

## Antibacterial Effects of Silver and Titanium Dioxide Nanoparticle Solutions on *Streptococcus mutans* on Thermoplastic Retainers

Nadya Amanda Paramita<sup>1\*</sup>, Maria Purbiati<sup>2</sup>, Nia Ayu Ismaniaty<sup>2</sup>

1. Orthodontics Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

2. Department of Orthodontics, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

### Abstract

Thermoplastic retainers with large retention areas can create a conducive environment for *Streptococcus mutans* colonization. Nanoparticle materials have recently been developed as antibacterial agents and have been scientifically proven to decrease bacterial colonization. This study compared the effectiveness of two types of nanoparticle solutions, silver nanoparticle (AgNP) and titanium dioxide nanoparticle (TiO<sub>2</sub>NP) as disinfectants for thermoplastic retainers by measuring the change in *S. mutans* colony forming units. This laboratory experiment counted the total number of *S. mutans* bacteria before and after treatment with the test nanoparticle solutions.

A set of 24 thermoplastic retainers were divided into three groups. The AgNP solution was applied to the first retainer group; the TiO<sub>2</sub>NP solution was applied to the second retainer group; and the last retainer group served as a control. There was a statistically significant decrease in the *S. mutans* colony counts after both nanoparticle solutions were applied to the thermoplastic retainers.

No significant difference in disinfectant effectiveness was found between the two types of nanoparticle solutions. Both AgNP and TiO<sub>2</sub>NP solutions were effective in reducing the *S. mutans* colony count on thermoplastic retainers.

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### Introduction

During orthodontic treatment, the retention stage aims to maintain the arch position of a targeted tooth that has been repositioned.<sup>1</sup> As Oppenheim pointed out in 1934,<sup>2</sup> retention is one of the most important and difficult phases of orthodontic treatment. Patients who have completed orthodontic treatment are required to use a retainer to stabilize the position of the corrective gear.<sup>3</sup> Recent clinical trials have shown that dental retention can be achieved with the use of both fixed and removable retainers.<sup>1</sup>

The long-term use of retainers may lead to an increase on dental surfaces of fungi, such as *Candida albicans*, and bacteria, such as pathogenic methicillin-resistant *Staphylococcus aureus* (MRSA), which could result in local or systemic infections.<sup>4</sup>

A study conducted by Turkoz et al<sup>2</sup> suggested that the use of thermoplastic retainers covering the palatal, lingual, labial and buccal tooth surfaces may act as plaque accumulation mediums in the oral cavity during retention periods, altering the oral microbial composition and preventing the cleansing effects of saliva on the teeth and mucosa, leading to enamel demineralization and gingival inflammation. Turkoz et al. also reported that the use of thermoplastic retainers could create conducive conditions for *S. mutans* and *Lactobacillus* colonization. Similarly, Battoni et al. found that removable orthodontic tools with larger retention areas resulted in increased adhesion and colonization of *S. mutans*.<sup>2,5</sup>

A variety of antibacterial agents are used to reduce the risk of enamel demineralization during orthodontic treatment. A recent approach involves adding nanoparticle materials to the retention device.<sup>6</sup>

\*Corresponding author:

Nadya Amanda Paramita  
Department of Orthodontics,  
Faculty of Dentistry, Universitas Indonesia, Jakarta,  
Indonesia E-mail: nadya.amandap@gmail.com

Nanoparticles are insoluble particles that are less than 100 nm in size. Compared with nanoscale particles, nanoparticles have a larger surface-to-volume ratio and form closer interactions with bacterial membranes, attacking microorganisms across a wider area. With an increasing number of bacterial strains becoming resistant to conventional antibiotics, metal nanoparticles offer an antibacterial alternative. Two options are silver nanoparticles (AgNP) and titanium dioxide nanoparticles (TiO<sub>2</sub>NP).<sup>7</sup>

AgNP and TiO<sub>2</sub> were used as antibacterial agents on orthodontal retainers by Nasrin et al. and Sodagar et al.<sup>6</sup> In these studies, AgNP were added to the acrylic resin of a Hawley retainer. The results suggested that the incorporation of AgNP with a size of 40 nm and a concentration of 500 ppm had a strong antimicrobial effect on *S. mutans* colony formation.<sup>6</sup>

The use of AgNP and TiO<sub>2</sub> on thermoplastic retainers has not been previously reported. The effectiveness of AgNP and TiO<sub>2</sub> applications on thermoplastic retainers to reduce *S. mutans* bacterial colonies that may cause enamel demineralization during the retention period needs to be assessed.

