ORIGINAL RESEARCH

Effect of Addition of Nano-TiO_2, Nano-SiO_2, and a Combination of Both, on Antimicrobial Activity of an Orthodontic Composite

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Abstract

Aim: This study aimed to assess the effect of addition of nano-titanium oxide (nano-TiO_2), nano-silicon dioxide (nano-SiO_2), and a combination of both, on antimicrobial activity of an orthodontic composite.

Materials and methods: Molds measuring 0.64 × 0.5 mm were used for the fabrication of composite disks. For this purpose, 0.5% and 1% nano-TiO_2, nano-SiO_2, and a combination of both (0.5% nano-TiO_2 and 0.5% nano-SiO_2), were mixed with Transbond XT composite (3M Unitek). A total of 180 composite disks were fabricated for eluted component, disk agar diffusion (DAD), and biofilm inhibition tests. The colony counts of *Streptococcus mutans* (*S. mutans*), *Streptococcus sanguinis* (*S. sanguinis*), and *Lactobacillus acidophilus* (*L. acidophilus*) and the diameters of growth inhibition zones were measured at 3, 15, and 30 days after exposure to the materials. Data were analyzed using one-way ANOVA and a *post hoc* test.

Results: None of the nano-TiO_2 and nano-SiO_2 concentrations had any significant effect on the growth inhibition zone. All tested concentrations of nano-TiO_2 and nano-SiO_2 decreased the colony count of all bacteria. The composite sample containing both nano-TiO_2 and nano-SiO_2 had the greatest efficacy for reduction of *S. mutans* and *S. sanguinis* colony counts at all three time points. Also, 1% nano-TiO_2 and 1% nano-SiO_2 had similar effects on *L. acidophilus* in eluted component test.

Conclusion: Addition of TiO_2 and SiO_2 nanoparticles conferred antimicrobial property to the tested orthodontic composite.

Clinical significance: Using orthodontic composite containing nonoparticles with antibacterial activity may prevent dental caries.

Keywords: Antimicrobial activity, Nano-SiO_2, Nano-TiO_2, Orthodontic composite.

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Introduction

Enamel demineralization is a common consequence of fixed orthodontic treatment. Brackets and orthodontic appliances enhance plaque accumulation and increase the count of oral bacteria during the course of orthodontic treatment.[1] Decalcification of enamel under fixed orthodontic appliances is a common finding in orthodontic treatment. Good oral hygiene maintenance by proper toothbrushing and use of fluoridated toothpaste was the first adopted strategy to prevent the occurrence of white spot lesions.[2] Despite the fact that oral hygiene measures are the first line of caries prevention, their efficacy highly depends on patient cooperation. Thus, they are not highly reliable. With this in mind, one possible strategy for caries prevention may be the addition of antimicrobial agents to orthodontic adhesives. Accordingly, some studies added fluoride and chlorhexidine to orthodontic adhesives. However, these additives adversely affected the mechanical properties of adhesives and only exerted a short-term antimicrobial effect.[3–6]

Streptococcus mutans (*S. mutans*) is among the most commonly isolated microorganisms from the cariogenic plaques accumulated on tooth surfaces. It is anaerobic at a pH of 5.5 and produces organic acids. *Lactobacillus acidophilus* (*L. acidophilus*) is another microorganism present in cariogenic plaques, which enhances the demineralization of the tooth structure. *Streptococcus sanguinis* (*S. sanguinis*) is another microorganism found in noncariogenic plaques at a pH of 5.5 and its presence in dental plaque indicates low cariogenic activity of plaque.[7]

Several methods have been employed to prevent the growth of biofilms that contribute to initiation of dental caries. The

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antimicrobial properties of some metal oxides such as magnesium, zinc, silver, and calcium have been previously confirmed.[8,9]

