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The laboratory comparison of shear bond strength and microscopic assessment of failure modes for a glass-ionomer cement and dentin bonding systems combined with silver nanoparticles

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Purpose: More than half of the cavity restorations are replaced due to bacterial microleakage. A need for disinfection agents arises. Application of silver nanoparticles (AgNPs) may be beneficial, yet their impact on the adhesives' shear bond strength to dentin remains unknown. *Methods*: The aim was to assess the shear bond strength to dentin of different dental materials combined with AgNPs. Failure modes were also examined using SEM/FIB, SEM/EDS and endodontic microscopes. *Results*: The results showed no impact of AgNPs addition to dental materials in terms of shear bond strength to dentin. A change of the failure mode of the self-etch bonding system, Clearfil SE Bond, combined with AgNPs was observed. The new failure modes depended upon the order of application of the materials onto dentin. *Conclusions*: The microscopic evaluation of the samples showed the presence of AgNPs agglomerations gathered on the dentin's surface. AgNPs connection with self-etching dentin bonding system may have a serious clinical impact.

Key words: *adhesion, silver nanoparticles, dentin bonding systems, glass-ionomer cement, bacterial microleakage*

1. Introduction

Dental caries is the most frequent infectious disease affecting the tooth's hard tissues. Its development and further progression are caused by Gram-positive bacteria, especially *Streptococcus* and *Lactobacillus*, which proliferate in the acidic environment of the dental plaque (pH $\leq$ 5.0). The operative treatment methods focus on the removal of the infected tissue and its' reconstruction with a restorative dental material, e.g., polymer resin. However, the seal between the tooth's tissues and the filling material is never ideal and, therefore, promotes bacterial microleakage and, in the end, caries recurrence. It is estimated that almost 57% of the polymer fillings are replaced within eight years' time [19]. The problem with the marginal gap remains unsolved. The cavity's mechanical shearing results in only partial eradication of bacterial remnants resting in the dentinal tubuli and within the smear layer. The bacteria may survive underneath the restorations up till 139 days producing toxins and other destructive products of their metabolism [7]. Thus, the treatment efficacy depends upon bacteria clearance, proper seal (elimination of the microleakage) and, possibly, antibacterial properties of restorative materials. Sadly, not all of the dental materials possess antibacterial properties. Glass-ionomer cements are proven to be successful in the remineralization process of the carious tissue. Due to their low pH

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during setting and fluoride ions content, they are also capable of reducing the number of bacteria in the cavity [15], [17].

The adhesion of the hydrophobic polymer materials to moist dentin is possible as different bonding systems of a dual chemical character are used to connect them. A minimal force of 17–20 MPa is needed to obtain a proper seal and minimize the stress caused by the polymerization shrinkage [14]. Drawbacks, such as post-operative sensitivity and marginal discoloration over time denote the lack of a good adhesion of the restorative material to the tooth's tissues [13]. Most frequently, the dentin bonding agents require an additional step called the acid etching (with 32–38% phosphoric acid) in order to remove the smear layer created on the dentin's surface and open up the dentinal tubuli. This is called the total-etch technique. The smear layer is a soft remnants of the dentin created on its surface as a result of the mechanical shearing. Some research states that it may be beneficial to use self-etching dentin bonding systems as an antibacterial agents. In self-etching systems, the separate acid-etching step is omitted as they etch and prime the hard tissues simultaneously. They infiltrate into the dentinal tubuli and impregnate the collapsed collagen fibrils of the smear layer incorporating it into the hybrid layer, which reduces the permeability of the dentin protecting it from bacterial toxins and free monomers elution [18], [20]. Moreover, the superficial dentin demineralization enhances the preservation of the hydroxyapatites connected to the collagen matrix, which counteracts the hydrolysis of the created bond [9].

Recently, the antibacterial properties of silver nanoparticles (AgNPs) have become the main course of the scientific research. Apart from being an anti-germs agents, AgNPs may also enhance the bond strength to dentin as well as other physical properties of the polymer materials [3]. The inhibitory effect of plaque accumulation on the experimental composite adhesives' (ECAs) surfaces has also been described and may play an important role in the suppression of the recurrent caries development and prolonging of the longevity of the restoration [6]. However, it is believed that the combination of AgNPs with the polymer resins creates the risk of an incomplete polymerization process, boost of the free monomers release and unaesthetic grayish discoloration of both the filling and the tooth [4], [6]. The literature on the ECAs' biophysical properties indicate that their antibacterial acting is due to shapes, sizes, concentrations and surface energy of the NPs [1], [4], [5], [12], [23]. On one hand, large contact area decides upon the antibacterial effectiveness of the ECAs, but on the other it may hasten the NPs' agglomeration process in which they could lose their unique properties [5], [10]. The agglomeration may affect the properties of other dental materials combined with the NPs. Being hydrophobic, the AgNPs are hard to dissolute in the environment of the Bis-GMA or TEGDMA monomers, so the silver ions release form the NPs may be compromised [6].

The aim of the study was to assess the shear bond strength of the dental materials combined with Nanocare Gold and their modes of failures in microscopic observations.

2. Materials and methods

Materials

Dental materials used in the study alone and following Nanocare Gold application (names, short names, manufacturers, specifications and application protocol) are shown in Table 1. Forty-two non-carious extracted human third molars were chosen for the study.

