Comparison of AgNP Antibacterial Effects with Resterilization Autoclave Technique in Used TADs Orthodontics

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Abstract

Repeatedly autoclaved TADs have shown surface alterations which might lead to instability, whereas there is possibility of reuse TADs for same patient to reduce costs. *Porphyromonas gingivalis* (PG), the most common type of bacteria found in peri-implantitis, causes failure of TADs insertion. Antibacterial properties of silver nanoparticles (AgNPs) has commonly used in orthodontic. However, no study has described the antibacterial effects of AgNPs to PG colonies on used TADs surfaces.

To evaluate the antimicrobial effects of AgNPs solution compared with autoclaving on the number of PG colonies in used TADs. 20 new TADs were separated into 2 groups : P1 (AgNPs solution) and P2 (autoclave). The PG antibacterial activity was evaluated by comparing the number of PG colonies on Brusella agar plates using digital electronic colony counter (103 CFU/mL) on T0 (after immersed in a plaque-forming solution containing PG ATCC 33277 and cultured under anaerobic conditions for 24 h) and T1 (after resterilized using 2 different methods and cultured under anaerobic conditions for 24 h). No PG colonies survived in both groups on T1. AgNPs solution was found to have antibacterial activity against PG equivalent to autoclaving.

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Introduction

Sterilization of instruments and devices used in medical treatment has become a required procedure to prevent bacterial infection. According to the World Health Organization, sterilization is defined as a process that kills biological agents that cause unfavorable contamination.[1] Autoclaving involves the use of steam in a range of 121 °C–134 °C,[1,2] and it has become the most widely used and reliable method of sterilization because it can kill all biological agents, by destroying enzymes and other essential cell constituents.

The technology in orthodontics at present has led to the development of implant-assisted orthodontics. Orthodontic implants or temporary anchorage devices (TADs) have become the most common type of anchorage because of their many advantages, including their low cost and simple surgical placement and removal. Their size is also an advantage because it enables their use in many anatomical regions, including interdental areas.[3,4] More than 80% of orthodontists in a recent survey reported that they had at least one active case involving TADs in their practice. Despite their wide functionality, failure of insertion might be a reason to reuse used TADs in the same patient. Used TADs should be sterilized before reuse. Sterilization using an autoclave, however, can alter the microstructure of TADs after one or more additional cycles, and this in turn could affect their integration and stability during use.[5,6] Most failure insertion cases of TADs involve peri-implantitis. Bacteria possibly associated with the development of peri-implantitis have been related to periodontal disease and include *Porpyromonas gingivalis* (PG). Both *Streptococcus mutans* and PG have been the most commonly observed bacteria in the peri-implant sulcus.[7,8]

Nanotechnology has revolutionized some areas in science and technology. The British

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Standards Institution defined nanoparticles as particles in the nanoscale range (dimension range from 1 to 100 nm). There are several metals that have nano-sized particles, such as silver, gold, alloys, magnesium, zinc, titanium, and copper. It is well known that silver ions and silver-based compounds are highly toxic to microorganisms, and they have shown strong biocidal effects on as many as 16 species of bacterial components and proven to be most effective against bacteria, viruses, and other eukaryotic microorganisms. Because of its bactericidal effects, silver has been widely used in the medical and orthodontics fields (for nano-coatings archwires/brackets or mixed with resin composites).[9–11]

The utility of TADs for orthodontists and the possibility of negative effects of resterilization using an autoclave has increased interest in new ways to sterilize TADs that do not cause adverse effects on these devices. The aim of this study was to evaluate and compare the antimicrobial effects of silver nanoparticles (AgNPs) solution and autoclave resterilization on the number of PG colonies in used TADs.

Materials and methods

This research was a laboratory experimental study that was approved by the Ethics Commitee of Universitas Indonesia, Faculty of Dentistry (Ethics No.08/Ethical Exempted/FKGUI/V/2017).

