2NPs nanoparticles on the percent changes of mean bacterial count, results showed that, there was a statistically significant reduction in the mean percent changes of mean bacterial count in all groups incorporating TiO_2NPs as compared to the control group, which showed a statistically significant increase in the mean percent changes of mean bacterial count.

Table (1) *Mean, standard deviation (SD) values and results for comparing the Log_{10} CFUs of mean bacterial count between different time periods within different groups.*

Group	Immediately		1 week		1 month		*P*-value
	Mean Log_{10}	SD	Mean Log_{10}	SD	Mean Log_{10}	SD	
Group A1	7.30 ^{A}	0.33	6.71 ^{A}	0.43	4.78 ^{B}	0.55	0.028*
Group A2	6.88 ^{A}	0.66	6.42 ^{A}	0.38	5.55 ^{B}	0.54	0.028*
Group A3	6.81 ^{A}	0.38	5.44 ^{A}	0.90	3.78 ^{B}	0.32	0.028*
Group A4	7.37	0.73	7.55	0.50	7.66	0.51	0.060

**: Significant at $P \leq 0.05$.*

Table (2) *Mean, standard deviation (SD) values, and results for comparing the percent changes of mean bacterial count between the four groups after 1 week and 1 month.*

Time	Group A1		Group A2		Group A3		Group A4		*P*-value
	Mean Log_{10}	SD	Mean Log_{10}	SD	Mean Log_{10}	SD	Mean Log_{10}	SD	
1 week	-70.93 ^{C}	15.30	-56.00 ^{B}	32.80	-84.64 ^{D}	23.07	66.18 ^{A}	39.11	0.040*
1 month	-91.38 ^{C}	0.73	-83.14 ^{B}	1.19	-99.82 ^{D}	0.23	115.91 ^{A}	40.05	0.017*

**: Significant at $P \leq 0.05$*

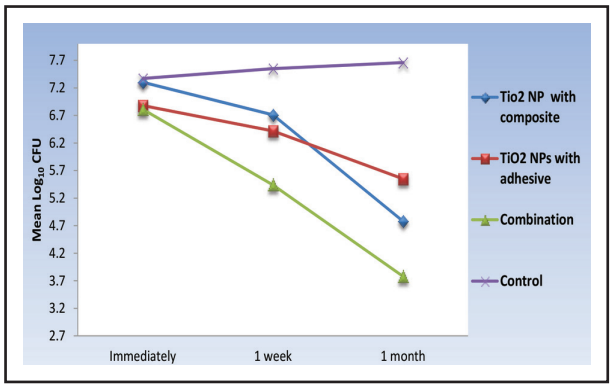

Fig. (1) Line chart reprsenting the mean values of Log10 CFU of mean bacterial counts at different time periods in the four groups.

DISCUSSION

Dental caries is a prevalent oral disease; its development is a complex interaction between the tooth and the acid producing bacteria colonized in dental plaque. The basic mechanism of caries is demineralization of enamel and dentin via acids generated by bacterial biofilm. Composite restorations cannot hinder bacterial colonization or combat the demineralization process. To overcome this problem, efforts include modifications of restorative material to enhance its properties. An approach to combat caries is reduction of cariogenic bacterial count. Various researchers have been attempted to develop new antibacterial resins and adhesives to reduce plaque accumulation on the surfaces of dental materials by incorporating bactericidal agents such as chlorhexidine, antibacterial monomer MDPB, and other antibacterial fillers [16-18].

However, incorporation of these agents causes the composite to suffer from mechanical problem, discoloration of composite matrix, short releasing period and toxic effects. With the revolution of nanotechnology, dental materials at nanoscale dimensions exhibit unusual properties with numerous applications. Therefore, multiple studies investigated the effect of different nanoparticles on the remineralization of tooth enamel and bacterial count [19,20].