Characteristics of nanoparticles

Chemical composition and evaluation of the NG's structure was earlier assessed using SEM/FIB, SEM/EDS and TEM microscopes [16]. Generally, NG comprises of tightly set NPs suspended in a liquid (isopropyl alcohol) and a solid (composition unknown) carriers. Because the material is soluble in water we assume that the solid carrier may be a hydrogel. The main element in the sample were silver (Ag; 91.34 weight%) and aluminum (Al; 4.53 weight%). Gold (Au) can be found in the material in a trace quantity (3.18 weight%) and is connected to a silver nanoparticle providing a conglomerate Au-AgNP. The cross-section of the Au-AgNP and its chemical composition revealed that it was a dual particle made of silver as a base with a dyscoid flake of gold on its surface (silver weight% 56.91; gold weight% 33.98 in Au-AgNP). The shape of the conglomerate is irregular and sized 41.6–100 nm (mean value (MV) 83.3 nm). Most of the AgNPs are spherical in shape and sized 48 nm (MV). Also, a truncated NPs sized 125.3 nm (MV) can be found. The smallest nanoparticle in the field of view was sized 5.8 nm. Knowing that there is 91.34 weight% of silver and by establishing the density and evaluating the volume of one drop of NG, weight in volume concentration was assessed. The data of the physicochemical analysis:

- mean density of the material 0.44 g/cm^3,
- mean weight of one drop of the material 0.0065 g,
- mean volume of one drop of the material 0.015 cm^3,
- AgNPs' concentration 3.96 μg/μl; 913400 ppm.

Methods

Shear bond strength (SBS) test determining the bond strength to dentin of the dental materials alone and combined with NG was performed using a universal testing machine (Instron Model 4411, Instron®, Norwood, MA, USA) with a knife-edged loading head just contacting the interface of the material and dentine column at a cross-head speed of 1 mm/min. Each group consisted of 6 specimens. The test groups were the ones with the materials applied following NG, whereas the control groups were the ones without NG. Later, the failure modes of the samples tested with the SBS test were evaluated. SEM/FIB (Quanta 3D FEG, FEI Europe, Eindhoven, The Netherlands), SEM/EDS (LEO 1430 VP, EM Systems Support, Ltd., Macclesfield, United Kingdom) and LED endodontic microscope (SmartOPTIC, Seliga Microscopes, Ltd., Łódź, Poland) were used. Two samples from each group were randomly chosen for the SEM appraisement. The rest of the samples were evaluated using the LED endodontic microscope at 16× magnification. The failure modes were described as follows: type I – adhesive (the fracture located in the border zone dentin–dental material or adhesive–restorative polymer resin), type II – cohesive (the fracture located within the material or dentin), type III – mixed adhesive–cohesive (the specimens show both types of fracture types I and II).

Sample preparation for the SBS test

Upon extraction, the teeth were carefully examined to ensure they were caries-free and ultrasonically cleaned from debris. The teeth were stored in a 0.1% thymol solution with 0.9% isotonic sodium chloride (5 °C) for up to 6 months till the beginning of the experiment. In order to obtain the base of the sample, the teeth's roots were mounted in a fast-setting acrylic resin Form-Plast® (Zhermapol, Ltd., Poland) up to 1 mm border before the cement–enamel junction. The prepared samples were kept overnight in distilled water (37 °C) so as to discard of the residual monomer. The crowns were cleansed with brush and pumice using the slow-speed handpiece and, using the diamond separating disc (Edenta ISO No. 806.104.355.514.220, Switzerland; 15.000 rotations/min) at slow-speed handpiece with continuous water cooling, were sectioned perpendicularly to the tooth's long axis. Approximately 2.0 mm of the tissue along with the cusps, without exposing of the pulps, was cut off and a middle portion of the dentin was gained. In order to create a smear layer and to polish the dentin's flat surface, 320, 600, 800-grit silicon carbide (SiC) paper was used under running water for 10 sec. per grit. The dental materials alone and following NG were applied on the prepared dentin surface using the application protocol given by the manufacturers (shown in Table 1). The order of application of the materials on dentin:

- The test group OB/NG: etchant – NG – OB – EVE;
- The test CSE_1/NG: CSE primer – NG – CSE adhesive – EVE;
- The test group CSE_2/NG: NG – CSE primer – CSE adhesive – EVE;
- The test group KM/NG: dentin conditioner – NG – KM.

In OB/NG group, the dentin surface was acid etched using Blue Etch® (Cerkamed, Poland) for 15 sec, rinsed with water for 15 sec, and air blow dried. Then NG was applied in the amount of five drops and was left undisturbed to evaporate for approx. three minutes. OB was then applied using brushing motions for 15 sec and air blow dried for 3 sec. The curing time was 20 sec. Then EVE was placed and light cured for another 10 sec. For OB group, the application protocol was alike, without using NG.

In CSE_1/NG, the dentin surface was primed with CSE primer for 20 sec and air dried. Then NG was applied in the amount of five drops and was left undisturbed to evaporate for approx. three minutes. Afterwards, the CSE adhesive was applied, air thinned and cured for 10 sec. Then EVE was placed and light cured for another 10 sec.

In CSE_2/NG the application protocol was alike, with the change of the order of each material application. For CSE group, the application protocol was alike but without NG.

In KM/NG, the dentin surface was conditioned with 10% polyacrylic acid solution for 15 sec, rinsed with water for 15 sec. Then NG was applied in the amount of five drops and was left undisturbed to evaporate for approx. three minutes. KM was prepared by mixing powder with liquid. The material sets chemically. For KM group, the application protocol was alike but without NG.

