Mechanical Properties of Silver Nanoparticles Containing Tissue Conditioners

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Abstract

Objective: The purpose of this study evaluates effects of the silver nanoparticles (AgNP) on mechanical properties of tissue conditioners.

Materials and Methods: Various concentrations (10, 20, 30, 40, 50 and 60 ppm) of silver nanoparticles were incorporated into the tissue conditioners, and divided into seven groups (one control group and six experimental groups; G1-G6). Seven concentrations out of ten trouser-leg designed tissue conditioners were made for tensile tear force (TTF) test. Seventy pairs of acrylic resin bars were fabricated from stainless steel mold and spacer designed for tensile bond strength (TBS) test. The tissue conditioners with silver nanoparticles of seven concentrations out of ten each were packed into the spacers to bond each ends of acrylic resin bars. All specimens of two studies were stored in water at 37 Celsius for 48 hours and thermocycled 1,000 times in water between 5 and 55 Celsius. The specimens were tested by a universal testing machine (Shimadzu EZ test, AK5N) until the specimens were torn for tensile tear force test and de-bonded between two acrylic resin bars of tensile bond strength. Data were analyzed using a one-way ANOVA with Tukey's HSD post-hoc tests.

Result: The results revealed increasing the concentrations of silver nanoparticles in G1-G6 gave an TTF but not different from control group ($p > 0.05$). All silver nanoparticles containing groups (TBS) had statistically significant higher bond strengths than the control groups ($p < 0.05$).

Conclusion: This study indicated that silver nanoparticles do not affect the tensile tear force of tissue conditioners while a low concentration of silver nanoparticles (G1-G6) have slightly affected the mechanical properties of tissue conditioners. Silver nanoparticles containing tissue conditioners appear to be a promising and innovative material for clinical uses.

Keywords: Sliver nanoparticles, Tensile bond strength, Tensile tear force, Tissue conditioners

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Introduction

Denture stomatitis is one of the most common oral diseases of the denture wearing. The etiology of denture stomatitis is based on multifactorial features such as malnutrition and poor oral hygiene, bacterial plaque accumulation, resin porosity, trauma and allergy (1-2).

Candida albicans are involved in most of the oral infections, but they are an opportunistic microbe comprising 40% to 80% of the microbial normal flora of healthy peoples and is responsible for causing 50% to 90% of denture stomatitis (3-4). Moreover, *in vitro* experiments have shown that none of the materials had an inhibitory effect on the growth of *Candida albicans* which could penetrate to the inner portions of the soft liner (5-6).

Soft lining materials such as tissue conditioners act as drug delivery agents to bring antiseptic agents to relieve denture stomatitis. Antifungal drugs, Nystatin and Amphoteracin B, have been commonly used for the treatment of denture stomatitis (7). However, antifungal drugs have a board spectrum target in humans which doctors use for treating patients. Although Nystatin and Amphoteracin B are good antifungal drugs, they are not effective when they are incorporated in the viscogel tissue conditioners (8).

Nowadays, there are several new antifungal agents such as metallic oxide powders (9), natural and herbal oils (10), and metal nanoparticles containing in soft lining materials have been made available for treatment. Silver nanoparticles (AgNP) (11-15) are beneficial because not only do they have the ability to against micro-organisms they are also non-toxic to humans. Furthermore, silver nanoparticles are used in low concentrations to against them (12-13,16).

Even though, silver nanoparticles incorporation into tissue conditioners may impair the physical and mechanical properties of tissue conditioners. Some the mechanical properties, TTF and TBS are interesting attention, as they are lead to the most common problems in these materials (17-19).

The overall purpose of this study is to evaluate TTF and TBS of silver nanoparticles contained on tissue conditioners.

Materials and Methods

Tissue conditioners (Visco-gel®, Dentsply Co.Ltd, Germany) were prepared by mixing a powder/liquid ratio of 3 g/2 ml according to the manufacturers' instructions (Fig 1a). Silver nanoparticles (Prime Nanotechnology Co.Ltd., Chulalongkorn University, Bangkok, Thailand) (10,000 ppm) (Fig 1b) was diluted into concentrations 10, 20, 30, 40, 50 and 60 ppm respectively then added into the liquid part of the Visco-gel tissue conditioners 2 ml with seven concentration of silver nanoparticles from experimental groups: G1-G6 (Table 1).

