

# Microshear Bond Strength, Ultramorphological, and Elemental Assessment of Gold–Silver Nanoparticle-treated Dentin Bonded to Resin Composite with Different Adhesive Modes

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## ABSTRACT

**Aim:** The aim of the present study is to evaluate the effect of Ag–Au NPs formulation as surface pretreatment by assessing the microshear bond strength, ultra-morphological, and elemental characteristics of gold–silver nanoparticle-treated dentin to resin composite with different adhesive modes.

**Materials and methods:** A total of 60 extracted sound human permanent molars were selected to test the microshear bond strength of composite resin to dentin (40 teeth) and 20 molars were used to assess the ultramorphological and elemental characteristics of silver–gold nanoparticles using environmental scanning electron microscope (ESEM) with energy-dispersive X-ray (EDX) analyzer. The specimens were randomly divided into two main groups according to measured tests either microshear bond strength or elemental analysis. For the bond-strength testing group, 40 teeth were equally distributed into two main groups (20 each) according to dentin-surface pretreatment with or without the use of silver–gold nanoparticles. Then, each group was further divided into two subgroups (10 specimens for each) according to adhesive mode. For ultramorphological and elemental analysis, 20 teeth were equally divided into similar previously mentioned groups according to treatment modalities (5 teeth per group). The multimode bonding agent used in this study was single-bond universal, 3M. The data were collected and statistically analyzed. The significance level was set at  $p \leq 0.05$ .

**Results:** The results showed that different etching modes had no statistically significant effect. Also, pretreatment had no statistically significant effect. The interaction between the two variables also had no statistically significant effect. Majority of samples in all groups had mixed failure mode. Ultramorphological examination of the tested samples treated with silver–gold nanoparticles revealed proper dispersion of nanoparticles in dentin.

**Conclusions:** The new formulation of silver–gold nanoparticles did not interfere with the bond strength and sealing ability of resin composite restoration in different adhesion modes.

**Clinical significance:** The cavity disinfection with silver–gold nanoparticles did not affect the marginal integrity of resin composite restoration. Further studies should be done to evaluate the impact of application of silver–gold nanoparticles in long duration with other adhesive systems in the clinical scenario.

**Keywords:** Adhesive modes, Bond strength, Cavity disinfection, Elemental analysis, NanoCare gold, Nanoparticles, Silver–gold nanoparticles, Surface pretreatment, Ultramorphological analysis.

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## INTRODUCTION

Resin composite restorations are consistently used in dental clinics owing to their bonding characteristics to the tooth structure dictating minimal tooth preparation in addition to their optical properties and color match that give them outstanding appearance.<sup>1</sup> The continuous improvement of adhesive technology is very crucial since resin composites relied heavily upon it in order to attain long-lasting durable and stable bonding with the tooth structure. This was the main concern and focus of researchers and manufacturers over the past years.<sup>2</sup> Presently, the available bonding systems are categorized into “etch-and-rinse” or “self-etch systems” with a combined or separate bonding and priming constituents, resulting into multimode adhesive systems with three or two or one-step bonding systems.<sup>3,4</sup>

However, several *in vivo* studies reported that resin composite restorations suffer from accumulation of more biofilm when compared with other restorative materials.<sup>5,6</sup> These biofilms were directly correlated to the material compositions and rheological properties that favor plaque accumulation, especially at the restoration margins. This might endanger the whole restorative

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system resulting in development of secondary caries. Plaques at the restoration margins could result in secondary caries and compromise the restoration’s longevity. Undeniably, secondary caries at the tooth-restoration margin are considered to be the

principal causative factor for failure of composite restoration. It was conveyed that more than half of restorations failed within 10 years and 50–70% of the restorations were replacements of the failed ones.<sup>7,8</sup>

The synthesis and characterization of nanoparticles by nanotechnology and their uses in conservative dentistry demonstrated to be capable to prohibit caries development through their antibacterial properties, moreover regulating biofilm adhesion and acid production, thus assisting in the remineralization mechanism. Recent studies revealed that incorporation of silver nanoparticles into composites/adhesives, with quaternary ammonium methacrylates (QAMs), was successful to defeat biofilms. Moreover, other nanoparticles, such as amorphous calcium phosphate (NACP), were able to aid in remineralization process and neutralize the acidity produced by cariogenic microorganisms by releasing calcium/phosphate ions. By combining NAg/QAM/NACP, a new class of composites and adhesives with antibacterial and remineralization double benefits was developed.<sup>9</sup>