With regard to obtaining a smooth surface of the polymer filling material and its standardized diameter, EVE and KM were applied onto dentin in previously sterilized translucent polyvinyl rings (internal diameter 6 mm, height 1 mm). All of the polymer materials were polymerized using bluephase®style polymerization lamp (Ivoclar Vivadent AG, Liechtenstein; light intensity

Table 1

Material (short name, manufacturer)	Type	Composition	Application	LOT number
Nanocare Gold® (NG) (Dental Nanotechnology S.A., Katowice, Poland)	Accessory material	Liquid carrier: isopropyl alcohol. Solid carrier: gel-like structure Silver nanoparticles (91.34% MW; shape mainly spherical of mean size 48 nm; concentration 3.96 µg/µl (913400 ppm)); Gold-silver nanoparticles (0.03% MW; shape mainly truncated of mean size 83.3 nm).	Disinfection agent (5 drops per cavity, must be left undisturbed to evaporate)	270213
OptiBond Solo Plus® (OB) (Kerr Italia S.r.l. Scafati, Salerno, Italy)	Total-etch dentin bonding system	Adhesive: Bis-GMA, GPDM, HEMA, silica, barium glass, sodium hexafluorosilicate, ethanol, water.	Acid etching with phosphoric acid (Blue Etch®, Cerkamed, Poland) for 15 sec. Rinsing with water for 15 sec. Air blow drying. Adhesive application for 15 sec and air blow drying for 3 sec. Curing time 20 sec. Restoration placement.	4534728
Clearfil SE Bond® (CSE) (Kuraray Noritake Dental, Inc., Kurashiki, Okayama, Japan)	2-step self-etching dentin bonding system	Primer: MDP, HEMA, hydrophilic dimethacrylate, *N*,*N*-diethanol-*p*-toluidine, water. Adhesive: MDP, bis-GMA, HEMA, hydrophobic dimethacrylate, dl-campherquinone, *N*,*N*-diethanol-*p*-toluidine, silanated silicate	Primer application for 20 sec. Air drying. Adhesive application and mild air blow drying. Curing time 10 sec. Restoration placement.	41948
Ketac Molar EasyMix® (KM) (3M ESPE Dental Products, St. Paul, USA)	Glass-ionomer cement	Powder**:** calcium (Ca), aluminum (Al), silicon (Si) fluorosilicate glass, pigments. Liquid**:** polycarboxylic acid, tartaric acid, water.	Dentin conditioning with 10% polyacrylic acid solution for 15 sec. Rinsing with water for 15 sec. Mixing powder with liquid (1:1 ratio per cavity) Chemical setting.	549021
Evetric® (EVE) (Ivoclar Vivadent, Liechtenstein)	Nanohybrid composite filling material A3 shade	Dimethacrylates (19–20 wt.%), fillers (barium glass, ytterbium trifluoride, mixed oxide, copolymers (80–81 wt.%; 55–57 vol.%, size 40–3000 nm)), addtitives, catalysts, stabilizers, pigments (<1 wt.%)	Curing time 10 sec.	T33059
Abbreviations: MDP: 10-methacryloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; bis-GMA: bisphenol A glycidyl dimethacrylate; GPDM: glycero-phosphate dimethacrylate; MW: molecular weight; wt.% – weight percent; vol.% – volume percent; sec. – seconds.				
Materials' short names alone CSE, OB, NG, KM, EVE and mixed with nanoparticles CSE_1/NG, CSE_2/NG, OB/NG, KM/NG.				

1100 mW/cm^2) and, after the polymerization process, the plastic rings holding EVE and KM were removed using a feather blade. For all materials, the adhesion surface area was round in shape and of 28.26 mm^2. After 24-hours storage in a distilled water, the specimens were further submitted to a thermocycling regimen between water baths (100 cycles at 5 and 55 °C, with a 30 sec dwell time in each bath and a max. 5 sec transfer time) and the SBS test was immediately conducted.

Specimens preparation for the microscopic evaluations

The specimens were sectioned longitudinally to the tooth's long axis using a diamond separating disc at slow-speed handpiece with continuous water cooling. The exposed dentin surface was polished with SiC paper (320, 600, 800, 1200), etched with phosphoric acid (60 sec), deproteinized with 2.0% sodium hypochlorite solution (120 sec) and dehydrated with 25, 50, 95% ethyl alcohol. The chosen specimens were examined and photographed in SEM.

Statistical analysis

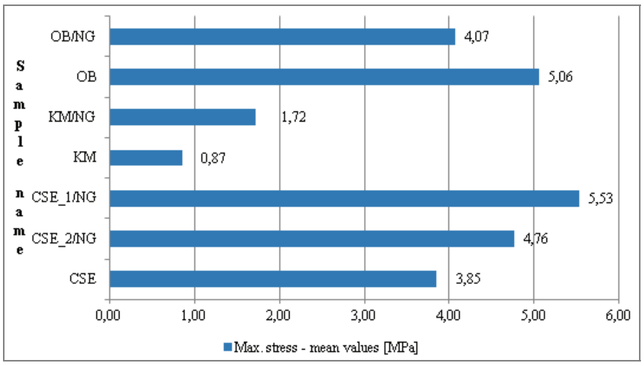
The statistical analysis was performed using a software package (SPSS 10.0 for Windows, SPSS, Inc., Chicago, USA). Means and standard deviations for the chosen parameters were calculated. The analysis comprised of the Shapiro–Wilk test (to confirm the normal distribution of the variables), Levene's test (to assess the equality of variances for multiple groups), the Bonferroni test (to make corrections for multiple comparisons) and *t* test for independent groups at a significance level of $p < 0.05$ for all analyses.

3. Results

The SBS study

In KM and KM/NG groups some of the specimens manifested spontaneous debonding directly after being mounted in the machine's grip and, for that reason, were excluded from both the statistical analysis and microscopic observations.

All of the results had normal distributions. The analysis confirmed the equality of the variances calculated for multiple groups in the Levene's test. Three other analyses of the variance were conducted to verify the research problem. The first one showed there were no statistically significant differences between the materials' groups concerning the diameter of the rings $F(6.30) = 1.15$; $p = 0.359$ $\eta^2 = 0.19$, the diameters of the rings for different dental materials were alike. Further analysis revealed that the materials were significantly different concerning max. load factor $F(6.30) = 4.64$; $p < 0.05$ $\eta^2 = 0.48$; the kind of the material was the factor influencing 48% of changes in values upon max. load. The Bonferroni test showed that KM was significantly weaker concerning max. load from CSE_2/NG ($p = 0.025$), CSE_1/NG ($p = 0.004$) and OB ($p = 0.004$). The last analysis of variance revealed that there were significant differences upon max. stress between the materials $F(6.30) = 4.63$; $p < 0.05$ $\eta^2 = 0.48$; the kind of the material was the factor influencing 48% of changes in values upon max. stress. The Bonferroni test showed that KM was significantly weaker concerning max. stress from CSE_2/NG ($p = 0.025$), CSE_1/NG ($p = 0.004$) and OB ($p = 0.004$) (Graph 1).