Nanotechnology has greatly advanced in the synthesis of dental materials especially composite resins. Ahn et al. showed that orthodontic adhesives containing silver nanofillers can prevent enamel demineralization without compromising the physical properties of the adhesive.[10] Evidence shows that nanocomposites and nanomonomers may be suitable for bonding because they provide clinically acceptable shear bond strength.[11] Also, it has been indicated that *S. mutans* is sensitive to silver, zinc oxide, and gold nanoparticles. Thus, these compounds can be clinically beneficial.[12]

Recent studies have confirmed the antimicrobial properties of titanium oxide (TiO_2) against *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *L. acidophilus*.[13] Haghi et al. evaluated the antimicrobial properties of TiO_2 nanoparticles (nano-TiO_2) against *E. coli* and showed that nano-TiO_2 created some pores in the bacterial cell wall that enhanced its permeability and caused death.[14] It shows photocatalytic properties in presence of photons with <388 nm wavelength, at which electrons are excited. Free radicals form with such a high level of energy, which can react with different organic materials and degrade them. In addition to the significant catalytic effect, other favorable properties of TiO_2 such as its white color, low toxicity, high stability, efficiency, availability, and low cost have made it a suitable antimicrobial additive for dental materials.[15]

Nonorganic carriers such as zeolite apatite and phosphate have been suggested to increase the durability of antimicrobial agents and enable their slow, sustained release. Silicon oxides (SiO_2) are suitable carriers due to their porous structure and surface adsorption. Nano-silicon dioxide (nano-SiO_2) has high surface activity, which enables the uptake of different ions and molecules. Sodagar et al. in 2016 evaluated the antimicrobial efficacy of addition of TiO_2/SiO_2 nanoparticles.[16] Poosti et al. in 2012 indicated optimal antimicrobial property following addition of TiO_2 nanoparticles to orthodontic composites.[17]

Considering all the above, this study aimed to assess the effect of addition of nano-TiO_2, nano-SiO_2, and a combination of both on antimicrobial activity of an orthodontic composite.

Materials and Methods

All procedures were done in the Medical Microbiology Department of Tehran University of Medical Sciences.

Fabrication of Nanocomposite Samples Containing SiO_2 and TiO_2

For the fabrication of these nanocomposite samples, nano-TiO_2 and nano-SiO_2 were purchased from Mehregan Chimie, Iran. Transbond XT composite paste (3M Unitek, Monrovia, CA) was mixed with nanoparticle powders according to a predetermined weight percentage and sonicated at 100 W and 30 kHz (Bandelin SONOPULS ultrasonic homogenizer, Berlin, Germany) in a dark environment for 5 minutes. The weight percentage of nanoparticles was determined according to a previous study.[13] Composites with 0.5% and 1% nano-SiO_2, 0.5% and 1% nano-TiO_2, and a combination of both (0.5% nano-TiO_2 and 0.5% nano-SiO_2) were prepared. In order to ensure equal distribution of nanoparticles in the composite, scanning electron microscopy was used.

Microbial Tests

Standard strain *S. mutans* (ATCC35668), *S. sanguinis* (ATCC10556) and *L. acidophilus* (ATCC314) were purchased from the National Institute of Genetic Engineering and Biotechnology of Iran and cultured on Mitis Salivarius-Mutans valinomycin (Merck, Germany) under aerobic conditions in presence of 5% sucrose agar, MM10 and 5% CO_2 (capnophilic conditions in a Gas-Pak), and MRS under anaerobic conditions, respectively, and incubated at 37°C for 48 hours. In order to prepare microbial suspensions, the bacteria were cultured on brain heart infusion agar (Merck, Germany) and incubated at 37°C. When the bacteria reached the logarithmic phase of growth confirmed by spectrophotometry (600 nm optical density, 0.08–0.1), bacterial suspensions containing 5.1×10^8 colony-forming units per milliliter (CFUs/mL) were prepared and the desired concentration was confirmed by culture (Figs 1 and 2).