### Materials and methods

This study was a laboratory experiment. A sample size calculation was used to determine the minimum number of test samples in each group. This calculation factor  $\alpha=5\%$  (type I error),  $\beta=20\%$  (type II error), standard deviation = 38 and a mean difference = 40.31. The results indicated that there should be eight samples in each group, with a test power of 80%. This study was a laboratory experiment to count the *S. mutans* colonies on the surface of thermoplastic retainers. The test samples were divided into three groups. The first group of eight retainers received an application of AgNP solution; the second group of eight retainers received an application of TiO<sub>2</sub>NP solution; and the last group of eight retainers served as the control (receiving an application of Aquadest).

This laboratory experiment calculated the number of *S. mutans* bacteria on thermoplastic retainers that had been treated with solutions of either AgNP, TiO<sub>2</sub> or the Aquadest control. The AgNP solution was prepared by mixing silver nitrate (AgNO<sub>3</sub>-) with sodium borohydride (NaBH<sub>4</sub>). The TiO<sub>2</sub> solution was obtained by mixing TiO<sub>2</sub> nanoparticles with sterile MQ

solution, followed by 30 minutes of ultrasound treatment and 20 minutes of autoclaving.

### Preparation of the Retainers

The retainers used in this study were made from Essix thermoplastic with a 0.004-inch depth and were attached to a non-vital premolar tooth that had been removed from patient. Prior to the attachment, the premolar teeth were immersed for 72 hours in a plaque-forming solution and incubated at 37°. Plaque-forming solution is consisted of 15 ml of sterile sucrose, 30 ml of Brain heart infusion broth and 5 ml of *S. mutans* cultured. The lingual surfaces of the premolars were then swabbed; each swab was placed in a sterile PBS solution and cultured on TYS20B medium. After the *S. mutans* proliferated, the amount of bacteria on the medium was calculated and designated as T0. The test solutions (AgNP, TiO<sub>2</sub>NP and Aquades) were applied to the retainers; then the retainers were reattached to the dental specimen and incubated for 72 hours at 37°. After 72 hours, the premolar teeth was again swabbed on the lingual surface, the swab was cultured on TYS20B medium (as discussed above), and the resulting calculation of *S. mutans* colonies was designated as T1.

### Statistical Analysis

Since the number of samples in each group was less than 50, the collected group data of the number of *S. mutans* colonies before and after treatment were tested with the Saphiro-Wilk test. The test results for each solution group before and after treatment were analyzed for normality. The data for the AgNP solution group before and after treatment, the TiO<sub>2</sub>NP solution group before treatment, and the control solution group were normally distributed ( $p>0.05$ ). The data for the TiO<sub>2</sub> solution group after treatment was not normally distributed ( $p$  value  $<0.05$ ). Due to their normal data distribution, the T-test was used with pairs to determine the significance of the difference in the number of *S. mutans* colonies before and after treatment in the AgNP solution group and the control solution group. As the TiO<sub>2</sub> nanoparticle solution group had an abnormal data distribution, the Wilcoxon test was used to analyze this data. Subsequent bivariate analysis compared both solution groups using the Mann Whitney test.

## Results

Before calculating the number of bacterial colonies, the researchers assayed the data reliability using inter-observer and intra-observer tests. The bacterial colony count calculations among the researchers under the supervision of the laboratory workers were tested using the Interclass Correlation Coefficient test. The inter-observer test was performed by taking ten samples. The initial calculations before the solution treatments obtained an  $r$  value = 0.870 ( $r > 0.8$ ), and the final calculations after the solution treatments resulted in  $r = 0.994$  ( $r > 0.8$ ). The intra-observer test was performed by the researcher by repeatedly counting the sample as many as ten times. The test results before the solution treatments yielded  $\alpha = 0.905$ , and the results after the solution treatments yielded  $\alpha = 0.996$  ( $r > 0.8$ ). Values of  $r > 0.8$  indicate good agreement on both inter-observer and intra-observer calculations.