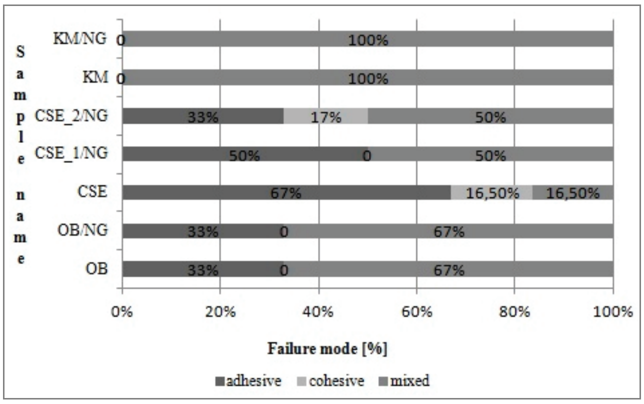
Graph 1. Maximal stress – mean values (the Bonferroni method)

The microscopic study

The microscopic study results showing types of failure for each group is presented in Table 2. Percentage share of failure types depending on the group is depicted in Graph 2.

Table 2. Results of microscopic study

Sample name	Sample number					
	1	2	3	4	5	6
OB	I	I	III	III	III	III
OB/NG	III	III	I	III	I	III
CSE	I	I	II	I	I	III
CSE_1/NG	III	I	III	I	III	I
CSE_2/NG	I	III	III	III	II	I
KM	III	III	III	(-)	(-)	(-)
KM/NG	III	III	III	III	(-)	(-)
Failure mode: I – adhesive; II – cohesive; III – mixed adhesive-cohesive; (–) spontaneous debonding						



Graph 2. Percentage frequency distribution of the failure modes for dental materials

For OB we observed the advantage of mixed failure mode, which was not influenced by NG (Fig. 1). Also, in KM and KM/NG, the mixed failure type was mostly shown, which was not influenced by NG (Fig. 2).

For CSE, the adhesive mode of failure was advantageous with the cohesive and mixed types occurring at the same frequency (Fig. 3). In CSE_1/NG the percentage share of the types of failure changed to only two modes – mixed and adhesive (50% : 50%). This experimental group lacked the cohesive mode of failure (Fig. 4). As for the CSE_2/NG, all types of failure were observed, but the percentage share of them changed in comparison to the control group (Graph 2). In CSE_2/NG, the mixed mode of failure was advantageous (Fig. 4). The SEM/EDS analysis of the elemental composition of CSE, CSE_1/NG and EVE specimens indicated the presence of such elements as barium, nickel, silicon and aluminum. In EVE, fluoride and gold ions were also found (Figs. 5, 6). Only for CSE_2/NG, gold could be observed on the whole surface of the sample and not only in CSE and EVE margins, which may indicate that NG was incorporated into the dentinal tubuli (Fig. 6).