Recently, the concept of cavity disinfection has been recuperated by introducing a new disinfecting agent called NanoCare Plus Silver Gold (Ag–Au NPs). NanoCare comprises silver nanoparticles and little amount of gold nanoparticles manufactured by Dental Nanotechnology, Katowice, Poland.<sup>10</sup> The most important applications practically of silver–gold nanoparticles are its antibacterial and antifungal characteristics in addition to enhancement of physical properties of restorative materials.<sup>11</sup> Moreover, a compatibility of nanocare provides an excellent advantage to be used in deep-cavity restoration.<sup>12,13</sup> By reviewing the literature, the antibacterial effect of AgNPs and their clinical impact on bond strength was grabbing the attention of some researchers.<sup>14–16</sup> The new formulation of NanoCare gold combining silver with gold nanoparticles increased the interest of researchers to assess their antibacterial effect as a cavity disinfectant.<sup>17</sup> Since there is limited number of literature discussing their effect on caries prevention,<sup>10–12,17</sup> only one clinical trial was carried out by one of the authors assessing the marginal integrity of resin composite restorations when using silver–gold nanoparticles as dentin surface pretreatment.<sup>18</sup> Therefore, it was found valuable to get a deeper analysis for the effect of this new formulation of Ag–Au NPs as surface pretreatment by assessing the microshear bond strength, ultramorphological, and elemental characteristics of gold–silver nanoparticle-treated dentin to resin composite with different adhesive modes. The null hypothesis in this laboratory study stated that there is no difference in bond strength of resin composite with and without the use of NanoCare gold as dentin-surface pretreatment.

## MATERIALS AND METHODS

### Specimen Preparation

In this study, a total sample size considered was 60 extracted sound human permanent molars, where a total of 40 extracted sound molars were selected to test the microshear bond strength of composite resin to dentin, and 20 molars were used to assess the ultramorphological and elemental characteristics of silver–gold nanoparticles using ESEM with EDX analyzer. The teeth were collected from clinics at the Faculty of Dentistry, Oral Surgery Department, Ahram Canadian University in January 2021. Selected teeth were extracted due to periodontal reasons and were free of caries, attrition, abrasion, erosion, cracking, or previous restoration. The teeth were cleaned of debris

using a rubber cup, pumice, and a low-speed handpiece, then thoroughly washed with running water and immersed in 0.5% solution of chloramine T for 1 week for sterilization. The teeth were mounted vertically in cold-curing acrylic resin 2 mm below cemento-enamel junction, using plastic circular molds. One of the proximal surface of mounted teeth was ground flat to expose dentin. A horizontal line was drawn 0.5 mm below the dentino-enamel junction (DEJ) determining the level of grinding to expose occlusal superficial dentin. The superficial occlusal dentin was then exposed by horizontally trimming the occlusal surface of each tooth crown with a low-speed diamond disk (Edetal Golden S.A.W., Switzerland) under running water. After trimming, the resulting surfaces were flattened and finished using 600-grit silicon carbide papers (waterproof silicon carbide paper, Atlas, UK) to create a standardized smear layer.<sup>19,20</sup>

### Study Grouping

The prepared specimens were randomly divided into two main groups according to measured tests either microshear bond strength or elemental analysis. For bond-strength testing group, 40 teeth were equally distributed into two main groups (20 each) according to dentin-surface pretreatment with or without the use of silver–gold nanoparticles. Then, each group was further divided into two subgroups (10 specimens for each) according to adhesive mode: etch and rinse or self-etch mode. So the tested groups obtained were group A with self-etch adhesive mode of dentine surface not pretreated by silver–gold nanoparticles, group B with self-etch adhesive mode of dentine surface pretreated by silver–gold nanoparticles, group C with total etch adhesive mode of dentine surface not pretreated by silver–gold nanoparticles, and finally group D with total etch-adhesive mode of dentine surface pretreated by silver–gold nanoparticles. For ultramorphological and elemental analysis, extra 20 teeth were equally divided into similar previously mentioned groups according to treatment modalities (5 teeth per group).