The study samples were 20 new TADs that were 7 mm in length and 1.2 mm in diameter. The samples were separated into two groups: P1 and P2. The P1 group was sterilized by using AgNPs solution,[12] and the P2 group was sterilized by using an autoclave. Before resterilization, all samples were immersed in a plaque-forming solution containing PG (ATCC 33277) and cultured under anaerobic condition for 24 h. The aim of this stage was to mimic the condition of TADs that had been used in a human oral cavity. After immersion and culturing, the materials on the TADs surfaces were obtained by swabbing with a sterile cotton pellet. The pellet with collected material was then cultured on a *Brucella* agar plate for 24 h under anaerobic conditions (T0). The number of PG colonies was counted by using an digital electronic colony counter (ECC) from Sun Lab Equipment, Neh Delhi, India. The number of PG colonies in T0 were subjected to intraobserver and interobserver tests to assess the reliability of the measurements.

P1 was resterilized in AgNPs solution for 180 min, and P2 was resterilized in an autoclave for 40 min at 121 °C (250 °F). The culturing steps above were repeated to grow any surviving PG colonies after resterilization. The number of PG colonies was counted by using an ECC and assessed by intraobserver and interobserver tests. Their antimicrobial activity was evaluated by comparing the number of PG colonies (10^3 CFU/mL) before and after resterilization. Data analysis was performed by using the SPSS v20.0 program with the Cronbach alpha (reliability test) and the Wilcoxon (univariate) and unpaired *t*-test (bivariate) if applicable. The level of statistical significance was set to $p < 0.05$.

Results

The Cronbach alpha test showed strong reliability for the measured numbers of PG colonies in this study (Tables 1 and 2). Figure 1 shows the numbers of PG colonies at T0 in both sterilization groups. The unpaired *t*-test proved that there was no statistically significant difference in the numbers of PG colonies between the sterilization groups (Table 3). The Shapiro–Wilk normality test showed that the distribution of T1 was abnormal in both groups and normal for T0 in both groups. Figure 3 shows that there were no surviving PG colonies on the *Brucella* agar plates in both groups after resterilization (T1). The Shapiro–Wilk normality test showed that the data distributions of T1 were abnormal in both groups.

	Cronbach Alpha	Reliability
Intraobserver	0.943	Strong
Interobserver	0.963	Strong
n total	20	

*Values: poor < 0.7; fair ≥ 0.7; strong ≥ 0.8

Table 1. Cronbach alpha test for reliability of the measured numbers of *Porphyromonas gingivalis* colonies (T0, P1).

	Cronbach Alpha	Reliability
Intraobserver	0.917	Strong
Interobserver	0.875	Strong
n total	20	

*Values: poor < 0.7; fair ≥ 0.7; strong ≥ 0.8

Table 2. Cronbach alpha test for reliability of the measured numbers of *Porphyromonas gingivalis* colonies (T0, P2)

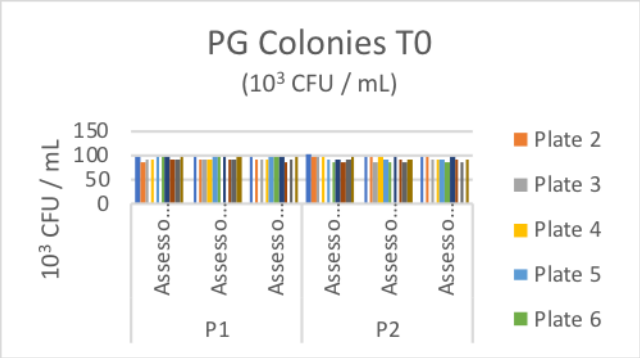

Figure 1. The number of *Porphyromonas gingivalis* colonies on P1 and P2 (T0)

Colonies of *Porphyromonas gingivalis**	N	Mean ± SD	p**
P1	10	93.30 ± 3.561	0.844
P2	10	92.20 ± 5.250	

*103 CFU/mL
**p < 0.05 = significant

Table 3. Unpaired *t*-test for differences in the PG colonies (T0) between P1 and P2

The *Wilcoxon* test showed that the number of PG colonies was reduced significantly (Tables 4 and 5). These results showed that the antimicrobial activity of AgNPs was as effective as that of autoclaving against PG (Figure 2).