It has been shown that, titanium dioxide has excellent mechanical properties and desirable color thereby may be considered ideal for incorporation into dental materials. Moreover, photocatalysis of titanium dioxide (TiO_2) has well known pathogenicidal effect by; inhibition of gram-positive and gram-negative bacteria [21, 22]. Therefore, it may be useful as an antibacterial agent incorporated into dental restoration especially when applied in nanometer size [23].

Extensive efforts have been undertaken to improve the performance of composite restoration using titanium dioxide nanoparticles to overcome the problem of secondary caries.[7,24] However, literature is sparse in evaluating the combined effect of incorporating titanium dioxide nanoparticles into both resin composite and adhesive. Thus the purpose of the present study was to evaluate the mineralization potential and the antibacterial activity of resin composite and adhesive both incorporated TiO_2NPs individually and in-combination. Since time may affect the treatment outcomes, thus the present study evaluated the antibacterial activity of composite restorations at different time intervals.

Regarding the effect of incorporation of TiO_2NPs into composite restorations on the mean percent changes of mean bacterial count, results revealed that, there was a statistically significant reduction in the mean percent changes of mean bacterial count with all restorations incorporating TiO_2NPs as compared to the control. Those effects are consistent with previous study [25]. in which, there was a statistically significant reduction in the mean percent changes of mean bacterial count with adhesive incorporating TiO_2NPs as compared to the control adhesive. This is also in accordance with another study [26]. which showed that, resin composite modified with TiO_2NPs was significantly effective in reducing *streptococcus mutans* count as compared to the control composite.

This finding was attributed to; first, photocatalysis of titanium dioxide upon exposure to light source,

when titanium dioxide absorbs ultraviolet (UV) radiation from sunlight or illuminated light source (fluorescent lamps), it produces pairs of electrons and holes.

The excited electrons can react with oxygen to produce a superoxide ion (O_2^-), while the positive holes can react with H_2O or OH^- to produce hydroxyl radicals ($OH^{\cdot}$). Further reactions can generate other reactive oxygen species (ROS) like hydroxyl peroxide (H_2O_2) and singlet oxygen (O_2). Hydroxyl radicals ($OH^{\bullet}$) and superoxide anions $(O_2)^-$ are particularly oxidative and can act on the cell wall or the cell membrane of the nearby bacteria causing leakage. After cell wall damage, oxidative stress is exerted on the cytoplasmic membrane causing an increase in the permeability and structural damage of the cell eventually leads to its death [22]. Second, the direct toxic effect of TiO_2NPs when it becomes in close contact to the microorganism. This effect enhanced when titanium dioxide particles are at the nano-scale. When particles are small in size, they may penetrate and diffuse easily into the cell [27].

However, the current findings are in a contrary with (Atbayga, in 2013) [28] who found that, all groups including TiO_2NPs did not exhibit antimicrobial activity regardless the microorganism type; Escherichia *coli* (*E. coli*) *Staphylococcus aureus* (*S. aureus*), and *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Streptococcus mutans* (*S. mutans*). Those differences could be explained by considering the difference between the current in vivo and their in vitro study. Apart of this could be related to the difference in the content, type, and technique of synthesis of TiO_2NPs.

Comparing the effect of incorporation of TiO_2NPs between different restorations, results showed that, composite restorations incorporating TiO_2NPs in both resin composite and adhesive, showed the highest statistically significantly reduction in the mean percent changes of mean bacterial count, which recorded (-84.64%). This may be attributed to the higher concentration of TiO_2NPs in the restoration, as this combination renders this restoration containing a higher concentration of TiO_2NPs than others. This may increase the antibacterial photocatalytic effect by increasing the formation of more reactive oxygen species. This explanation could be supported by previous study [29], in which, poly (methyl methacrylate) (PMMA) incorporating 1% TiO_2NPs was significantly reduced *S. mutans* count as compared to poly (methyl methacrylate) (PMMA) incorporating 0.5% TiO_2NPs. This was explained by, increasing the concentration of TiO_2NPs, the antibacterial photocatalytic effect increases. On the other hand, composite restorations incorporating TiO_2NPs in adhesive only showed the lower statistically significantly reduction in the mean percent changes of mean bacterial count (-56%) than that incorporated TiO_2NPs in composite only (-70.93%).