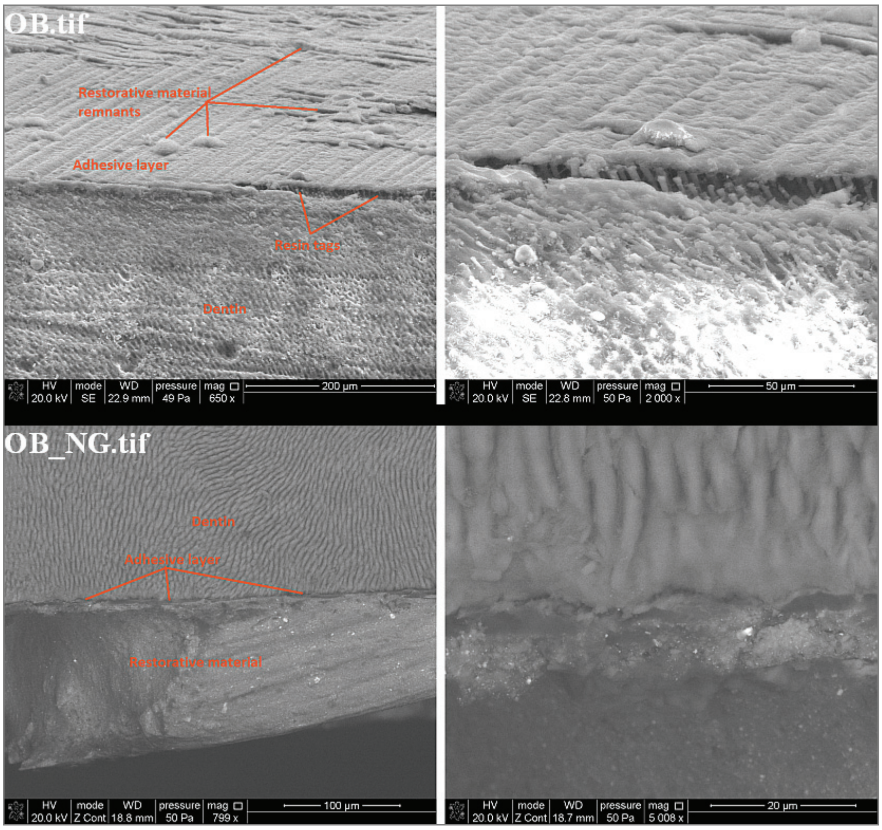


Fig. 1. Failure types for OB and OBNG materials

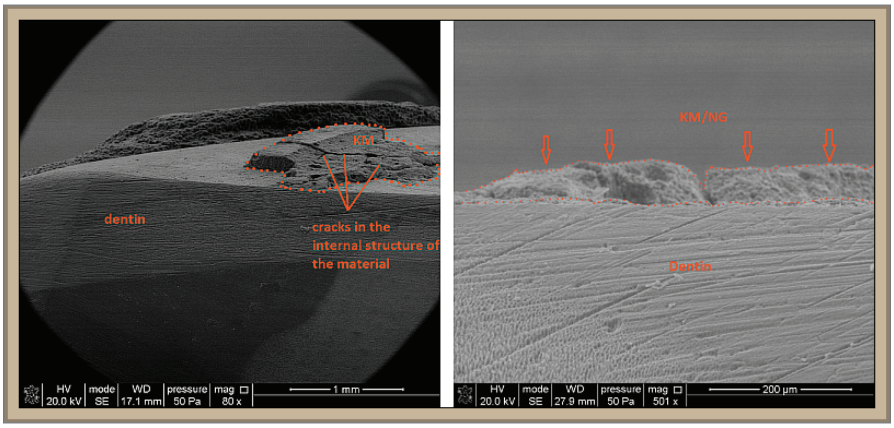


Fig. 2. Failure types for KM and KMNG materials

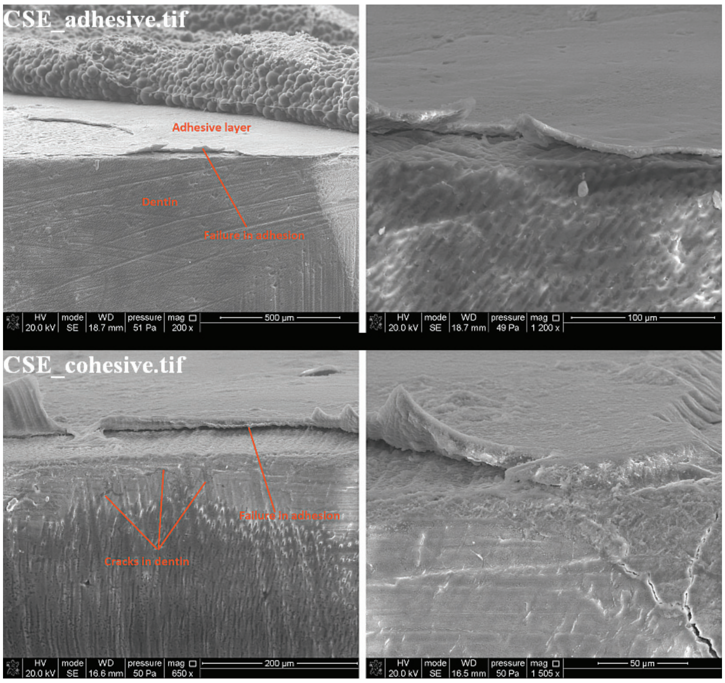


Fig. 3. Failure type for CSE

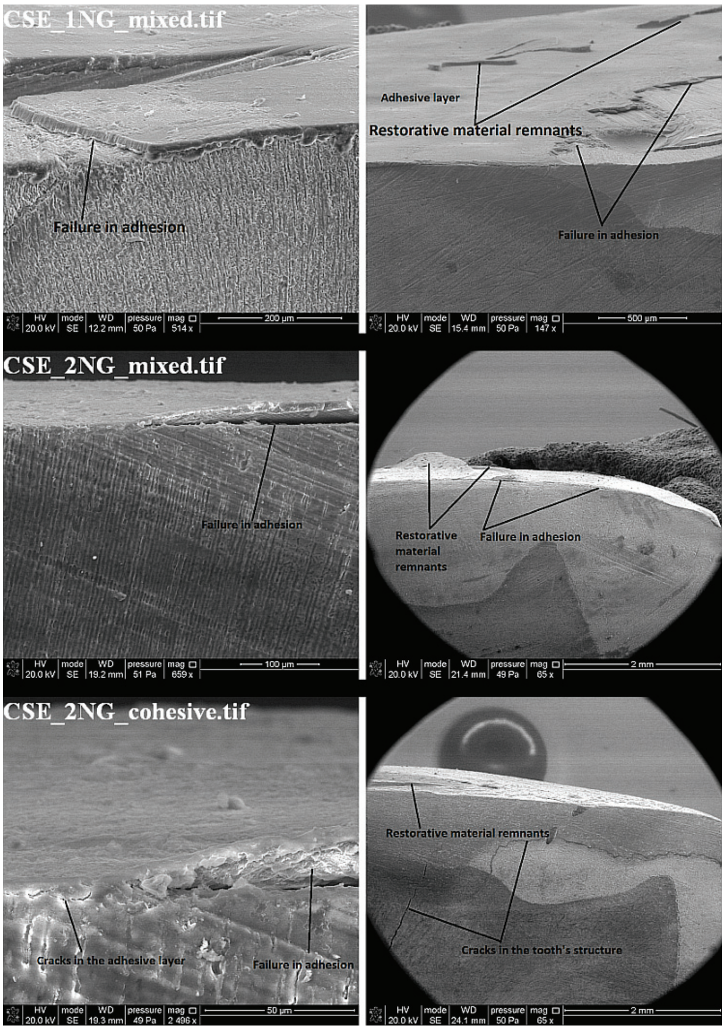
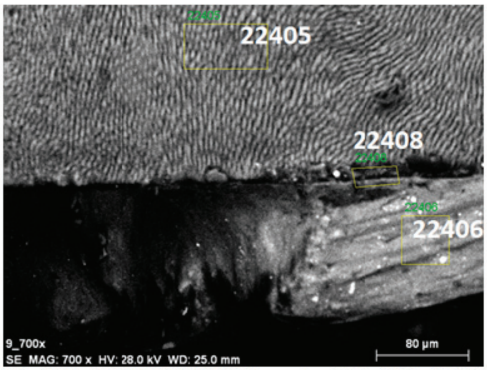


