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# Long-term Antibacterial Properties and Bond Strength of Experimental Nano Silver-containing Orthodontic Cements

**LI Fujun, LI Zubing*, LIU Gumei, HE Hong**
*(Hospital of Stomatology, Wuhan University, Wuhan 430079, China)*

**Abstract:** The purpose of this study was to evaluate the long time antibacterial properties and shear bond strength of experimental nano silver-containing cements (NSC). Nano silver base inorganic antibacterial powder was added to the reinforced glass ionomer cement at five different weight ratios to obtain a series of nano silver-containing cements, then the antibacterial properties of three orthodontic cement products and five NSC samples were evaluated by the direct contact test (DCT) and the agar diffusion test (ADT). The DCT, which was based on turbidness determination of bacterial growth in 96-well microtiter plates, was performed in both fresh and aged for 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, and 8 weeks tested materials. The shear bond strengthes of three orthodontic cement products and five NSC samples were examined using a universal testing machine. The ADT results indicated that there were no significant differences between NSCs and ORTHO LC fresh specimens. In the DCT experiment, all fresh silver nanoparticles-containing tested samples presented powerful antibacterial properties, but they gradually lost the effective antimicrobial agents with the extension of aging time. Finally, none of the tested materials maintained its antibacterial property after aging for 8 weeks. A gradually decreasing trend of bond strength presented with the increasing incorporation of nano silver base inorganic antibacterial powder into the glass ionomer cement, even though all the tested material specimens reached the ideal bond strength range. We may conclude that NSCs can contribute to decrease the demineralization rate around brackets without compromising bond strength.

**Key words:** long-term; antibacterial properties; bond strength; nano silver-containing cements

## 1 Introduction

Enamel demineralization is a publicly recognized complication of orthodontic treatment with a fixed appliance. The placement of fixed orthodontic appliances creates a favorable environment for the proliferation of caries-associated microorganisms, with the higher risk of caries prevalence[1]. Clinical observations have indicated that the common sites for demineralization are at the areas adjacent to the orthodontic bonding agents[2].

For the purpose of decreasing the demineralization rate during orthodontic treatment, various antimicrobial agents have been incorporated into orthodontic adhesives. Fluoride and chlorhexidine are the most common preventive additives for orthodontic use. Although initially strong, the released amounts of fluoride and chlorhexidine do not last for long periods[3-5]. In addition, antibacterial adhesives show a higher bond failure rate because incorporation of antibacterial agents affects their mechanical properties[6-8]. Despite some advances in orthodontic bonding materials and techniques in recent years, the enamel demineralization and dental caries around bracket remain unresolved. To keep long time antibacterial activity without affecting the bond strength, silver nanoparticles were incorporated into resin-modified glass ionomer cement (RMGIC, GC Fuji ORTHO LC; GC Corporation, Tokyo, Japan) in this study to acquire experimental nano-silver containing orthodontic cements.

The silver ions exhibited high bactericidal activity against oral streptococci[9]. Several studies have demonstrated that silver nanoparticle-containing adhesives achieved strong antibacterial effects against


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LI Fujun(李福军): Ph D Candidate; E-mail:lfj6666@163.com
*Corresponding author: LI Zubing(李祖兵): Prof.; Ph D; E-mail: lizubing@sina.com
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microcosm biofilms[10] and the oral pathogenic species of Streptococcus mutans[11].

In order to clinical acceptance, the new adhesives must provide long-term antimicrobial activity and display enough bond strength when compared with conventional adhesives. The purpose of this study was to compare the antibacterial effects and the bond strength of experimental nano silver-containing orthodontic cements, with those of the orthodontic adhesives currently available.

# 2 Experimental

## 2.1 Materials

To obtain the nano silver-containing cement(NSC), nano silver base inorganic antibacterial powder ($AgNaZr_2(PO_4)_3 \cdot H_2O$, AGP-ZP003 Shanghai Huzheng Nano Technology Co., Ltd, Zirconium phosphate as the carrier, silver nanoparticles diameter 5-10 nm according to scanning electron microscopy result, silver content=3% according to product description), was added to the reinforced glass ionomer cement (GC Fuji ORTHO LC; GC Corporation, Tokyo, Japan) with the weight ratio of 1:99, 3:97, 5:95, 10:90 and 15:85 respectively, which were named NSC1, NSC2, NSC3, NSC4 and NSC5, respectively. Then NSCs were mixed gently by hand in agate mortar for 7 hours to ensure homologous silver nanoparticle dispersion,

The following orthodontic adhesives were tested as control: reinforced glass ionomer cement and two composite resin cements (Transbond XT and Transbond Plus; 3M Unitek Dental Products, Monrovia, Calif).