(a) (b)

Fig 1. (a) Tissue conditioners, (b) Silver nanoparticles from Prime Nanotechnology.

Table 1. The classification of experimental groups vs control group.

Sample group	Silver nanoparticles concentration (ppm)	Silver Volume (ml)
Control	0	0.000
G1	10	0.002
G2	20	0.004
G3	30	0.006
G4	40	0.008
G5	50	0.010
G6	60	0.012

Tensile tear force test

Ten specimens of each concentration from 7 groups were provided trouser-leg tear test (20). The specimens were 50 mm long, 10 mm wide and 1 mm thick with an initial cut length of 25 mm With blade number 15 and were allowed to set at 25 Celsius for 30 minutes (Fig 2). The thermocycling test (Model: TC 301, Medical and Environmental Equipment Research Laboratory, KMITL, Thailand) was set up to a dwelling time of 30 seconds and a resting time of 6 seconds with temperatures of 5 and 55 Celsius. Then the specimens were tested on a universal testing machine (Shimadzu, EZ test, Japan) at a cross-head speed of 5 mm/min, load cell 500 Newtons until the specimens start tear and recorded maximum tensile tear force. (Fig 4a)

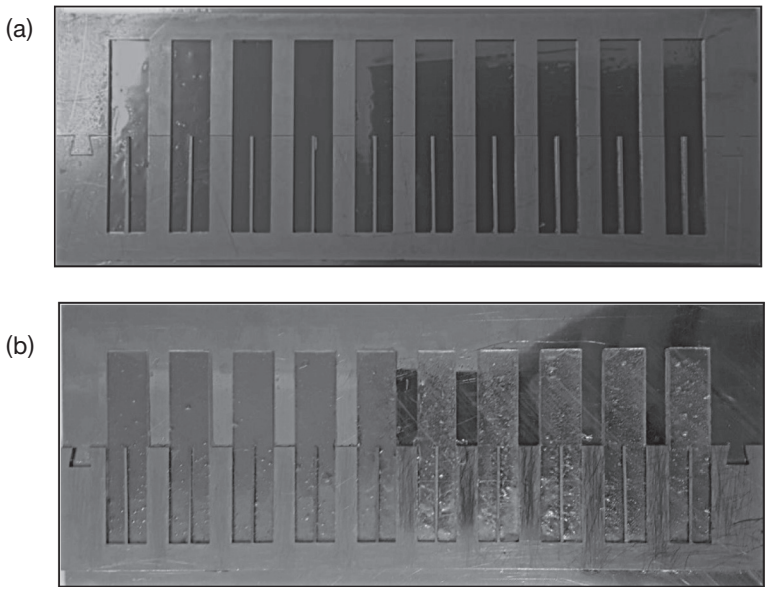

Fig 2. (a) Stainless steel mold for tear strength test specimen fabrication.
(b) Tissue conditioners were packed into stainless.

Tensile bond strength test

Stainless steel mold was made for specimens with 40 mm/long, 10 mm/wide, and 3 mm/thick and a stainless spacer bar (80 mm/long; 3 mm/wide; and 3 mm/thick) (21) (Fig 3a). Acrylic resin was mixed according to the manufacturer's instructions, pressed into the mold and processed in a water bath at 75 Celsius for 9 hours. After polymerization, the stainless spacer and acrylic specimens were removed and the acrylic resin bars were trimmed. The surface to be bonded was smoothed and cleaned by wet abrasive papers sized 220, 500, 800 and 1,000 grit, respectively. The tissue conditioners containing silver nanoparticles were packed into the space after the spacer bar removal, the mold was re-assembled and the tissue conditioners were processed according to the manufacturer's instructions (Fig 3b). After setting at 25 Celsius for 30 minutes, the specimen was removed from the mold and aged in thermocycling test.

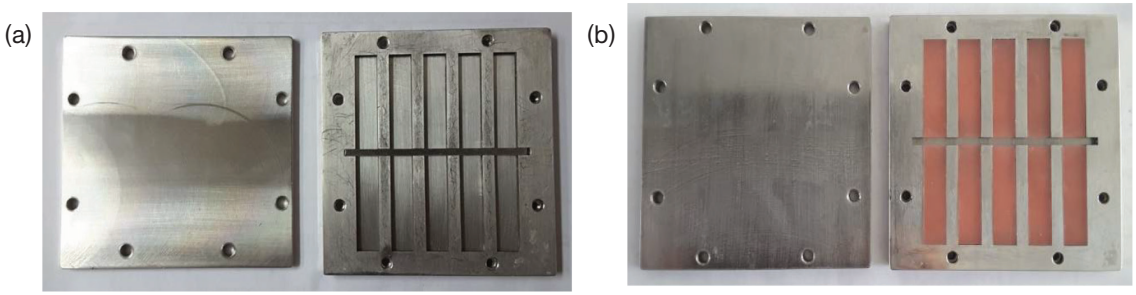

Fig 3. (a) Stainless bars and spacer designed for tensile strength test specimen fabrication
(b) Tissue conditioners was packed into the space bar in stainless mold.

