Effect of Addition of Nano-TiO₂, Nano-SiO₂, 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-TiO₂), nano-silicon dioxide (nano-SiO₂), and a combination of both, on antimicrobial activity of an orthodontic composite.

**Materials and methods:** Molds measuring $0.64 \times 0.5$ mm were used for the fabrication of composite disks. For this purpose, 0.5% and 1% nano-TiO₂, nano-SiO₂, and a combination of both (0.5% nano-TiO₂ and 0.5% nano-SiO₂) 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-TiO₂ and nano-SiO₂ concentrations had any significant effect on the growth inhibition zone. All tested concentrations of nano-TiO₂ and nano-SiO₂ decreased the colony count of all bacteria. The composite sample containing both nano-TiO₂ and nano-SiO₂ had the greatest efficacy for reduction of *S. mutans* and *S. sanguinis* colony counts at all three time points. Also, 1% nano-TiO₂ and 1% nano-SiO₂ had similar effects on *L. acidophilus* in eluted component test.

**Conclusion:** Addition of TiO₂ and SiO₂ nanoparticles conferred antimicrobial property to the tested orthodontic composite.

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

**Keywords:** Antimicrobial activity, Nano-TiO₂, Nano-SiO₂, 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. They, thus, 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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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. Evidence shows that nanocomposites and nanomonomers may be suitable for bonding because they provide clinically acceptable shear bond strength. Also, it has been indicated that is sensitive to silver, zinc oxide, and gold nanoparticles. Thus, these compounds can be clinically beneficial. Recent studies have confirmed the antimicrobial properties of titanium oxide (TiO2) against Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and L. acidophilus. Haghi et al. evaluated the antimicrobial properties of TiO2 nanoparticles (nano-TiO2) against E. coli and showed that nano-TiO2 created some pores in the bacterial cell wall that enhanced its permeability and caused death. 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 TiO2 such as its white color, low toxicity, high stability, efficiency, availability, and low cost have made it a suitable antimicrobial additive for dental materials.

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 (SiO2) are suitable carriers due to their porous structure and surface adsorption. Nano-silicon dioxide (nano-SiO2) 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 TiO2/SiO2 nanoparticles. Poosti et al. in 2012 indicated optimal antimicrobial property following addition of TiO2 nanoparticles to orthodontic composites.

Considering all the above, this study aimed to assess the effect of addition of nano-TiO2, nano-SiO2, 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 SiO2 and TiO2
For the fabrication of these nanocomposite samples, nano-TiO2 and nano-SiO2 were purchased from Mehrigan 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. Composites with 0.5% and 1% nano-SiO2, 0.5% and 1% nano-TiO2, and a combination of both (0.5% nano-TiO2 and 0.5% nano-SiO2) 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% CO2 (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 × 108 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-SiO2, 0.5% and 1% TiO2, a combination of 0.5% TiO2 and 0.5% SiO2, 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 × 104 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 added to the middle row of the well. The biofilm was 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.

Disk Agar Diffusion (DAD) Method
A bacterial suspension containing 5.1 × 104 CFUs/mL was prepared 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 SiO2 and TiO2 were placed in microtubes containing 1 mL of sterile artificial saliva composed of 2 g NaCl, 0.2 g KCl, 0.453 g CaCl2·2H2O, 0.345 g
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NaH₂PO₄·2H₂O, 0.0025 g Na₂S·9H₂O, 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 $5.1 \times 10^5$ 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

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**Table 1:** Colony count of bacteria (CFUs/mL) in presence of composite disks containing 0.5 and 1% nano-TiO₂ and nano-SiO₂ and a combination of 0.5% nano-TiO₂ and 0.5% nano-SiO₂ and the control group in biofilm inhibition test

|     | TiO₂ (%) | SiO₂ (%) | 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 |
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 |
|------------|-------------|-----|---------|---------|---------|-----------------|
| 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 | 4.32685         |
| 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         |
| 0.5        | 0           | 3   | 38.7    | 46.8    | 41.8333 | 4.3501          |
| 0.5        | 0           | 15  | 38.7    | 46.8    | 41.8333 | 4.3501          |
| 0.5        | 0           | 30  | 38.7    | 46.8    | 41.8333 | 4.3501          |
| 0.5        | 0.5         | 3   | 31.3    | 38.4    | 35.3    | 3.63456         |
| 0.5        | 0.5         | 15  | 35.8    | 40      | 37.6667 | 2.13854         |
| 0.5        | 0.5         | 30  | 38.8    | 42.4    | 40.1333 | 1.97315         |
| 0.5        | 0.5         | 3   | 20      | 27.5    | 24.5667 | 3.75011         |
| 0.5        | 0.5         | 15  | 22      | 31.2    | 26.2    | 4.65188         |
| 0.5        | 0.5         | 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         |
| 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         |
| 0.5        | 0           | 3   | 34.2    | 40.2    | 37.3    | 3.005           |
| 0.5        | 0           | 15  | 34.2    | 40.2    | 37.3    | 3.005           |
| 0.5        | 0           | 30  | 34.2    | 40.2    | 37.3    | 3.005           |
| 0.5        | 0.5         | 3   | 29.2    | 33.6    | 31.6    | 2.22711         |
| 0.5        | 0.5         | 15  | 30.5    | 38.2    | 33.3    | 4.25793         |
| 0.5        | 0.5         | 30  | 33.2    | 40.4    | 36.4667 | 3.646           |
| 0.5        | 0.5         | 3   | 19.5    | 24.3    | 21.3333 | 2.59294         |
| 0.5        | 0.5         | 15  | 21     | 29.2    | 25.4333 | 4.14045         |
| 0.5        | 0.5         | 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 TiO2 and SiO2 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% TiO2 and 1% SiO2 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-SiO2 and nano-TiO2, especially in combination with each other, to an orthodontic composite resin enhanced its antimicrobial properties. By an increase in concentration of added nano-SiO2 and nano-TiO2, the antimicrobial property increased as well.

Ahn et al. showed that orthodontic adhesives containing silver nanoparticles 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-SiO2 and nano-TiO2 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-SiO2 and nano-TiO2 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 TiO2/SiO2 nanoparticles.16 Poosti et al. in 2012 indicated optimal antimicrobial property following addition of TiO2 nanoparticles to orthodontic composites. Sodagar et al.13 showed that TiO2 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 TiO2 used. Lower concentrations of TiO2 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-SiO2 and nano-TiO2 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-SiO2 and nano-TiO2 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-TiO2 (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-TiO2. The fabrication process of the disks can cause differences in the results because TiO2 and, in a less extent, SiO2 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. 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-SiO2 and nano-TiO2 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 SiO2 and TiO2 alone. However, for L. acidophilus, 1% concentration of nano-SiO2 and 1% concentration of nano-TiO2 alone were as effective as the combination of 0.5% nano-SiO2 and 0.5% nano-TiO2. Sodagar et al.16 reported results similar to ours with the difference that in their study, TiO2 showed a generally higher antimicrobial activity than SiO2 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 TiO2, UVA does not reinforce the antimicrobial activity of SiO2 (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 SiO2 in comparison with TiO2 while composite does not cause such a change.

**Conclusion**

Addition of TiO2 and SiO2 nanoparticles to the Transbond XT orthodontic composite confers antimicrobial property to it.

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