*S mutans*, the primary cariogenic bacterium, was used as the test microorganism. *S mutans* was grown aerobically from frozen stock cultures in brain heart infusion (BHI) broth containing 0.5% bacitracin at 37° C. Bacteria were used at their late-logarithmic to early-stationary phase.

## 2.2 Agar diffusion test (ADT)

The ADT was designed to test whether the adhesives containing an antimicrobial agent could permeate through agar to produce an inhibition zone. Each plate was spread on the surface of the plate with 100 μL of freshly grown *S mutans* (OD 0.6 at 650 nm). Seven 5-mm diameter holes were punched on the agar surface of each plate, and nano silver base inorganic antibacterial powder water mixture, NSCs and ORTHO LC control cement respectively were introduced and polymerized immediately. The plates were incubated at 37 ℃ for 48 hours and then inspected for the presence of inhibition zones around the tested materials. Bacterial inhibition zone was measured in 2 perpendicular locations and expressed in millimeters. The ADT for each material was repeated 3 times.

Similar experiments were performed in which the above used NSCs and ORTHO LC materials were aged for 2 days, 1 week, and 2 weeks. Aging was performed with phosphate-buffered saline (PBS) containing 25 μg/mL bacitracin, which was replaced every 48 hours.

## 2.3 Direct contact test (DCT)

The DCT[12, 13] was based on turbidness determination of bacterial growth in 96-well microtiter plates (96-well, flat-bottom). The sidewalls of eight continuous wells were coated evenly with a measured amount of the tested material while the plate was held vertically. The material was light polymerized in accordance with the manufacturer' guidance. Special care was taken to avoid the material's flow to the bottom, which would interfere with the measurement of OD, and the up edge of the well, which would lead to the fast evaporation of medium after covering the lid. A 10 μL bacterial suspension (about $1.0\times10^6$ colony-forming units) was dropped in the test material, then the plate was incubated in vertical position at 37 ℃ for 1 hour. During the incubation period, the medium was evaporated to acquire a thin layer of bacteria, insuring immediate contact between the tested materials and the bacteria. Then, 220 μL of BHI medium with 25 μg/mL bacitracin were added to each of the wells and gently mixed for 1 minute. The positive control was consisted of eight uncoated wells in the same microtiter plate and processed as in the experiment wells. The

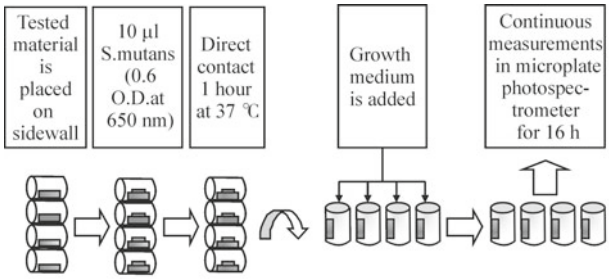


Fig.1 Schematic diagram of DCT experimental procedure. Test was performed in 96-well microtiter plate. Plate is held vertically, and sidewall of well was evenly coated with tested material. A 10 μL bacterial suspension was dropped into the test material. Evaporation of medium (1 hour at 37 ℃) insures immediate contact between bacteria and tested materials. Keeping plate horizontally, growth medium was added to each well and then gently mixed for 1 minute. The turbidness of each well was recorded at 650 nm every 30 minutes for 16 hours with a microplate spectrophotometer

negative control was consisted of eight wells coated with the tested materials, containing equal volumes of uninoculated medium. The turbidness of each well was recorded at 650 nm every 30 minutes for 16 hours with a microplate spectrophotometer (Power Wave XS2; Bio-Tek Instrument, USA). To reduce the influence of temperature change on the growth of bacteria, we tried our best to shorten the measuring time and control room temperature close to 37 ℃. Automatic vibration for 1 minute before each measurement guaranteed a homogeneous mixture of bacterial suspension. The experimental procedure is shown in Fig.1.