The specimens were divided into seven groups out of ten; control, 10, 20, 30, 40, 50 and 60 ppm. The thermocycling test was set up to a dwelling time of 30 seconds and a resting time of 6 seconds with temperatures of 5 and 55 Celsius. Then the specimens were tested using a universal testing machine at a cross-head speed of 5 mm/min, load cell 500 Newtons until the specimens started tearing down, while recording maximum tensile strength. (Fig 4b)

(a) (b)

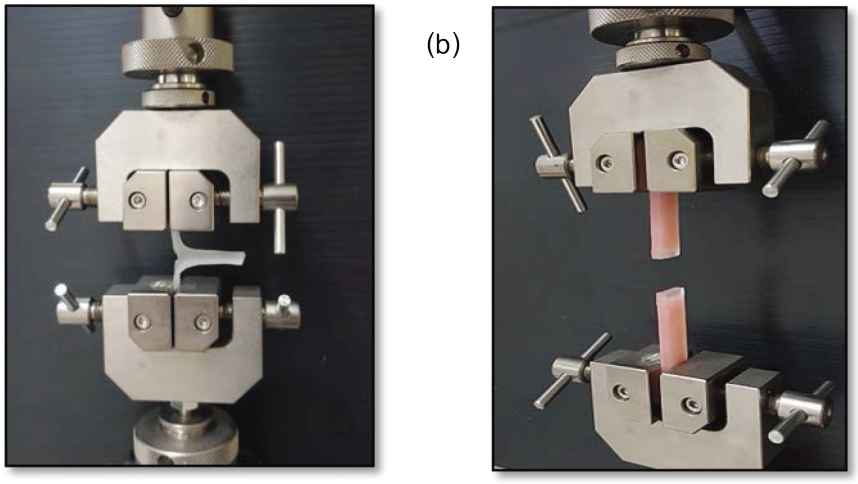

Fig 4. (a) The specimens were tested using universal testing machine for tensile tear strength. (b) The specimens were tested using universal testing machine for tensile bond strength.

In order to analyze the TTF and TBS values data, a statistic software package of SPSS version 20.0 (IBM SPSS Inc., Chicago, Illinois, USA) was used in this study. The normality distribution and homogeneity of variance were tested using the Kolmogorov-Smirnov and Levene's test, respectively. Means and standard deviations of the results from the TTF and TBS test were calculated and analyzed statistically using a one-way ANOVA with Tukey's HSD tests employed to determine the direction of significance. Alpha was set at 0.05.

Results

Tensile tear force test (TTF)

The average of TTF is shown in Table 2. The experiment group of tissue conditioner (G1-G6) have a higher trend of TTF values than the control group. However, all groups with silver nanoparticles are not significantly different from control group ($p > 0.05$).

Table 2. Average and standard deviation of tensile tear force.

Concentration of silver nanoparticles (ppm)	Average of maximum force (SD) Newton
Control	2.02 (0.17)
G1 (10 ppm)	2.04 (0.18)[a]
G2 (20 ppm)	2.06 (0.27)[a]
G3 (30 ppm)	2.05 (0.29)[a]
G4 (40 ppm)	2.03 (0.20)[a]
G5 (50 ppm)	2.12 (0.19)[a]
G6 (60 ppm)	2.03 (0.22)[a]

The same small letter indicates no significantly significant differences compared with control group ($p > 0.05$).

Tensile bond strength test (TBS)

The average of TBS values is shown in Table 3. The experiment group of tissue conditioner (G1-G6) groups have a higher trend of TBS values than the control group. All groups with silver nanoparticles are significantly different from control group ($p < 0.05$).

Table 3. Average and standard deviation of tensile bond strength.