Fabrication of Composite Samples

Molds measuring 5 mm in diameter and 0.64 mm thickness were placed on glass slabs. Composites containing 0.5% and 1% nano-SiO_2, 0.5% and 1% TiO_2, a combination of 0.5% TiO_2 and 0.5% SiO_2, and pure composite without nanoparticles (control group) were applied in the molds. Each sample was light-cured. After light curing, the samples were removed from the molds and sterilized by gamma radiation (25 kGy). A total of six groups were evaluated. Each test was repeated in triplicate to ensure accuracy.

Biofilm Inhibition Test

In order to form microbial biofilms, 500 µL of each bacterial suspension containing 5.1×10^8 CFUs/mL was added to the wells of a 48-well microplate containing composite disks. They were then incubated at 37°C for 48 hours. Next, in order to eliminate the planktonic and loosely attached bacteria, composite disks were rinsed with 5 mL of sterile saline under aseptic conditions and placed in microtubes containing 1 mL of sterile saline. In order to eliminate the bacterial biofilm from the surface of composite disks, microtubes containing disks were vortexed at high speed for 1 minute. Next, serial dilutions of bacterial biofilm were prepared in 96-well microplates; 10 µL of each dilution was inoculated in brain heart infusion broth and spread-cultured. The plates were then incubated at 37°C for 48 hours and the colony count (CFUs/mL) in each group was calculated using the Miles and Misra method.[18]

Disk Agar Diffusion (DAD) Method

A bacterial suspension containing 5.1×10^5 CFUs/mL was prepared of the bacteria in Mueller Hinton broth culture medium (Merck, Germany) and spread-cultured on Mueller Hinton agar (Merck, Germany). After culture, disks containing different concentrations of the nanoparticles were placed on the surface of the culture medium inoculated with the microorganisms. The disks had 2 cm distance from each other and the plates were incubated at 37°C for 24 hours. The diameter of the growth inhibition zones around the disks was measured by a caliper.

Eluted Component Test

Composite disks containing different concentrations of SiO_2 and TiO_2 were placed in microtubes containing 1 mL of sterile artificial saliva composed of 2 g NaCl, 0.2 g KCl, 0.453 g $CaCl_2 \cdot 2H_2O$, 0.345 g