Calibration experiments were executed in the same plate to establish the bacterial growth rate for each experiment so as to compare the data between plates. For this reason, 10 μL of bacterial suspension ($1.0\times 10^6$cells) was placed on each sidewall of 3 wells in a 96-well microtiter plate. Then, 275 μL of fresh medium was added and gently stirred for 1 minute. After that, 55 μL were transferred to an adjacent well containing 220 μL of fresh medium. This process was repeated 7 times.

Similar experiments were executed in which the tested materials were aged for 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, and 8 weeks. Aging process was performed with phosphate-buffered saline containing 25 μg/mL bacitracin, which was renewed every 48 hours.

The linear portion of the growth curve, which is relevant to bacterial growth rate, was expressed as a linear mathematical slope. Analysis of one-way ANOVA and Games-Howell multiple comparison procedures (SPSS for Windows version 13.0) were applied on the slopes of these linear portions.

### 2.4 Teeth preparation and grouping

A total of 160 freshly extracted upper premolar teeth were used. The teeth were selected on visual observation for soundness of the coronal portion, absence of caries, no cracks on the labial surface, and not subjected to any chemical agents. The teeth were stored in distilled water at room temperature. After embedded in dental stone, a mounting jig was used to align the facial surfaces of the teeth perpendicular with the bottom of the mold. This kept the buccal surface of the tooth parallel to the applied force during the shear test. Following mounting, the teeth were cleaned and polished with pumice and rubber prophylactic cups for 10 seconds, sprayed with water, and dried with compressed air.

Orthodontic upper premolar metal brackets of the Masel Titan9000™ Roth were used. The average surface area of the bracket base was determined to be 10.5 $mm^2$. This was determined by randomly measuring five bracket bases.

Each bracket was positioned on the least-curved part of the labial enamel surface and was under a constant load of 3 kg applied by a plunger-type loading device, to standardize the procedure, as described by Bishara *et al*[14]. The excess adhesive was removed from the margins of the bracket with a dental probe. Each bracket was light cured as per manufacture's instruction.

One hundred and sixty enamel specimens were randomly subdivided into eight subgroups: group 1, ORTHO LC (control); group 2, Transbond XT; group 3, Transbond Plus; group 4, NSC1; group 5, NSC2; group 6, NSC3; group 7, NSC4; and group 8, NSC5.

### 2.5 Shear bond strength test

Debonding of the brackets was carried out 24 hours after bracket bonding on an Instron Universal testing instrument (8841,Instron Corp ) using a looped rectangular, 0.018 × 0.025–inch, stainless steel wire at a crosshead speed of 1 mm/min[15]. The wire passed beneath the bracket wing with the labial surface perpendicular to the horizontal plane. This was not pure shear because the load was applied some distance from the bonding interface. The bond force were recorded by a computer in Newtons and then divided by the bracket base area, 10.5 $mm^2$, and converted to megapascals (MPa) (1 MPa =1 $N/mm^2$). One-way ANOVA and LSD Multiple Comparisons (SPSS for Windows version 13.0) were applied on the shear bond strength.

## 3 Results and discussion

We evaluated the antibacterial properties of 3 different orthodontic cements and 5 nano silver-containing cement specimens, using both the ADT and the DCT. The fresh and aged specimens were evaluated.

### 3.1 Agar diffusion test

Agar diffusion test is the popular method for some materials to analyze their antibacterial properties, despite the acknowledged limitations, such as, its ability to measure only the activity of soluble components, its unable to display the change of antibacterial effect as time goes by.

After 48 hours incubation, about 8 mm bacterial inhibition zones are observed around NSCs and ORTHO LC fresh specimens, with no significant

differences between them. The results of the present study indicate that 8 mm bacterial inhibition zones may be mainly attributed to the fluoride, which will be released gradually from conventional glass ionomer and resin-modified glass ionomer and present antibacterial activity in vivo[16,17]. Another explanation for this result may be the silver ion non-releasing property from reinforced glass ionomer cement[18] or the amount of silver ion from NSCs permeating into agar is not enough to produce bacterial inhibition zone. Distinctively, one 13 mm bacterial inhibition zone is seen around the nano silver antibacterial powder, which indicates that enough amount of silver ion release into the environment. However, no inhibition zones was observed around any of the specimens aged for 2 days, 1 week, and 2 weeks, which should be interpreted as the fast consumption of fluoride and silver ion existing in the surface of the material, and the amount of fluoride and silver ion permeating into agar is not enough to produce a bacterial inhibition zone.