Time	N	Colonies of *Porphyromonas gingivalis**		p**
		Mean	SD	
sT0	10	93.3	3.561	.005
T1	10	0	0	

*103 CFU/mL
**p < 0.05 = significant

Table 4. *Wilcoxon* test of P1 at T0 and T1

Time	N	Colonies of *Porphyromonas gingivalis**		p**
		Mean	SD	
T0	10	92.2	5.350	.005
T1	10	0	0	

*103 CFU/mL
**p < 0.05 = significant

Table 5. *Wilcoxon* test of P2 at T0 and T1

Figure 2. The trend in reduction of *Porphyromonas gingivalis* (PG) colonies in the P1 and P2 sterilization groups. Both groups show the same pattern

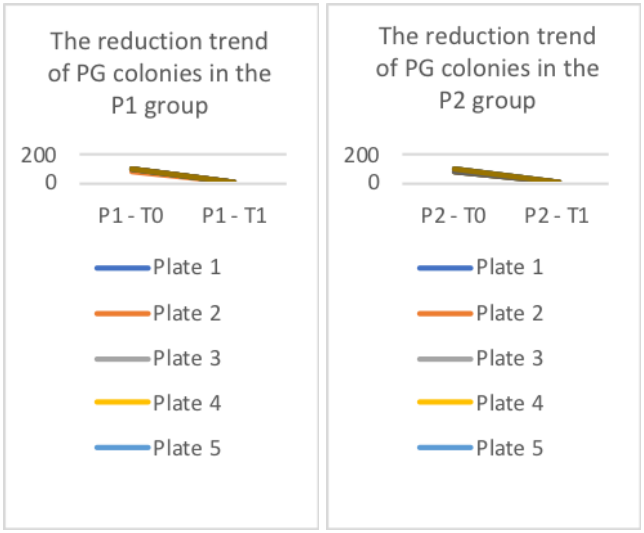

Figure 3. *Brucella* agar plates in both groups (T1-P2 left and T1-P1 right) show no surviving PG colonies after resterilization

Discussion

The antimicrobial properties of AgNPs and silver ions have been known for decades.[13,14] Silver not only has suppressive effects on bacterial growth but also efficiently destroys existing microorganisms. Similar to other nanometals, silver in the nano-size range of 1–10 nm has shown the greatest biocidal activity against germs. This study used 20 nm silver particles in consideration of its stability during antimicrobial activities. The *in vitro* experiments in this study used PG ATCC 33277 samples because that strain is an anaerobic Gram-positive red complex type commonly found in the peri-implantitis sulcus and is one of the bacteria that can cause failure of TADs insertion. In this study, the authors ensured that only PG was cultured by using ATCC 33277 and *Brucella* agar plates as the specific PG media.[15]

The mechanism of the inhibitory effects of silver ions on microorganisms is poorly unknown.

Some studies have reported that the positive charge on the silver ions was crucial for its antimicrobial activity through the electrostatic attraction between negatively charged cell membranes of microorganisms and positively charged nanoparticles. Other studies have reported that the antimicrobial activity of AgNP was dependent on the concentration of AgNP itself. Amro et al. proposed another mechanism in which metal depletion might cause formation of irregulary shaped pits in the outer membrane and change membrane permeability; this mechanism would involve progressive release of lipopolisaccharide molecules and membrane proteins. This condition might lead the organism to death.[14,16] The results of the present study are consistent with those of many other studies that have shown that AgNP has a strong bactericidal effect against PG, similar to that of autoclaving.[5,6]

The negative effect of resterilization using an autoclave cannot be avoided. Eliades et al. observed morphological and surface structural alterations in used TADs, but no material structural changes in the form of defects or pores were documented. In contrast, Mattos et al. found that used TADs showed altered surface characteristics and a wider range of fracture torque values. Further, Mattos suggested that reuse of TADs should not be recommended because there was not enough evidence of variables that can affect their expected resistance to fracture.[6,17,18]

The results of this study should prompt further research into identifying new methods of resterilizing used TADs. Unfortunately, in this study, the effect of immersion in AgNPs solution on TADs other than of the antimicrobial effects was not investigated. Future studies could use a digital torque gauge or scanning electron microscopy to study the effects of immersion of AgNPs solution on TAD composition and stability.

Conclusions

The antibacterial effect of AgNPs was compared with that of autoclave resterilization against PG by measuring the number of surviving PG colonies. Both resterilization methods showed that no PG colonies survived on TADs.

Declaration of Interest

The authors report no conflict of interest.

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