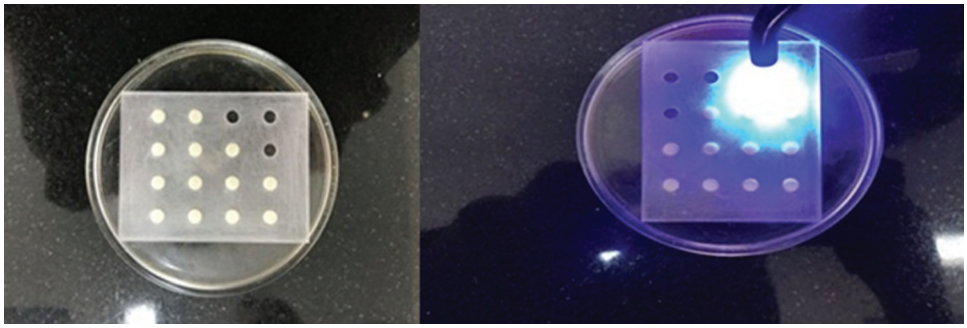

Fig. 1: Fabrication of composite disks

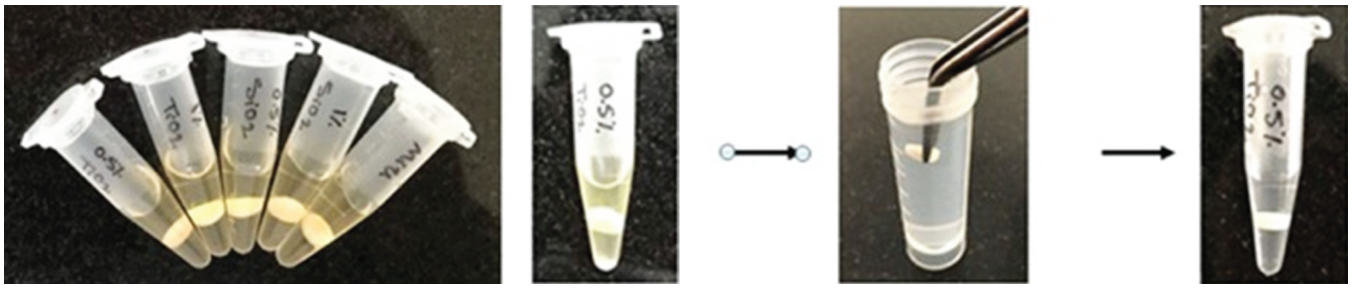

Fig. 2: Antimicrobial properties in biofilm inhibition test (left) and eluted component test (right)

Table 1: Colony count of bacteria (CFUs/mL) in presence of composite disks containing 0.5 and 1% nano-TiO_2 and nano-SiO_2 and a combination of 0.5% nano-TiO_2 and 0.5% nano-SiO_2 and the control group in biofilm inhibition test

	TiO_2 (%)	*SiO_2 (%)*	*Minimum*	*Maximum*	*Mean*	*Std. deviations*
S. mutans	0	0	23	27	25	2
	0	0.5	21	24	22.67	1.528
	0	1	16	20	18	2
	0.5	0	18	22	20	2
	0.5	0.5	12	13	12.33	0.577
	1	0	13	19	15.67	3.055
L. acidophilus	0	0	33	40	35.67	3.786
	0	0.5	31	35	32.67	2.082
	0	1	25	30	27.33	2.517
	0.5	0	26	33	29	3.606
	0.5	0.5	15	20	17.67	2.517
	1	0	21	29	24.67	4.041
S. sanguinis	0	0	27	32	29.67	2.517
	0	0.5	24	29	26.67	2.517
	0	1	19	23	21.33	2.082
	0.5	0	21	25	23	2
	0.5	0.5	11	16	13.33	2.517
	1	0	17	20	18.33	1.528

$NaH_2PO_4 \cdot 2H_2O$, 0.0025 g $Na_2S \cdot 9H_2O$, and 0.5 g urea. Next, on days 3, 15, and 30, 50 µL of the contents of the microtubes were transferred to new microtubes containing 50 µL of each bacterial suspension with a final concentration of 5.1×10^5 CFUs/mL. The microtubes were incubated at 37°C and shaken at 300 rpm for 24 hours. Eventually, the colony count (CFUs/mL) of each sample was calculated by serial dilution in 96-well microplates and subsequent spread-cultured on brain heart infusion agar using the Miles and Misra method.[18]

Statistical Analysis

The results of antimicrobial tests were analyzed using the one-way ANOVA. Pairwise comparisons were carried out using a *post hoc* test.

Results

Biofilm Inhibition Test

Table 1 shows the descriptive results. All tests were performed in triplicate for each group. One-way ANOVA showed that *S. mutans*, *S. sanguinis*, and *L. acidophilus* colony counts significantly decreased in

Table 2: Colony count (CFUs/mL) of bacteria in presence of composite disks containing 0.5 and 1% nano-TiO_2 and nano-SiO_2 and a combination of 0.5% nano-TiO_2 and 0.5% nano-SiO_2 and the control group in eluted component test