### 3.2 Direct contact and aging tests

A key event in the initiation of enamel demineralization is microbial adhesion to the teeth. It is widely accepted that enamel demineralization can be better controlled by changing the microenvironment around bonded orthodontic devices[19, 20]. Orthodontic adhesives are reported to have a higher retaining capacity of cariogenic streptococci than bracket materials[21]. On that account, reducing the adhesion of cariogenic streptococci to orthodontic adhesives is essential in preventing enamel demineralization.

In DCT experiment, test microorganism and the tested materials have a direct contact, after which the amount of the bacterial growth is quantified. 8 samples of each tested material were performed for DCT testing. The slope was countered according to the linear part of the curve, which represents the logarithm growth period. One-way ANOVA Games-Howell comparison was taken to compare the difference in bacterial growth rate (slope) between the adhesive material in a combination of time ($P$<0.001).

The results of bacterial suspension optical density regarding the direct contact and material aging test after 0 h(fresh material), 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, and 8 weeks are depicted in Figs.3-10, respectively. Each point on the curve represents the mean value measured from 8 wells containing the same test material.

The calibration growth curve is described in each experiment to maintain the quantitative nature of the DCT. For this purpose, bacteria were diluted by a factor of 5 (Fig.2); each point on the curve is the average of 3 wells.

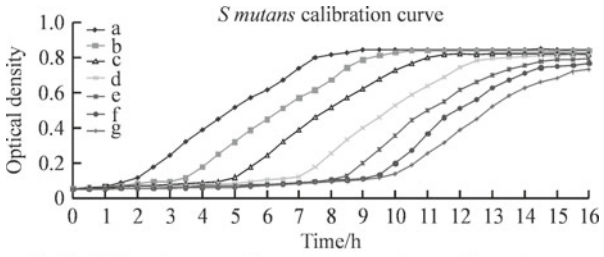


Fig.2 Calibration experiments were performed in each plate to establish bacterial growth under experimental conditions. Each point on growth curve is average of optical densities measured in three wells at same time. Gradient dilution has no influence on bacterial growth rate and density of stationary phase

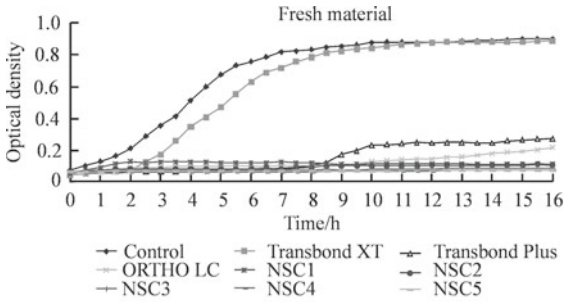


Fig.3 Bacterial growth after direct contact with fresh material. Each point on curve is average of optical densities (OD) measured in 8 separate wells at same time

In the fresh-material experiment, as can be seen in Fig.3, all silver nanoparticles-containing tested materials entirely inhibit formation of any bacterial colony and present powerful antibacterial properties. However, Transbond XT, as is observed in the negative control wells, has none antibacterial effect; Transbond Plus and ORTHO LC show strong antibacterial effect in the previous 8-9 hours, and then present insufficient antibacterial ability.

In order to evaluate the sustainability of the Ag-NPs efficacy in the resin matrix, the aging test was performed. Figs.4-6 and Table 1 demonstrate the gradual process of Transbond Plus, ORTHO LC, NSC1, NSC2 and NSC3 losing their antibacterial ability, which is interpreted as the gradual loss of effective antimicrobial agents after an aging period of 1 day ,1 week and 2 weeks. Moreover, as it is evident in Figs.7-9 and Table 1, after an aging period of 3-6 weeks, NSC4 gradually lost its antibacterial ability, on the contrary, NSC5 still keep the most potent antibacterial properties. None of the tested materials maintained its antibacterial property after aging for 8 weeks (Fig.10, Table 1). A steeper slope and an