Groups	R Values	Information
<i>Inter-observer</i>		
Before treatment	0.870	$r > 0.8$
After treatment	0.994	$r > 0.8$
<i>Intra-observer</i>		
Before treatment	0.905	$r > 0.8$
After treatment	0.996	$r > 0.8$

**Table 1.** Calculation of The Inter-Observer and Intra-Observer Tests Before and After Treatments.

After calculating the number of *S. mutans* bacterial colonies, the average number of bacterial colonies in the AgNP solutions before the treatments was 114 CFU/ $\mu$ L with a standard deviation of 11.19 CFU/ $\mu$ L. The smallest bacterial colony count was 102 CFU/ $\mu$ L, and the largest bacterial colony count was 137 CFU/ $\mu$ L. Based on the interval estimation calculations, a 95% confidence interval could be applied to the results, indicating that the average number of bacterial colonies in the AgNP solution before the treatments was between 104.65 CFU/ $\mu$ L and 123.35 CFU/ $\mu$ L. In the TiO<sub>2</sub> solutions before the treatments, the average number of bacterial colonies was 116.87 CFU/ $\mu$ L with a standard deviation of 38.69 CFU/ $\mu$ L. The smallest bacterial colony count was 31 CFU/ $\mu$ L, and the largest bacterial colony count was 161 CFU/ $\mu$ L. Based on the interval estimation calculations, a 95% confidence interval could be applied to the results, indicating that the average number of bacterial colonies in the TiO<sub>2</sub> solutions before the

treatments was between 84.53 CFU/ $\mu$ L and 149.22 CFU/ $\mu$ L.

In the AgNP solutions after the treatments, the average number of bacterial colonies was 44.75 CFU/ $\mu$ L with a standard deviation of 41.71 CFU/ $\mu$ L. The smallest bacterial colony count was 4 CFU/ $\mu$ L, and the largest bacterial colony count was 120 CFU/ $\mu$ L. Based on the interval estimation calculations, a 95% confidence interval could be applied to the results, indicating that the average number of bacterial colonies in the AgNP solutions after the treatments was between 9.88 CFU/ $\mu$ L and 79.62 CFU/ $\mu$ L. In the TiO<sub>2</sub> solutions after the treatments, the average number of bacterial colonies was as much as 33.13 CFU/ $\mu$ L with a standard deviation of 28.85 CFU/ $\mu$ L. The smallest bacterial colony count was 6 CFU/ $\mu$ L, and the largest bacterial colony count was 98 CFU/ $\mu$ L. Based on the interval estimation calculations, a 95% confidence interval could be

applied to the results, indicating that the average number of bacterial colonies in the TiO<sub>2</sub> solutions after the treatments was between 9.01 CFU/ $\mu$ L and 57.25 CFU/ $\mu$ L.

Antibacterial Nanoparticle on Thermoplastic Retainer	Mean (CFU/ $\mu$ L)	SD	Minimum - Maximum	95% CI
<i>Before Treatment</i>				
AgNP Solution	114	11.1	102–137	104.65–123.35
TiO <sub>2</sub> Solution	116.87	38.6	31–161	84.53–149.22
Control Group	124.5	29.4	99–149	99.9–149.1
<i>After Treatment</i>				
AgNP Solution	44.75	41.7	4–120	9.88–79.62
TiO <sub>2</sub> Solution	33.13	28.8	6–98	9.01–57.25
Control Group	157.4	27.0	125–200	134.8–178

**Table 2.** *S. Mutans* Colony Count Calculations Before and After Treatments for Each Group.

To analyze the differences in the number of *S. mutans* colonies after the AgNP and TiO<sub>2</sub> solutions were applied to the retainers, a Mann-Whitney test was performed. The results showed that the average bacterial colony count in the AgNP solutions after the treatments was greater than that of the TiO<sub>2</sub> solutions after the treatments (8.69 > 8.31). The analysis also calculated that  $p = 0.875$  and identified a mean alpha of 5%, indicating that there was no significant difference between the average

number of bacterial colonies in the AgNP solutions after the treatments compared to that in the TiO<sub>2</sub> solutions after the treatments.