Fig. 4. Failure type for CSE_1NG and CSE_2NG



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Mass percent (%)

Spectrum       C     O     F   Na   Mg    Al    Si     P   Cl    K    Ca   Ni   Ba   Au
-----------------------------------------------------------------------------------------
 22405      0.67 43.67     - 0.65 0.05  1.25  0.02 11.82 0.10 0.23 41.54    -    -    -
 22406      6.31 20.42 23.43 1.90 1.45 11.80 16.16  2.00 0.20 0.08  3.10 3.84 8.15 1.16
 22408      4.70 51.70     - 1.17 0.45  4.33  0.95  8.74 0.22 0.38 26.83 0.54    -    -
-----------------------------------------------------------------------------------------
Mean value: 3.89 38.59  7.81 1.24 0.65  5.79  5.71  7.52 0.17 0.23 23.82 1.46 2.72 0.39
Sigma:      2.90 16.24 13.53 0.63 0.72  5.43  9.06  5.02 0.06 0.15 19.40 2.08 4.70 0.67
Sigma mean: 1.68  9.38  7.81 0.36 0.42  3.13  5.23  2.90 0.04 0.09 11.20 1.20 2.72 0.39
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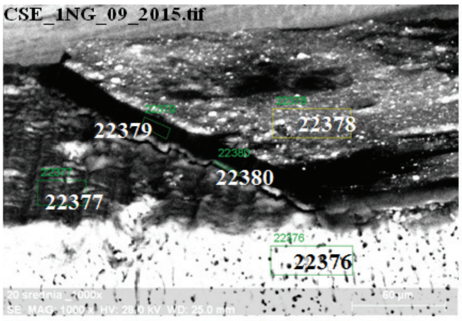
Fig. 5. Chemical analysis of elemental composition of CSE (SEM/EDS)



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Mass percent (%)

Spectrum        C     O     F   Na   Mg    Al    Si     P   Cl    K    Ca   Ni   Ba   Au
------------------------------------------------------------------------------------------
 22376       2.51 57.40     - 0.95 0.39  0.23  0.19  9.12 0.23 0.26 28.73    -    -    -
 22377       0.32 27.04     - 3.24 0.78  6.45  0.97 16.70 0.35 0.45 43.70    -    -    -
 22378       2.24 22.31 23.05 1.97 1.24 12.30 18.99  2.80 0.27 0.20  4.08 3.01 6.22 1.34
 22379      19.53 58.74     - 2.90 0.76  1.15  6.22  3.14 0.37 0.07  7.12    -    -    -
 22380       2.29 38.58     - 5.05 1.47  1.80  2.19 13.96 0.33 0.38 33.96    -    -    -
------------------------------------------------------------------------------------------
Mean value:  5.38 40.81  4.61 2.82 0.93  4.39  5.71  9.14 0.31 0.27 23.52 0.60 1.24 0.27
Sigma:       7.96 16.84 10.31 1.53 0.42  5.04  7.78  6.25 0.06 0.15 17.25 1.35 2.78 0.60
Sigma mean:  3.56  7.53  4.61 0.69 0.19  2.25  3.48  2.80 0.03 0.07  7.72 0.60 1.24 0.27
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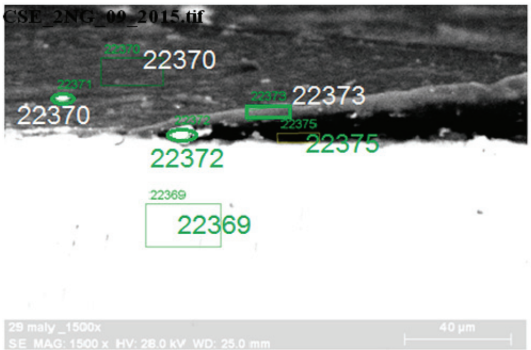

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Mass percent (%)

Spectrum        C     O   Na   Mg    Al   Si     P   Cl    K    Ca   Cu   Au
------------------------------------------------------------------------------
 22369       1.73 53.39 1.11 0.59  0.50 0.12 11.06 0.25 0.20 30.67 0.19 0.19
 22370       9.14 27.84 0.94 1.00 43.12 0.56  4.06 0.63 0.21  7.89 1.95 2.68
 22371       9.33 29.86 0.99 1.02 40.44 0.65  4.31 0.71 0.24  8.69 1.54 2.21
 22372       0.82 42.85 1.91 0.77  3.31 0.61  3.80 0.32 0.39 44.52 0.32 0.38
 22373      18.25 42.78 2.28 0.72 12.84 9.00  3.44 1.42 0.58  7.00 0.36 1.33
 22375       7.71 53.08 1.14 0.35  4.00 1.26  6.89 0.35 0.40 24.41 0.19 0.23
------------------------------------------------------------------------------
Mean value:  7.83 41.63 1.40 0.74 17.37 2.03  5.59 0.61 0.34 20.53 0.76 1.17
Sigma:       6.31 10.97 0.56 0.25 19.37 3.43  2.95 0.44 0.15 15.34 0.78 1.08
Sigma mean:  2.57  4.48 0.23 0.10  7.91 1.40  1.20 0.18 0.06  6.26 0.32 0.44
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Fig. 6. Chemical analysis and elemental composition of CSE_1/NG and CSE_2/NG (SEM/EDS)