**Table 1 Bacterial growth rate as demonstrated by slope of linear portion of growth curve**

| Tested material | Fresh material | 1 day | 1 week | 2 weeks | 3 weeks | 4 weeks | 6 weeks | 8 weeks |
|---|---|---|---|---|---|---|---|---|
| Control | 0.142±0.004 | 0.149±0.002 | 0.157±0.010 | 0.155±0.009 | 0.155±0.009 | 0.158±0.002 | 0.157±0.009 | 0.154±0.009 |
| Transbond XT | 0.140±0.003 | 0.145±0.002 | | | | | | |
| Transbond Plus | 0.073±0.002 | 0.096±0.001 | 0.124±0.007 | | | | | |
| ORTHO LC | 0.015±0.001 | 0.056±0.002 | 0.127±0.010 | 0.100±0.006 | 0.095±0.008 | 0.105±0.002 | 0.141±0.004 | 0.232±0.012 |
| NSC1 | 0.000±0.000 | 0.058±0.001 | 0.057±0.006 | 0.077±0.006 | 0.126±0.007 | 0.091±0.002 | 0.147±0.005 | 0.231±0.012 |
| NSC2 | 0.000±0.000 | 0.059±0.001 | 0.084±0.005 | 0.044±0.006 | 0.137±0.008 | 0.100±0.002 | 0.144±0.012 | 0.248±0.012 |
| NSC3 | 0.000±0.000 | 0.053±0.001 | 0.078±0.006 | 0.039±0.006 | 0.049±0.005 | 0.111±0.007 | 0.135±0.009 | 0.226±0.012 |
| NSC4 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.037±0.004 | 0.010±0.002 | 0.120±0.008 | 0.199±0.017 |
| NSC5 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.171±0.011 |
| 1-way ANOVA | $P < .001$ | $P < .001$ | $P < .001$ | $P < .001$ | $P < .001$ | $P < .001$ | $P < .001$ | $P < .001$ |

Each number in table is average± standard deviation of the slope of bacterial growth in 8 separate wells in same microtiter plate.
Vertical lines connect values that do not differ significantly (Games-Howell comparison).

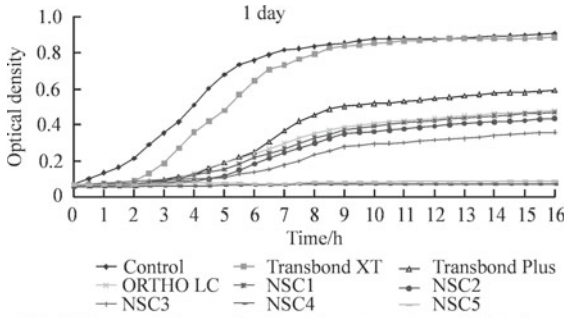


Fig.4 Bacterial growth after direct contact with 1-day-aged material. Each point on curve is average of optical densities (OD) measured in 8 separate wells at same time

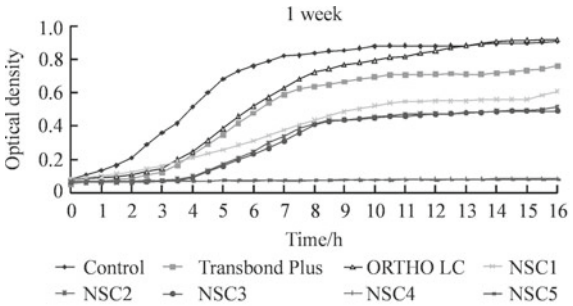


Fig.5 Bacterial growth after direct contact with 1-week-aged material. Each point on curve is average of optical densities (OD) measured in 8 separate wells at same time

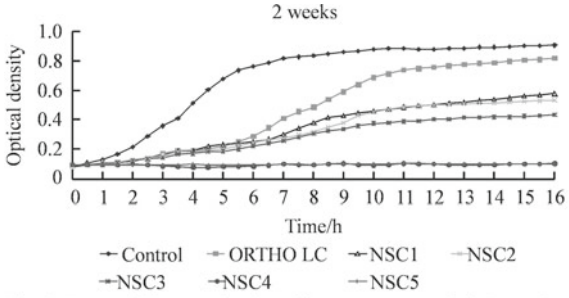


Fig.6 Bacterial growth after direct contact with 2-week-aged material. Each point on curve is average of optical densities (OD) measured in 8 separate wells at same time