Antibacterial Nanoparticle on Thermoplastic Retainer	n	Mean Rank (CFU/ $\mu$ L)	P Value
After Treatment			
AgNP Solution	8	8.69	0.875*
TiO <sub>2</sub> Solution	8	8.31	

**Table 3.** The Mann-Whitney Test Results Comparing the Number of *S. Mutans* Colonies After Treatments with Silver and Titanium Dioxide Nanoparticle Solutions.

### Discussion

This study demonstrated the effectiveness of a TiO<sub>2</sub>nanoparticle solution as a dental disinfectant. The number of *S.mutans* colonies before TiO<sub>2</sub> treatment was significantly more than after the treatment. Adams et al. suggested that TiO<sub>2</sub>could inhibit bacterial growth at high concentrations.<sup>8,9</sup>In the current study, the TiO<sub>2</sub>concentration was 1%. Nano particle size is generally not considered to be a significant factor in antibacterial potency. The TiO<sub>2</sub> nanoparticles used in this study were <100 nm.

Comparisons between the AgNP and TiO<sub>2</sub> treatment groups did not reveal any statistically significant differences (p> 0.05), the solutions had similar effectiveness. In contrast, Sierra et al. (2009) reported that AgNP were more effective against *S. mutans* than other metal nanoparticles, such as gold and zinc, and that AgNP were more effective than traditional disinfectants commonly used in dentistry, such as chlorhexidine.<sup>9</sup> The similarity in the antibacterial effectiveness of the AgNP and TiO<sub>2</sub> solutions used in this study may be due to a variety of factors, including the concentration and size of the nanoparticles, the presence of light activation and the species of the target. Regarding the last factor, several studies have shown that AgNP have higher antibacterial properties against Gram-negative rod-shaped bacteria than Gram-positive coccus bacteria, like *S. mutans*. This difference may be related to structural differences in the bacterial cell walls, as Gram-positive bacteria have higher peptidoglycan concentrations.

TiO<sub>2</sub> acts as a photocatalyst, utilizing light to produce an active material that kills bacteria. This antibacterial reaction is more effective when the TiO<sub>2</sub>particles are exposed to ultraviolet light, as reported by Sodagar et al., where the addition of 1% TiO<sub>2</sub> nanoparticles to an acrylic resin reduced the number of bacteria.<sup>7</sup>TiO<sub>2</sub> nanoparticles were more effective against *S .mutans* if they were applied outdoors for one hour under sun exposure or under UVA light with an intensity of 1 mW/cm<sup>2</sup>. Similarly, Wang et al. showed increased TiO<sub>2</sub>antibacterial activity against *S. aureus* after the nanoparticles were exposed to UV radiation. In contrast, Liu et al. studied TiO<sub>2</sub> antibacterial activity against *Escherichia coli* bacteria and found no difference in the antibacterial activity of nanoparticles that were exposed to ordinary light and those that were exposed to UV light.<sup>10</sup>In the present study, the TiO<sub>2</sub> antibacterial effectiveness was assessed under laboratory room lighting.

The similar antibacterial effectiveness of both solutions used in this study indicates that both may be used as antibacterial agents in thermoplastic retainers. However, since this was an in vitro study, further research is required. In addition, this study did not test nanoparticle solutions of varying concentrations, so further research is needed to determine the most effective nanoparticle concentration to inhibit bacterial growth.

### Conclusions

The application of AgNP and TiO<sub>2</sub> solutions to thermoplastic retainers was statistically effective in decreasing the number of *S. mutans* colonies on the retainers. There was no statistically significant difference between the AgNP and TiO<sub>2</sub> solutions in their ability to decrease the number of *S.mutans* colonies; both were similarly effective in reducing the number of *S.mutans* colonies.

### Declaration of Interest

The authors report no conflict of interest and this research was approved and funded by the HBAH PITTA for directorate research and community engagement at the Universitas Indonesia.

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