The bonding agent used in this study was single-bond universal, 3M in self-etch and etch-and-rinse modes. The standard composition and manufacturer of the materials used in the study are shown in Table 1.

Regarding pretreated groups, the exposed dentin surfaces were pretreated using double coats of NanoCare plus silver gold, while in other groups, the exposed dentin surfaces were not pretreated with NanoCare plus silver gold. For the self-etch mode, the dentin-bonding systems were applied directly to dentin following the manufacturer's instructions by applying 2 consecutive coats with agitation for 20 seconds using bond microbrush followed by gentle air thinning using air syringe and then light cured for 10 seconds using a light-curing device (B-Cure Woodpecker Co., Ltd., Guilin Guangxi, China) that has an output of 1200 MW/cm<sup>2</sup>. However, for the etch-and-rinse mode, dentin was etched for 20 seconds with 37% phosphoric acid gel (N-etch, Ivoclar Vivadent Inc., Schaan, Liechtenstein), and the acid-etch agent was then rinsed with water for approximately 20 seconds, and the excess moisture dried off with a gentle stream of air with triple-way syringe. The bonding agent was then applied as previously described and light cured for 10 seconds.

### Building up of Resin Composite for Testing Microshear Bond Strength

The resin composite was built up using a polyethylene tube (BioFlon IV cannula, India). The height of the tube was 3 mm and its outer

**Table 1:** Materials composition of adhesives, resin composite, and NanoCare gold investigated in the present study

| Tested materials and manufacturers  | Composition  |
|---|--|
| NanoCare Plus Silver Gold® (NanoCare) (Dental Nanotechnology, Katowice, Poland) | Silver nanoparticles (AgNPs) and small amount of gold nanoparticles (AuNPs)  |
| N-Etch (Ivoclar Vivadent Inc., Schaan, Liechtenstein)                           | 37% Phosphoric acid  |
| Single-bond universal 3M, USA   | HEMA, methacryloxydecyl dihydrogen phosphate (MDP phosphate) monomer, Bis-GMA, dimethacrylate resin, ethanol, water, fillers, initiator, silane, vitrebond copolymer   |
| Filtek (Z350 XT) nanofilled universal restorative material 3M, USA              | Fillers: Non-agglomerated 20 nm silica filler, non-agglomerated 4–12 nm Zirconia filler, aggregated zirconia/silica cluster. Filler loading 55.6% by volume and 72.5% by weight. Matrix: BISGMA, UDMA, TEGDMA, PEGDMA, and BIS-EMA |

and inner diameters were 1.3 and 1 mm, respectively, to allow the maximum number of specimens to be bonded to the same dentin substrate. All composite tubes were cured using the same LED curing unit at zero distance. According to the manufacturer's instructions, each composite tube was cured for 20 seconds (Table 1). Tubes around the composite cylinders were removed by gently cutting each tube using a surgical scalpel blade no. 11. Four cylinders were placed on each molar, perpendicular to the prepared dentin surface.<sup>21</sup>

### Microshear Bond Strength Tests

After 1 week of storage in distilled water at 37°C, each acrylic-embedded tooth cylinder was fixed on the compartment of the universal testing machine (Instron model 3345, England) with a load cell of 500 N. Data were recorded using computer software (Bluehill 3, Instron). A loop prepared from an orthodontic wire (0.14 mm in diameter) was wrapped around the bonded microcylinder assembly as close as possible to the base of the microcylinder. The wire was aligned with the loading axis of the upper movable compartment of the testing machine. A shearing load was applied through the specimens. The shear load was applied at a cross-head speed of 0.5 mm/min until bonding failure occurs. The microshear bond strength values (expressed in MPa) were calculated from the maximum load at failure divided by the bonded-surface area.<sup>4</sup> The fracture load was recorded and the shear bond strength was calculated according to the following equation:  $\sigma = F/A$ , where,  $\sigma$  is the microshear bond strength in megapascals (MPa),  $F$  is the failure load in Newtons (N), and  $A$  is the surface area in square millimeters ( $\text{mm}^2$ ), where,  $A = \pi r^2$ ,  $\pi = 3.14$ ,  $r$  = radius of each composite cylinder = 1 mm.