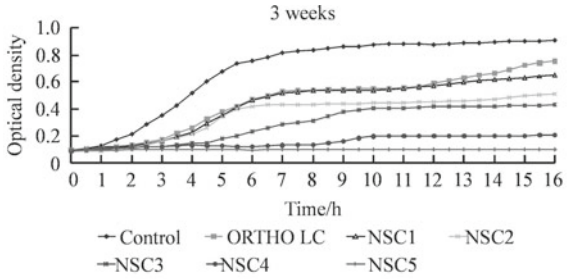


Fig.7 Bacterial growth after direct contact with 3-week-aged material. Each point on curve is average of optical densities (OD) measured in 8 separate wells at same time

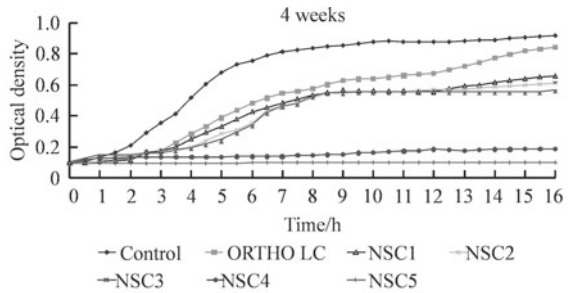


Fig.8 Bacterial growth after direct contact with 4-week-aged material. Each point on curve is average of optical densities (OD) measured in 8 separate wells at same time

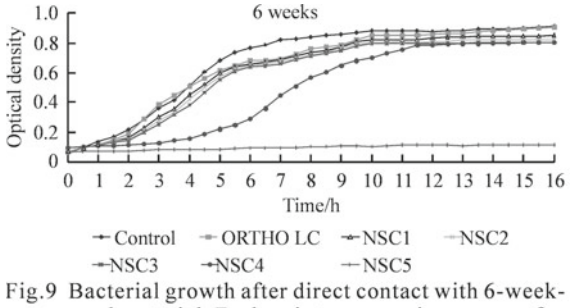


Fig.9 Bacterial growth after direct contact with 6-week-aged material. Each point on curve is average of optical densities (OD) measured in 8 separate wells at same time

**Table 2 Comparison of shear bond strength of eight tested cements**

| Material | *n* | Environment | Surface treatment | Mean SBS±SD/MPa | 1-way ANOVA, *P* value |
|---|---|---|---|---|---|
| ORTHO LC | 20 | Dry | Unconditioned | 9.58±1.73 | – |
| Transbond XT | 20 | Dry | 37% phosphoric acid | 10.91±1.53 | 0.197 |
| Transbond Plus | 20 | Dry | 37% phosphoric acid | 11.15±2.00 | 0.172 |
| NSC1 | 20 | Dry | Unconditioned | 9.47±1.74 | 1.000 |
| NSC2 | 20 | Dry | Unconditioned | 9.30±1.66 | 0.999 |
| NSC3 | 20 | Dry | Unconditioned | 9.07±1.61 | 0.975 |
| NSC4 | 20 | Dry | Unconditioned | 8.64±1.32 | 0.530 |
| NSC5 | 20 | Dry | Unconditioned | 7.80±0.99 | 0.008* |

SD indicates standard deviation; SBS is shear bond strength
* The mean difference is significant at the 0.05 level
One-way ANOVA and LSD multiple comparisons (ORTHO LC as reference group)
Test of homogeneity of variances , $P>0.05$

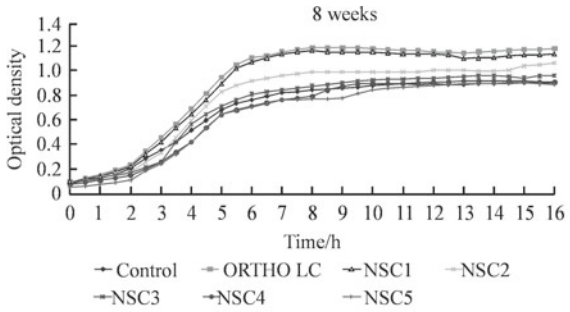


Fig.10 Bacterial growth after direct contact with 8-week-aged material. Each point on curve is average of optical densities (OD) measured in 8 separate wells at same time

elevation relative to the control group in the measured optical density curve of ORTHO LC, NSC1, NSC2 and NSC3 are observed in the 8-week-aged material experiment, which is interpreted as the material's dissolution into the bacterial suspension.

Turbidity or optical density value was used to evaluate the antibacterial effect of resin composites containing different antibacterial composition[22]. This method, however, has an important limitation as it consider both vital and dead bacteria in the bacterial suspension[23].