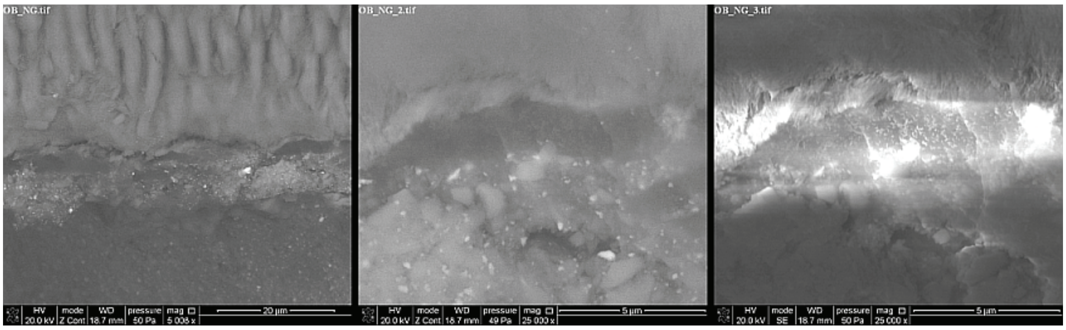

Fig. 7. NG material gathered on the dentin for OB and OBNG samples

NG, gathered in larger agglomerations, was also present in the dentin–adhesive border zone (Fig. 7).

4. Discussion

Antibacterial substances, which are frequently added to the dental materials, are chlorhexidine, fluoride and sodium hypochlorite, yet the research of the subject will not clearly indicate if and how any of these substances would influence the SBS to dentin [1]–[10]. Some of the studies indicate that there are no negative aspects of the disinfectant agents addition to the polymer adhesives [22]–[25], other alarm that the efficacy of ECAs depends upon dentin characteristics and chemical structures of both the disinfectant and the adhesive [7], [21]. Thermocycling is the method used for an artificial ageing of the filling. The number of cycles as well as the temperatures used may vary. The need for the right temperatures interval in *in vitro* studies seems to be more important factor than the number of cycles [2]–[8]. Burger proved that there was no difference in the results for 100, 500, 1.000, 2.000 and 4.000 cycles [8]. Our study was designed to provide 100 cycles at various temperatures that would mimic the conditions in the oral cavity influencing the restorative material's work within the first 24 hours.

Metal nanoparticles may enhance the mechanical properties of the polymer materials in terms of wear resistance and surface hardness [10]. Particles sized between 1–100 nm are qualified as nanoparticles and possess a large active surface, which enables them to transfer loads acting on the polymer's surface. Thanks to this, the material gains stiffness and physical resistance [10]. The impact of AgNPs concentrations and sizes remains controversial. Some reports indicate that the addition of 0.3% AgNPs may significantly raise the ECAs' compressive strength and elasticity modulus, whereas, ironically, others report that with when 0.6% AgNPs addition, these parameters would significantly drop [5]. Nowadays, AgNPs are eagerly used in dentistry due to their antibacterial action towards cariogenic bacteria. In cooperation with fluoride ions, AgNPs induct the fluoroapatites synthesis [9]. Furthermore, the ECAs enriched with AgNPs are less prone to bacterial biofilm accumulation on their surfaces without any negative impact on the polymer's physical characteristics [5]–[10], [21]. AgNPs are also added to the GICs, as they were proven to enhance their biological and physical features (in terms of compressive strength, elasticity and creep resistance) [21]. Therefore, AgNPs' presence in dental materials science as a disinfection agent seems to be beneficial. Yet, it has been shown that 0.0125% AgNPs addition to the polymer resin may induce large elution of the free monomers and photo initiators (TEGDMA, Bis-GMA, *dl*-camphorquinone) [4], as well as barium and strontium ions [24]. This, in return, be the cause of faster hydrolysis of the created bond and negatively influence the material's cytotoxicity. Most *in vitro* scientific studies upon ECAs with AgNPs are designed in a way that fabricated AgNPs powder is added to the polymer and ultrasonically dispersed [3]–[5], [23]. The literature states that the discoloration of the fillings and/or the tooth tissues can be noticed over time. The discoloration of the polymer filling or the tooth's tissue was not the primary goal of this study, yet the authors did not notice any signs of it, which was probably due to the short time of the study. Only few studies focus on AgNPs addition to the adhesives [1]. In Ahn study, the adhesion force to the etched enamel of an orthodontic adhesive enriched with AgNPs was evaluated and no significant differences could be observed [1]. In our study, we also have not found any significant differences between the SBS test results obtained for the experimental materials in comparison with the control groups. The NG addition seems not to have any impact neither on the adhesives nor GIC's adhesion to non-carious dentin. There is also no data concerning direct AgNPs application on the dentin's surface.

NG is an accessory material dedicated for cavity disinfection before the adhesive systems application. Our own research on its physico-chemical structure and composition revealed that the NPs are dispersed in two carriers and confirmed the scarce content of gold bound to AgNPs creating larger conglomerates [16]. Therefore, the impact of gold on the SBS test seems to be minimal and, therefore, was omitted. The isopropyl alcohol acts as a surface-active agent, whereas the solid carrier may play in an important role in the prevention of NPs agglomeration.

In SEM/EDS observations, gold was found within the samples of CSE_1/NG and CSE_2/NG, yet the content of silver was not depicted. This observation is unexpected, as the weight% of silver in NG sample was found to be high (91.34 weight%), in comparison to the gold content being minor (3.18 weight%) [16]. Gold was connected to the silver nanoparticle providing a conglomerate of both metals [16]. The specimen's preparation technique for the SEM evaluation may vary, depending on the determined factor (elementary composition and/or surface evaluation). The most common is the replica technique, in which the sample's replica is taken and evaluated. However, it enables only the surface evaluation of the specimen. In this study, the adhesive surfaces with and without NG's presence, were vital to be shown. Also, NG's distribution within the adhesive surfaces was crucial to understand this material's impact on the SBS of the dental materials. The lack of silver NPs in the specimens in SEM/EDS may be due to the specimen's preparation technique in which the sodium hypochlorite was used. As the solid carrier's chemical characteristics remain unknown, sodium hypochlorite could have modified NG in such a way that only chemically inert conglomerates Au-AgNP remained. In the conglomerate Au-AgNP, gold is situated on the surface of a silver nanoparticle thus it may have overlaid the silver peaks in the EDS detector. There's also a high probability that the larger part of NG was lost along with the rest of the materials (EVE, KM and the adhesives) during the SBS test. Furthermore, the display area of the specimens could have been insufficient for SEM/EDS detector, with a resolution up to 2 μm, to investigate the region where NG was entrapped between OB and dentin and where there was a high probability of detecting both silver and gold (Fig. 7).