### Failure-mode Analysis of Debonded Specimens

After microshear testing, the debonding sites of the fractured dentin specimens from all groups were prepared for ESEM evaluation of different failure modes, where their roots were cut off up to the

cemento-enamel junction, by a high-speed motor with cooling system. Then the specimens were air-dried. They were then examined using ESEM attached with EDXA (Main Defense Chemical Laboratory, Cairo, Egypt) to determine the mode of failure. For ESEM examination, the collected specimens were examined at 20 kV using the secondary-electron LFD detector under the magnification of 22× and 35× with a spot size 5 mm. Based on the percentage of substrate area (adhesive–resin composite–dentin) observed on the debonded cylinders and tooth-bonding sites, the types of bond failure were recorded as (i) cohesive failure and (ii) mixed failure.

### Building up of Composite for Ultramorphological and Elemental Analysis

The resin composite was built up using splitted Teflon circular disc with internal diameter of 5 mm and 2 mm height, that was placed on the occlusal dentin after curing of the bonding agent in all specimens. In total, 2 mm composite increments were directly packed inside the mold and light cured for 20 seconds. The mold was then removed and the specimen was stored in water for 24 hours. Specimens were then sectioned into two halves in buccolingual direction with a low-speed diamond disk (Edetal Golden S.A.W., Switzerland) under running water. After trimming, the resulting surfaces were flattened and finished using 600-grit silicon carbide papers (waterproof silicon carbide paper, Atlas, UK) to create a standardized smear layer. Their roots were then cut off up to the cemento-enamel junction, by a high-speed motor with cooling system. Specimens were then air-dried and examined using ESEM attached with EDX analyzer to determine the amount of nanoparticles inside the dentinal tubules as well as their depth of penetration. For ultramorphological elemental-analysis examination, the collected specimens were examined at 20 kV using the secondary-electron LFD detector under the magnification of 2000× and 4000× with a spot size 30–50  $\mu\text{m}$ . Silver–gold nanoparticles were analyzed with EDX analyzer.

### Statistical Analysis

For microshear bond strength analysis, the mean and standard deviation (SD) values were calculated for each group in each test. Data were explored for normality using Kolmogorov–Smirnov and Shapiro–Wilk tests, and bond strength data showed parametric (normal) distribution, while failure data showed nonparametric (not-normal) distribution. For parametric data, independent sample  $t$ -test was used to compare between two groups in nonrelated samples. For nonparametric data, Mann–Whitney test was used to compare between two groups in nonrelated samples. The significance level was set at  $p \leq 0.05$ .

### Pretest Failure and Failure-mode Analysis

Categorical data were presented as frequency and percentage values and were analyzed using Fisher's exact test. Numerical data were presented as mean and SD values. Shapiro–Wilk's test was used to test for normality. Data of microshear bond strength without the inclusion of pretesting failed samples and of EDEX analysis were normally distributed and were analyzed using one-way ANOVA test followed by Tukey's *post hoc*. Data of microshear bond strength with the failed samples included had nonparametric distribution and were analyzed using Kruskal–Wallis test followed by Dunn's *post hoc* test with Bonferroni correction. The significance level was set at  $p < 0.05$  within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows.

## RESULTS

### Bond Strength Evaluation

#### Effect of Dentine Surface Pretreatment using Silver–Gold Nanoparticles

*Self-etch adhesive mode:* There was no statistically significant difference between the two groups, where  $p = 0.752$ . The highest mean value was found in group A, while the lowest mean value was found in group B (Table 2).

*Total-etch adhesive mode:* There was no statistically significant difference between the two groups, where  $p = 0.602$ . The highest mean value was found in group C, while the lowest mean value was found in group D (Table 2).

#### Effect of Etching

*Without pretreatment groups:* There was no statistically significant difference between groups, where  $p = 0.621$ . The highest mean value was found in group C, while the lowest mean value was found in group A (Table 2).

*With pretreatment groups:* There was no statistically significant difference between groups, where  $p = 0.726$ . The highest mean value was found in group D, while the lowest mean value was found in group B (Table 2).