Maybe, the colony count method is a desirable one to accurately reflect the number of live bacteria at the end of the specific time intervals[24]. In spite of this, optical density value, to some extent, still can reflect the growth of bacteria.

### 3.3 Shear bond strength test

The findings from this study indicate that the brackets bonded with all the tested material specimens reach the ideal bond strength range of 6 to 8 MPa[25], which is adequate for clinical use (Table 2). However, a gradually decreasing trend of bond strength is seen with the increasing incorporation of nano silver base inorganic antibacterial powder into the glass ionomer cement. A recent study suggests that incorporation of silver nanoparticles seems to have minimal effect on shear bond strength[18]. Zirconium phosphate is opaque against visible light and insoluble in the liquid component of ORTHO LC, so, when incorporated into the resin composite, it would adversely affects the curing process. Hence, the gradually decreasing trend of bond strength in this study may be attributed to the incorporation of Zirconium phosphate, which can not form an effective polymer with the resin composition. As it is represented in Table 2, the results show that the NSC5 group with 15% nano silver antibacterial powder has a great difference with the ORTHO LC control group.

The results of the present study indicate that the growth inhibitory effect of NSCs on the cariogenic streptococci probably is not due to silver ion release but due to direct contact with cariogenic streptococci. The internal silver ion slow-releasing property can help to maintain bond strength during a long period because the release of the active agent can cause vacuolation of the matrix and induce weakening of the composites.

Possible disadvantage of incorporating silver nanoparticles is the color, which can produce a potential limitation, especially when aesthetic brackets are used[18]. In this study, the NSCs after polymerization present very light grey color and not likely cause color appearance. Further studies will be necessary to investigate physical properties and potential ability of preventing enamel demineralization using animal model of the experimental cement system.

## 4 Conclusions

This study demonstrated that incorporation of Ag-NPs provided beneficial antibacterial properties while the enough bond strength was maintained. The

inhibition of bacterial growth on the surfaces of cement maybe effectively prevent or decrease the occurrence of enamel demineralization around orthodontic brackets where most white spot lesions are present. We may conclude that NSCs can contribute to decrease the demineralization rate around brackets without compromising bond strength.

## References


[1] Corbett JA, Brown LR, Keene HJ, Horton IM. Comparison of Streptococcus Mutans Concentrations in Non-banded and Banded Orthodontic Patients[J]. *J. Dent. Res*., 1981, 60: 1 936-1 942

[2] Sukontapatipark W, El-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial Colonization Associated with Fixed Orthodontic Appliances. A Scanning Electron Microscopy Study[J]. *Eur. J. Orthod*., 2001, 23: 475-484

[3] Ribeiro J, Ericson D. In vitro Antibacterial Effect of Chlorhexidine Added to Glass-ionomer Cements[J]. *Scand. J. Dent. Res*., 1991, 99: 533-540

[4] Cohen WJ, Wiltshire WA, Dawes C, Lavelle CL. Long-term in vitro Fluoride Release and Re-release from Orthodontic Bonding Materials Containing Fluoride[J]. *Am. J. Orthod. Dentofacial Orthop*., 2003, 124: 571-576

[5] Evrenol BI, Kucukkeles N, Arun T, Yarat A. Fluoride Release Capacities of Four Different Orthodontic Adhesives[J]. J Clin Pediatr Dent, 1999, 23: 315-319

[6] Ashcraft DB, Staley RN, Jakobsen JR. Fluoride Release and Shear Bond Strengths of Three Light-cured Glass Ionomer Cements[J]. *Am. J. Orthod. Dentofacial Orthop*., 1997, 111: 260-265

[7] Jedrychowski JR, Caputo AA, Kerper S. Antibacterial and Mechanical Properties of Restorative Materials Combined with Chlorhexidines[J]. *J. Oral. Rehabil*., 1983, 10: 373-381

[8] Addy M, Handley R. The Effects of the Incorporation of Chlorhexidine Acetate on Some Physical Properties of Polymerized and Plasticized Acrylics[J]. *J. Oral. Rehabil*., 1981, 8: 153-163

[9] Yamamoto K, Ohashi S, Aono M, Kokubo T, Yamada I, Yamauchi J. Antibacterial Activity of Silver Ions Implanted in $SiO_2$ Filler on Oral Streptococci[J]. *Dent. Mater.*, 1996, 12: 227-229