Concentration of silver nanoparticles (ppm)	Average of maximum strength (SD) MPa
Control	0.63 (0.07)
G1 (10 ppm)	0.79 (0.11)[*, a, b]
G2 (20 ppm)	0.92 (0.30)[*, a, b, c]
G3 (30 ppm)	0.91 (0.12)[*, a, b, c]
G4 (40 ppm)	0.97 (0.09)[*, b, c]
G5 (50 ppm)	0.96 (0.13)[*, b, c]
G6 (60 ppm)	1.17 (0.07)[*]

The asterisk indicates statistically significant compared with control group ($p < 0.05$), The same small letter indicats no significant differences between groups.

Failure mode after de-bonding

Adhesive failure mode, cohesive failure mode and mixed failure mode were seen in this study (Fig 5) and their percentage of failure modes are shown in Fig 6.

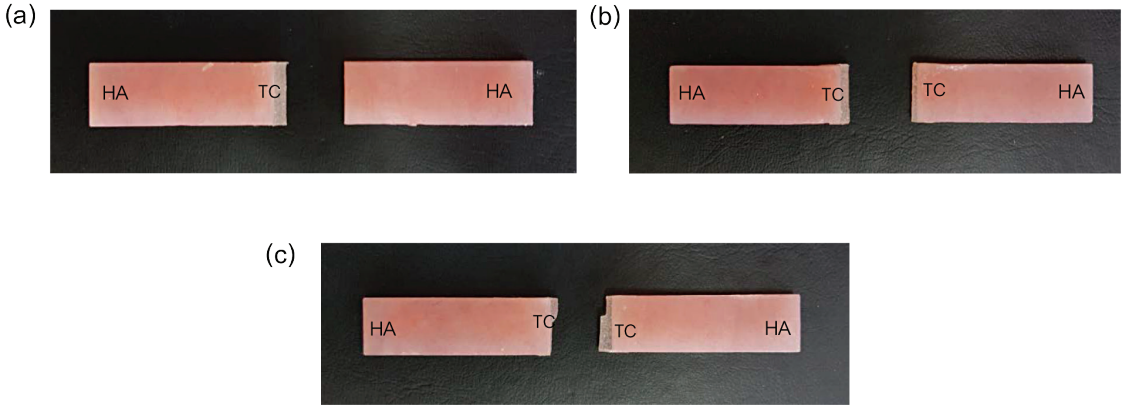

Fig 5. Failure mode after debonding (a) adhesive failure mode, (b) cohesive failure mode and (c) mixed failure mode (HA = Heat-cured acrylic resin, TC = Tissue conditioners).

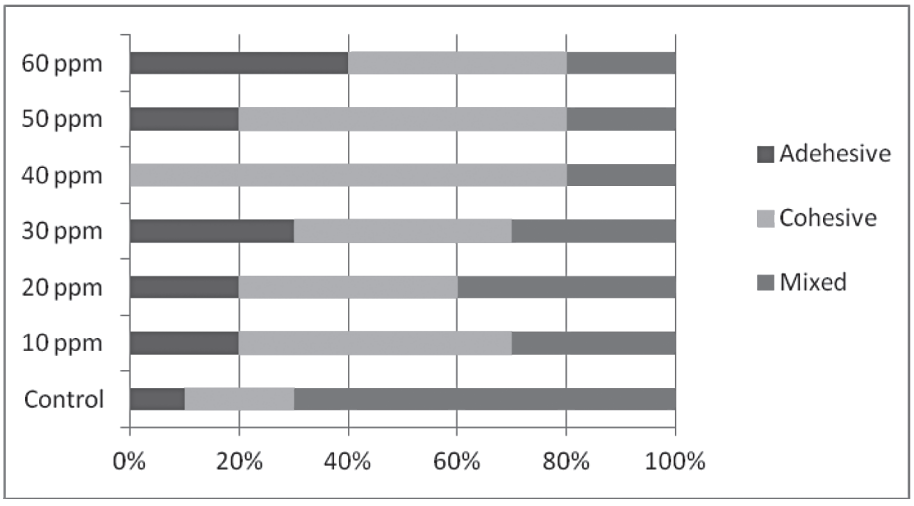

Fig 6. Percentage of failure mode after debonding in thermocycling groups.