Data in Table 3 show the results of two-way ANOVA analysis for the interaction of different variables. The results showed that different etchings had no statistically significant effect. Also, pretreatment had no statistically significant effect. The interaction between the two variables also had no statistically significant effect.

### Pretest Failure Analysis

With and without the inclusion of pretesting failed samples, there was no significant difference between tested groups ( $p > 0.05$ ), with group C, having the highest mean value, followed by group D, then group A, while the lowest value was found in group B. The results of intergroup comparisons of microshear bond strength are presented in Table 4.

### The Correlations between Microshear Bond Strength Values and Mode Failure

Majority of samples in all groups had mixed failure mode (Fig. 1) and the difference between tested groups was not statistically significant ( $p = 0.948$ ). The results of intergroup comparisons of failure modes are presented in Table 5.

There was no association between failure mode and microshear bond strength ( $p = 0.515$ ). The association between failure mode and microshear bond strength is presented in Table 6.

**Table 2:** The mean and SD values of bond strength of different groups

| Variables            | Bond strength |      |       |                       |               |      |       |                       |          |
|----------------------|---------------|------|-------|-----------------------|---------------|------|-------|-----------------------|----------|
|                      | Bond          |      |       |                       | Etch and bond |      |       |                       |          |
|                      | Mean          | SD   | Range | Variation coefficient | Mean          | SD   | Range | Variation coefficient | p-value  |
| Without pretreatment | 13.33         | 1.83 | 4.47  | 13.76                 | 13.86         | 2.03 | 7.14  | 14.67                 | 0.621 ns |
| With pretreatment    | 12.94         | 2.14 | 5.25  | 16.50                 | 13.34         | 1.72 | 5.02  | 12.84                 | 0.726 ns |
| p-value              | 0.752 ns      |      |       |                       | 0.602 ns      |      |       |                       |          |

ns, nonsignificant ( $p > 0.05$ )

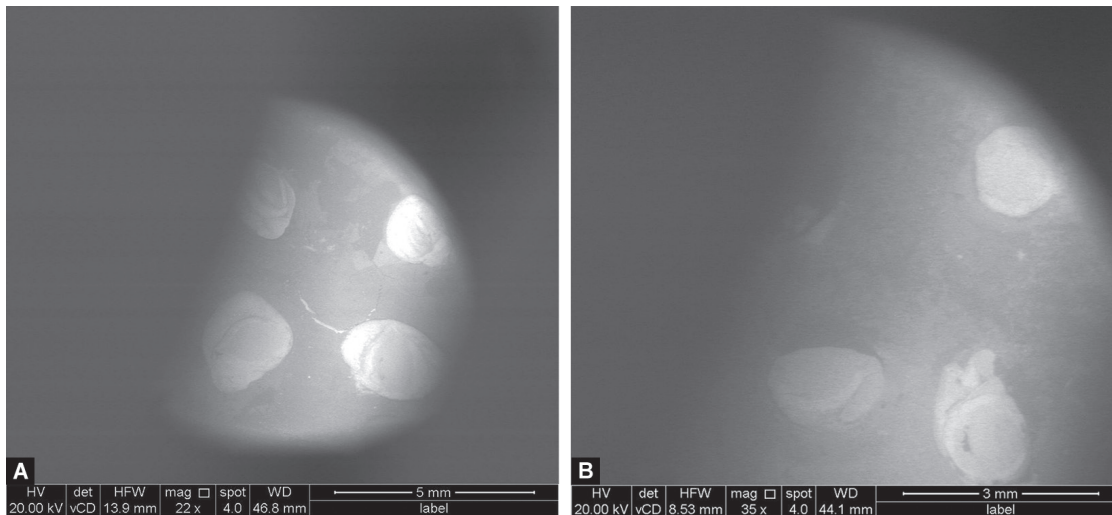
**Table 3:** Results of two-way ANOVA for the effect of different variables

| Source                 | Type III sum of squares | Df | Mean square | F        | Sig.  |
|------------------------|-------------------------|----|-------------|----------|-------|
| Corrected model        | 2.917                   | 3  | 0.972       | 0.260    | 0.853 |
| Intercept              | 4607.875                | 1  | 4607.875    | 1234.681 | 0.000 |
| Etching                | 1.371                   | 1  | 1.371       | 0.367    | 0.550 |
| Pretreatment           | 1.307                   | 1  | 1.307       | 0.350    | 0.560 |
| Etching × pretreatment | 0.024                   | 1  | 0.024       | 0.006    | 0.937 |
| Error                  | 85.837                  | 23 | 3.732       |          |       |
| Total                  | 4964.517                | 27 |             |          |       |
| Corrected total        | 88.753                  | 26 |             |          |       |