[10] Zhang K, Melo MA, Cheng L, Weir MD, Bai Y, Xu HH. Effect of Quaternary Ammonium and Silver Nanoparticle-containing Adhesives on Dentin Bond Strength and Dental Plaque Microcosm Biofilms[J]. *Dent. Mater.*, 2012, 28(8): 842-852

[11] Besinis A, De Peralta T, Handy RD. The Antibacterial Effects of Silver, Titanium Dioxide and Silica Dioxide Nanoparticles Compared to the Dental Disinfectant Chlorhexidine on Streptococcus Mutans Using a Suite of Bioassays[J]. *Nanotoxicology*, 2012, 15: 1-16

[12] Weiss EI, Shlhav M, Fuss Z. Assessment of Antibacterial Activity of Endodontic Sealers by A Direct Contact Test[J]. *Endod. Dent. Traumatol*., 1996, 12: 179-184

[13] Beyth N, Domb AJ, Weiss EI. An in vitro Quantitative Antibacterial Analysis of Amalgam and Composite Resins[J]. *Journal of Dentistry*, 2007, 35:201-06

[14] Bishara SE, Ajlouni R, Laffoon J, Warren J. Effects of Modifying the Adhesive Composition on the Bond Strength of Orthodontic Brackets[J]. *Angle Orthod*., 2002, 72: 5 464-5 467

[15] LI Fujun, PENG Youjian, PENG Bin. The Evaluation of A Resin-modified Glass Ionome Cement for Bonding Orthodontic Brackets[J]. *Journal of Wuhan Uiversity of Technology-Mater.Sci.Edit*., 2009, 24(6): 986-991

[16] Rix D, Foley TF, Banting D, Mamandras A. A Comparison of Fluoride Release by Resin-modified GIC and Polyacid-modified Composite Resin[J]. *Am. J. Orthod. Dentofacial Orthop*., 2001,120: 398-405

[17] Fujimaki M, Rosa OPS, Torres SA, Costa B, Cury JA. Relationship Between Fluoride and Aluminum Release by Dental Materials and Its Antibacterial Effect[J]. *J. Dent. Res*., 2000,79: 294

[18] Sug-Joon Ahna, Shin-Jae Leea, Joong-Ki Kookb, Bum-Soon Limc. Experimental Antimicrobial Orthodontic Adhesives Using Nanofillers and Silver Nanoparticles[J]. *Dent. Mater*., 2009, 25(2): 206-213

[19] Skold-Larsson K, Borgstrom MK, Twetman S. Effect of An Antibacterial Varnish on Lactic Acid Production in Plaque Adjacent to Fixed Orthodontic Appliances[J]. *Clin. Oral. Invest*., 2001, 5: 118-121

[20] Madlena M, Vitalyos G, Marton S, Nagy G. Effect of Chlorhexidine Varnish on Bacterial Levels in Plaque and Saliva During Orthodontic Treatment[J]. *J. Clin. Dent*., 2000, 11: 42-46

[21] Lim BS, Lee SJ, Lee JW, Ahn SJ. Quantitative Analysis of Adhesion of Cariogenic Streptococci to Orthodontic Raw Materials[J]. *Am. J. Orthod. Dentofacial Orthop*., 2008, 133: 882-886

[22] Beyth N, Houri-Haddad Y, Baraness-Hadar L, Yudovin-Farber I, Domb AJ, Weiss EI. Surface Antimicrobial Activity and Biocompatibility of Incorporated Polyethylenimine Nanoparticles[J]. *Biomaterials*, 2008, 29: 4 157-4 163

[23] Guggenheim B, Giertsen E, Schupbach P, Shapiro S. Validation of An in vitro Biofilm Model of Supragingival Plaque[J]. *Journal of Dental Research*, 2001, 80: 363-370

[24] Tavassoli Hojati S, Alaghemand H, Hamze F, Ahmadian Babaki F, Rajab-Nia R, Rezvani MB, Kaviani M, Atai M. Antibacterial, Physical and Mechanical Properties of Flowable Resin Composites Containing Zinc Oxide Nanoparticles[J]. *Dent. Mater*., 2013, 29(5):495-505

[25] Reynolds IR. Composite Filling Materials as Adhesives in Orthodontics[J]. *Br. Dent. J*., 1975, 138: 383