This could be explained by two reasons: First, diminish of reactive oxygen species production by the adhesive layer which is not directly exposed to the light source as composite surface. This explanation could be supported by previous study[8], in which, the reduction in bacterial count by resin composite containing TiO_2NPs was maintained for a significant time following UV irradiation compared to that which did not receive UV pre-treatment. Furthermore, another study [29], demonstrated that, photocatalysis of TiO_2NPs incorporated with acrylic resin in the dark was significantly reduced when compared to that exposed to UV light. Second, diminish of the non photocatalytic effect of titanium dioxide in the adhesive layer which was not in a direct contact with the microorganism as compared to the composite surface. This holds true, specifically that Verdier et al, in 2014[30], reported that reduction in the distance between TiO_2NPs and the bacteria, increases the inactivation of microorganisms by non-photocatalytic effect (direct contact).

Concerning the effect of time on the changes of the mean values of Log_{10} CFUs of mean bacterial count using different restorations, results showed that, after 1 week, there was no statistically

significant reduction in the mean values of Log_{10} CFUs of mean bacterial count compared to immediately (after restoration). This was recorded with all restorations including TiO_2NPs. This could be related to the effect of UV light. A previous study [30], showed that, there was no statistically significant biofilm reduction with nanofilled composite incorporating 10 wt% TiO_2NPs after 3 days of inoculation with *Streptococcus sobrinus* under laboratory fluorescence light. This attributed to that; TiO_2NPs are apparently unable to inhibit the bacterial growth in the absence of UV light.

However, another study[31], showed that, there was a statistically significant reduction in *Staphylococcus aureus* count using a heat cured acrylic resin incorporating TiO_2 NPs after 1 week. Those differences could be attributed to the difference of the microorganism type, or by considering the differences between the in vitro and in vivo studies. Part of this, is the change in the UV intensity, which could be explained by the uneven exposure of the patients in the current study to the sunlight compared to the fixed intensity synthetic UV light source used in the other in vitro study. This might affect the rate of photocatalysis of TiO_2NPs as previous study,[14] which showed that, the solar UVA intensity is about 4 mW/cm^2 on sunny days and drops by about 10 times on cloudy days.

In the present study, after 1 month, there was a statistically significant reduction in the mean values of Log_{10} CFUs of mean bacterial count as compared to immediately (after restoration) as well as 1week after the restoration.This effect was recorded with all restorations including TiO_2NPs. This is matching with other study [32], which showed that, the photocatalytic ability of soft liners incorporating TiO_2NPs was significantly increased by time and was maintained for a period of 30 days. This attributed to that; the formation of reactive oxygen species (ROS) upon exposure of TiO_2NPs to UV light which continues when light is available.

However, this is in contrary with previous study[33], in which, TiO_2-nanotube coated rod which was implanted in the rat tibia presented an evidence of acute pyogenic infection in the shape of an abundant neutrophilic exudate at 2 weeks, with chronic inflammatory cell infiltration emerge in large numbers after 3 weeks, and intramedullary necrosis including abscess and osteonecrosis demonstrated in the medulla of the metaphysis with mild chronic inflammation after 4 weeks. This inflammatory effect was attributed to bacterial colonization on the surface of the implant rod. This difference could be related to the difference in the substrate and the absence of light irradiation of the implant rod used in this study compared to the current study.

CONCLUSIONS

Composite restorations incorporating TiO_2NPs enhanced the antibacterial activity especially when blended TiO_2NPs in both resin composite and adhesive. There was time dependent improvement in the antibacterial activity of composite restorations incorporating titanium dioxide nanoparticles.

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