Bonding to enamel may be easily obtained by the etching process with phosphoric acid, yet adhesion to dentin is more complicated due to numerous dentinal tubuli filled with the dentinal fluid. Therefore, adhesion to dentin requests the smear layer modification (self-etching adhesives) or complete removal (total-etch adhesives) and may be assessed by a mechanical retention through resin tags created upon the adhesives' filling in the dentinal tubuli [11]. The research on self-etching adhesives indicates that they may acquire lower physical resistance parameters than the total-etch adhesives due to the dentin wetness. Also, the created bond (hybrid layer) may be faster hydrolyzed [2]. In Lopes study [12], the greatest adhesion to dentin was obtained for OB system which was explained by the ethanol (acting as a bonding agent between the hydrophilic tissue and hydrophobic polymer structure) and inorganic filler contents (enhancing the elasticity modulus and minimizing the polymerization shrinkage). However, other studies opt for the lack of significant differences between OB and CSE systems in terms of adhesion forces to sound and carious dentin [11]. Furthermore, the failure mode for CSE would mainly be adhesive with a change to mixed when connected to carious tissue [11]. In our study, we did not find any significant differences between the adhesion forces for OB and CSE. Also, the advantage of the adhesive failure type for CSE was found, which would change with the NG application. Even though the NG's manufacturer advises that the disinfection agent should be applied onto the etched dentin, the authors decided to create two different modes of NG's application for the CSE groups. In CSE_1/NG, the change to mixed failure mode was observed (characteristic of CSE connected to carious tissue) and the lack of the cohesive mode. For CSE_2/NG, all of the failure mode types were found, with the evident advantage of the mixed type.

The adhesion is a phenomenon of a surface bonding between two substances brought about to be touched. Cohesion is a type of adhesion where two identical substances are connected. In terms of definition, cohesion will occur between dentin particles, inside the adhesive and the restorative materials' molecules. In the case of the total-etch bonding systems, the retention to dentin is strictly mechanical through the resin tags, but, in the case of the self-etching systems, the smear layer is bound to the hybrid layer, therefore "the smear layer-adhesive connection" may be regarded as a chemical bond. Taking into account the adsorption theory (Sharpe and Schornhorn, 1963) explaining the adhesion as a chemical interaction between the molecules and atoms of each substrate and the energy of linkages created between them, two different types of adhesion may be elicited – mechanical and specific. The mechanical adhesion relies on micro-connections between the substrates and its force is directly proportional to the substrates' surface development values. The substances increasing the free surface energy and lowering the surface tension also have an impact on the surface development values. The specific adhesion is created on the boundary of two touching bodies as an effect of the primary (covalent bonds) and secondary forces (Van der Waals forces). This type of adhesion is possible only when the bodies stand very close to each other and in dentistry is obtained by the dampening of the adhesion surface (dentin) with a liquid substrate (the adhesive primer). NG may act in range of both these types of adhesion because the solid carrier allows it to gather on the dentin's surface, which, inevitably, enlarges the bonding area for the adhesive system. Isopropyl alcohol influences the dentin's surface energy. Nevertheless, the thickness of the created bond may have a negative impact on the adhesion because thicker layer diminishes the forces of the specific adhesion by separating the connecting bodies. Thus, this may be the reason for the decrease of the SBS to dentin of OB/NG and KM/NG in comparison to their controls. The adhesion to dentin of both these materials is mostly based on the mechanical retention within the dentinal tubuli. As for the CSE system, we proved the failure type changed depending on the order of application of the materials. When NG was applied after the primer, the only CSE's failure modes seen were mixed and adhesive (with the same frequency). In a clinical practice, this result interpolates to the mechanical crash of either the filling or the filling along with the tooth's hard tissues. On the other hand, when NG was applied onto the dentin surface before the primer, the failure modes were the same as the control's but with different frequency – the frequency of the adhesive modes decreased by 34% in comparison to the control. As the adhesive failures are considered to be responsible for the negative features of the resin materials (post-operative sensitivity, pulpal damage and recurrent caries), such results may be beneficial in clinical practice. Even though the values of SBS test were not statistically significant, the authors noticed that the values of the max. load and max. stress values in the CSE_2/NG's experimental group were higher than those of the control one. We assume that the isopropyl alcohol applied directly onto the dentin's surface decreases its surface tension and increases the values of the surface development. Therefore, CSE_2/NG's SBS to dentin was higher than the CSE_1/NG's, which in dental practice may result in an increased danger of the tissue cracking. The authors reckon that the availability of NG's application in clinical practice must be supplemented with the material's antibacterial properties and therefore the research will be continued.

5. Conclusions

The study revealed that the KM's results of the SBS to dentin were significantly different in comparison to CSE_1/NG, CSE_2/NG and OB in terms of max. load and m. stress values. The microscopic evaluation of the samples showed the presence of AgNPs agglomerations gathered on the dentin's surface. The NG addition did not influence the adhesives' bonding strength to dentin, yet the failure modes obtained for CSE changed after the NG application. CSE's new types of failure were dependent upon the order of NG application protocol, which may have a clinical impact on predicting the mechanical failure of the filling and/or tooth's tissue. The study needs to be continued in terms of NG's antibacterial features.

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