Discussion

Previous studies Eurwongpanich et al., 2018 found that the silver nanopaticles was added to tissue conditioners to improve the antimicrobial effects of this material. Furthermore, This study examined the cytotoxicity of silver nanopaticles incorporated on the tissue conditioners at concentration levels of 10, 20, 30, 40, 50 and 60 ppm. They found that slight cytotoxicity effects of silver nanopaticles solution occurred at high concentration groups (exceeding 50 ppm) in 24 hours after mixing the tissue conditioner samples (19). However, It has not been reported that mechanical property change when silver nanopaticles were added to tissue conditioners. Therefore, our study investigated further on the mechanical properties of tissue conditioners added by silver nanopaticles.

Chladek, et al., 2013 studied in antifungal activity of denture soft-lining material modified by silver nanoparticles at six concentrations (10, 20, 40, 80, 120 and 200 ppm). They found that the average antifungal efficacy value (AFE) for samples with 40 ppm contained in silver nanoparticles was mean 31.5 (22). However, additional increases in concentration of AgNP resulted in less dynamic AFE value outputs, although there were still visible AFE value changes. Consequently, the appropriate strength of AgNP concentration was the 40 ppm concentration group. Chladek, et al., 2012 observed tendency of AgNP to form large aggregations of more than several hundred nm in the material (exceeding 80 ppm) (13). It can lead to a decrease in antimicrobial effectiveness of AgNP by the reduction of effective surface to contact microorganisms. So, the optimumal concentration should not exceed 80 ppm which the result from our study gave the similar optimal concentration.

At present, few studies have focused on mechanical property (TTF) of tissue conditioners with silver nanoparticles. This study, however, found that silver nanoparticles do not increase tensile tear force. The tear occurs in the tissue conditioner material even with or without silver nanoparticles. Oguz et al., 2007 explained that the high tear force and tensile bond strength are the desired mechanical properties of soft lining material. While the tensile strength indicates the maximum stress that can be applied uniformly on a material or piece, the tear force or tear resistance is the resistance of material to the tearing force or cut after tension is applied (23). In this study, silver nanopaticles did not significantly increase tensile tear force ($p > 0.05$) when concentration of silver nanopaticles increased. Even more, the tear occurred in the tissue conditioner material with or without silver nanopaticles. Furthermore, many factors might influence on mechanical properties of tissue conditioner, improve strength of polymer bonding, and thus provide good tensile strength. These include the increasing cycle of thermocycling, reduction of free unreaction monomers and adequate time for redistribution of molecules during stress, which would provide the high tear force and tensile bond strength (21).

This study, different concentrations of silver nanoparticles from 10 to 60 ppm resulted in the statistically significant difference of higher TBS values ($p < 0.05$), comparing to the control group. The highest concentration of the silver nanoparticles (60 ppm) have increased the maximum TBS (1.17 MPa). This implies more-strong bonding between tissue conditioners and resin acrylic bars when increase the addition of silver nanoparticles.

The result could be explained by the flowability of silver nanoparticles in the tissue conditioners, which allows the material to readily adapt to the bonding surfaces and creates a union together (17).

The adhesion between acrylic resin and tissue conditioner is important clinically. Three failure modes occurred after de-bonding in this study (TBS). The control group showed less adhesive failure than the others (Fig 6). These results occurred maybe due to the nanoparticles distribution on the bonding surface. Khumsup et al., 2017 found that when increased in silver nanoparticles concentration resulted in an increased hardness and a decrease in both water sorption and water solubility (24). Therefore, the increased hardness of tissue conditioner in high concentration groups may influence on the bonding surface between tissue conditioner and acrylic resin denture base. It is well known that silver nanoparticles made the tissue conditioners apart easily. Thus, the cohesive and mixed failure modes were more seen in all groups when silver nanoparticles containing tissue conditioners increased. On the other hand, the 40-ppm group, adhesive failure mode was not observed. It may result from experimental error such as number of specimens. In addition, tensile tear force tended to increase in high concentration nanoparticles condition. The stiffer tissue conditioner maybe transmitted the external force to the bonding site, which changed mode of failure to adhesive failure. However, further study is required to evaluate and confirm.

Conclusion

Within limitation of this study indicated the tensile tear force and the tensile bond strength values of tissue conditioners slightly increase when adding the low concentration (40 ppm) of silver nanoparticle. This concentration has less cytotoxicity and it is suitablility for further application. It appears that more adhesive failure bonding occur when adding more silver nanoparticles. Silver nanoparticles containing tissue conditioners appear to be a promising and innovative material in clinical uses.

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