**Table 4:** Intergroup comparisons for microshear bond strength (MPa)

| Measurement | Mean ± SD                 |                           |                           |                           | p-value |
|-------------|---------------------------|---------------------------|---------------------------|---------------------------|---------|
|             | Group A                   | Group B                   | Group C                   | Group D                   |         |
| No PTF      | 13.33 ± 1.83 <sup>A</sup> | 12.94 ± 2.14 <sup>A</sup> | 13.86 ± 2.03 <sup>A</sup> | 13.34 ± 1.71 <sup>A</sup> | 0.852   |
| PTF         | 6.67 ± 7.07 <sup>A</sup>  | 5.39 ± 6.79 <sup>A</sup>  | 11.34 ± 5.89 <sup>A</sup> | 7.78 ± 6.99 <sup>A</sup>  | 0.194   |

Different superscript letters indicate a statistically significant difference within the same horizontal row



**Figs 1A and B:** ESEM evaluation of failure mode showing mixed failure at (A) 22× and (B) 35× magnifications

**Table 5:** Intergroup comparison of failure modes

| Failure mode | Group A | Group B | Group C | Group D | p-value |
|--------------|---------|---------|---------|---------|---------|
| Cohesive     |         |         |         |         | 0.948   |
| n            | 2       | 2       | 4       | 2       |         |
| %            | 33.3%   | 40.0%   | 44.4%   | 28.6%   |         |
| Mixed        |         |         |         |         |         |
| n            | 4       | 3       | 5       | 5       |         |
| %            | 66.7%   | 60.0%   | 55.6%   | 71.4%   |         |

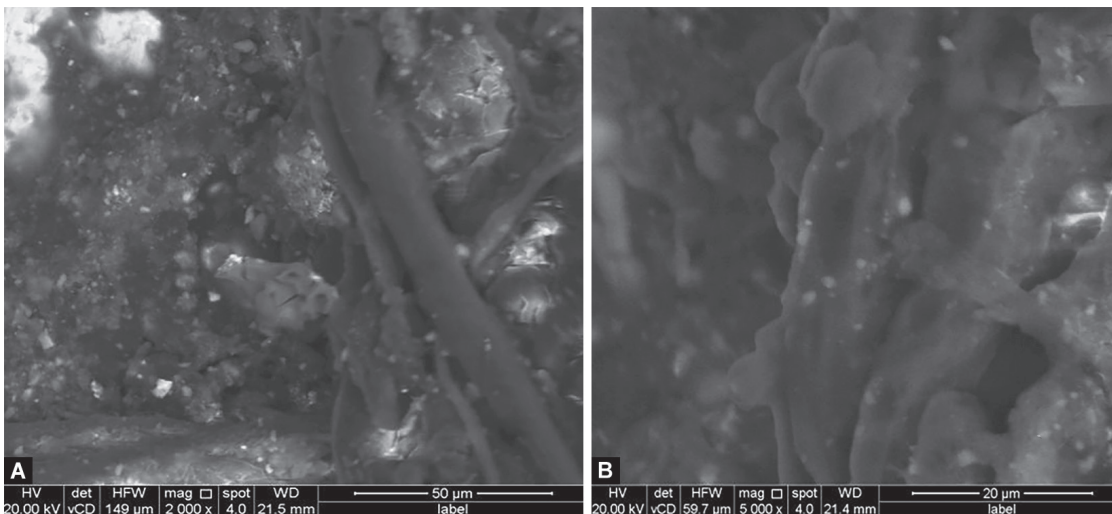
$p < 0.05$

**Table 6:** Association between mode of failure and microshear bond strength (MPa)

| Mean ± SD    |              | p-value |
|--------------|--------------|---------|
| Cohesive     | Mixed        |         |
| 13.75 ± 1.88 | 13.26 ± 1.86 | 0.852   |