	TiO_2 (%)	*SiO_2 (%)*	*Day*	*Minimum*	*Maximum*	*Mean*	*Std. deviations*
S. mutans	0	0	3	32.2	38.1	35.7667	3.13741
	0	0	15	32.2	38.1	35.7667	3.13741
	0	0	30	32.2	38.1	35.7667	3.13741
	0	0.5	3	28	33.1	30.1667	2.63502
	0	0.5	15	30.1	36.2	32.6	3.19531
	0	0.5	30	30	40.1	34.3	5.2144
	0	1	3	16.2	22	18.7667	2.95691
	0	1	15	20.8	28.3	24.5667	3.75011
	0	1	30	25.3	29.1	27.3	1.90788
	0.5	0	3	23.2	28.1	26.3	2.69629
	0.5	0	15	27.4	34.1	30.3333	3.42685
	0.5	0	30	28.4	33.1	31.2333	2.49466
	1	0	3	12.1	18.3	15.3	3.10483
	1	0	15	19.5	25.6	22.4	3.06105
	1	0	30	20.1	27.8	24.7	4.06325
	0.5	0.5	3	10	12.1	11.1	1.05357
	0.5	0.5	15	11.5	16.7	15.6	2.74044
	0.5	0.5	30	12.3	18.1	15.3	2.90517
L. acidophilus	0	0	3	38.7	46.8	41.8333	4.3501
	0	0	15	38.7	46.8	41.8333	4.3501
	0	0	30	38.7	46.8	41.8333	4.3501
	0	0.5	3	31.3	38.4	35.3	3.63456
	0	0.5	15	35.8	40	37.6667	2.13854
	0	0.5	30	38.8	42.4	40.1333	1.97315
	0	1	3	20	27.5	24.5667	3.75011
	0	1	15	22	31.2	26.2	4.65188
	0	1	30	31.8	37.4	33.7667	3.15013
	0.5	0	3	28.1	35.4	31.6667	3.65285
	0.5	0	15	36.2	40.4	38.7667	2.25019
	0.5	0	30	26	33	29	3.60555
	1	0	3	19.3	25.1	22.4	2.92062
	1	0	15	21.2	26.4	23.7	2.60576
	1	0	30	28.3	35.6	31.1667.	30.89401
	0.5	0.5	3	12.3	17.6	15	2.65141
	0.5	0.5	15	16.5	21.4	19.6333	2.72091
	0.5	0.5	30	19.5	26.1	23.6	3.57911
S. sanguinis	0	0	3	34.2	40.2	37.3	3.005
	0	0	15	34.2	40.2	37.3	3.005
	0	0	30	34.2	40.2	37.3	3.005
	0	0.5	3	29.2	33.6	31.6	2.22711
	0	0.5	15	30.5	38.2	33.3	4.25793
	0	0.5	30	33.2	40.4	36.4667	3.646
	0	1	3	19.5	24.3	21.3333	2.59294
	0	1	15	21	29.2	25.4333	4.14045
	0	1	30	27.2	33.1	30.1667	2.95014
	0.5	0	3	25.1	32.4	27.6333	4.13078
	0.5	0	15	26.7	31.7	29.4	2.52389
	0.5	0	30	32.2	38.8	35.2	3.34066
	1	0	3	16	21.2	18.4333	2.61598
	1	0	15	18.4	25.3	21.2667	3.5949
	1	0	30	25.4	30	27.6667	2.30072
	0.5	0.5	3	10.1	16.3	13.5333	3.15331
	0.5	0.5	15	12.8	17.2	15.5	2.36432
	0.5	0.5	30	16.2	20	18.0667	1.90088

all composite groups containing nanoparticles ($p < 0.001$). Addition of a combination of TiO_2 and SiO_2 nanoparticles had the greatest effect on all three groups of bacteria.

DAD Test

None of the composite samples containing nanoparticles created growth inhibition zones in any microorganism culture.

Eluted Component Test

The colony count of all bacteria significantly decreased in all composite groups containing nanoparticles ($p < 0.001$). Addition of a combination of both types of nanoparticles to composite had the greatest effect on all three groups of bacteria. However, 1% TiO_2 and 1% SiO_2 had the same effect on *L. acidophilus* as the combination of the two nanoparticles. The release of nanoparticles decreased with time. Table 2 shows the results.

Discussion

Nanotechnology has been used in the fabrication of dental composite resins with improved long-term antimicrobial activity and excellent mechanical properties.[11] One possible strategy for caries prevention after orthodontic treatment may be the addition of antimicrobial agents to orthodontic adhesives.

The results of this study confirmed that addition of nano-SiO_2 and nano-TiO_2, especially in combination with each other, to an orthodontic composite resin enhanced its antimicrobial properties. By an increase in concentration of added nano-SiO_2 and nano-TiO_2, the antimicrobial property increased as well.

Ahn et al. showed that orthodontic adhesives containing silver nanofillers can prevent enamel demineralization without compromising the physical properties of the adhesive.[10] As a result of the present study, the growth inhibition zone of microorganisms around the composite disks by the DAD test revealed that addition of nano-SiO_2 and nano-TiO_2 to composite had no significant effect on the growth inhibition zone of microorganisms around the composite disks, and the diameter of the growth inhibition zone around all disks was zero (similar to the control group). In other words, the concentrations of nano-SiO_2 and nano-TiO_2 added to the composite in this study did not have any inhibitory effect on the bacteria in this test. This finding is probably due to the inability of these concentrations of nanoparticles to penetrate and spread in the solid agar medium. Sodagar et al. in 2016 evaluated the antimicrobial efficacy of addition of TiO_2/SiO_2 nanoparticles.[16] Poosti et al. in 2012 indicated optimal antimicrobial property following addition of TiO_2 nanoparticles to orthodontic composites.[17] Sodagar et al.[13] showed that TiO_2 nanoparticles in 1, 5, and 10% concentrations were effective in formation of growth inhibition zone in *S. mutans* and *S. sanguinis* cultures. It seems that the difference between our findings and those of Sodagar et al.[13] is due to the concentration of TiO_2 used. Lower concentrations of TiO_2 in our study (0.5 and 1%) are probably responsible for absence of growth inhibition zones.

In the biofilm inhibition test, counting the colonies in presence of each concentration of nanoparticles revealed that nano-SiO_2 and nano-TiO_2 caused a significant reduction in *S. mutans*, *S. sanguinis*, and *L. acidophilus* colony counts. Also, significant differences were noted in this respect between different concentrations of nanoparticles. In other words, the reduction in colony count was dose-dependent, and higher concentrations of nanoparticles caused a greater reduction in colony count. Also, addition of a combination of nano-SiO_2 and nano-TiO_2 conferred the highest antimicrobial activity to the composite against all three bacterial types. Similar to our study, Sodagar et al.[13] used the biofilm inhibition test and showed that *S. mutans* and *S. sanguinis* colony counts significantly decreased in presence of all concentrations of nano-TiO_2 (1, 5, and 10%). However, in contrast to our results, they demonstrated that *L. acidophilus* colony count significantly decreased only in presence of 10% concentration of nano-TiO_2. The fabrication process of the disks can cause differences in the results because TiO_2 and, in a less extent, SiO_2 are photo-sensitive and should be prepared in the dark. This factor can affect the results and explain the differences between the results of Sodagar et al.[13] and our study.

The eluted component test evaluates the antimicrobial effects of the solution into which, nanoparticles have been probably released from the nanocomposite disks over time. This test indicates the substantivity and durability of antimicrobial property. The current results revealed that all three bacterial groups had a significant difference with the control group in presence of both nano-SiO_2 and nano-TiO_2 in 0.5% concentrations. Also, the three bacterial groups had a significant difference with the control group in presence of 0.5 and 1% concentrations of SiO_2 and TiO_2 alone. However, for *L. acidophilus*, 1% concentration of nano-SiO_2 and 1% concentration of nano-TiO_2 alone were as effective as the combination of 0.5% nano-SiO_2 and 0.5% nano-TiO_2. Sodagar et al.[16] reported results similar to ours with the difference that in their study, TiO_2 showed a generally higher antimicrobial activity than SiO_2 while in the present study, no significant difference was noted in this respect between the two. This difference may be due to the different lighting conditions in the study by Sodagar et al., because in contrast to TiO_2, UVA does not reinforce the antimicrobial activity of SiO_2 (compared with the dark). Also, the monomer used in the fabrication of the disks in their study could have caused some changes resulting in a reduction in the antimicrobial activity of SiO_2 in comparison with TiO_2 while composite does not cause such a change.

Conclusion

Addition of TiO_2 and SiO_2 nanoparticles to the Transbond XT orthodontic composite confers antimicrobial property to it.

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