### Ultramorphological and Elemental Analysis in Silver–Gold Nanoparticle-treated Groups

Ultramorphological examination of the tested samples treated with silver–gold nanoparticles revealed deep penetration of nanoparticles inside the dentinal tubules and along with resin tags for specimens with etching mode (Fig. 2). While for specimens with self-etch mode, the silver–gold nanoparticles were properly dispersed in the hybrid layer (Fig. 3). The numerous spherical nanoparticles (round, discoid) have mean size of 48 nm. Uniform dispersion and no agglomeration could be detected (Figs 2 and 3). Moreover, elemental analysis (Figs 4 to 6) revealed that the average of silver–gold nanoparticles within the specimen was  $2.82 \pm 0.26\%$  for Au NPs in self-etch group and  $2.46 \pm 0.57$  for etch-and-rinse group, while  $1.77 \pm 0.47$  wt% for Ag NPs in self-etch and  $1.31 \pm 0.29$  wt% in etch-and-rinse group. For gold and silver, group B had higher mean value of weight percentage than group D, yet the difference was not statistically significant ( $p > 0.05$ ). The results of intergroup comparisons of elements' weight percentage are presented in Table 7. Thus, the above-mentioned results revealed that silver–gold nanoparticles did not interfere with the bond



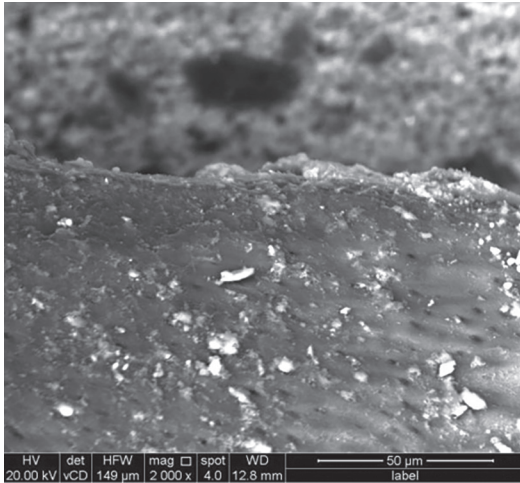
**Figs 2A and B:** Ultramorphological evaluation of Ag–Au NPs penetration inside dentinal tubules using ESEM at (A) 2000× and (B) 5000×

strength and sealing ability of resin composite restoration in different adhesion modes.

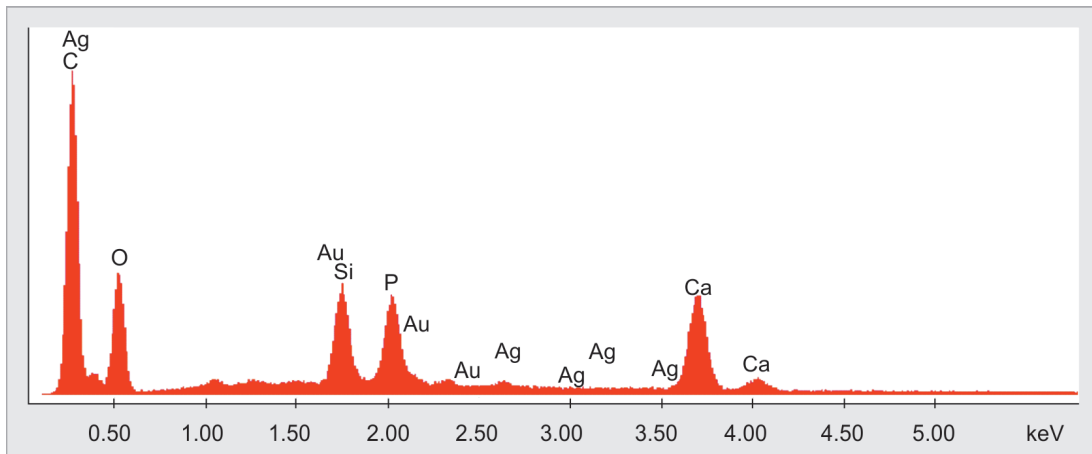
**DISCUSSION**

The bond performance of direct esthetic restorations is very crucial in determining the durability of these restorations. In

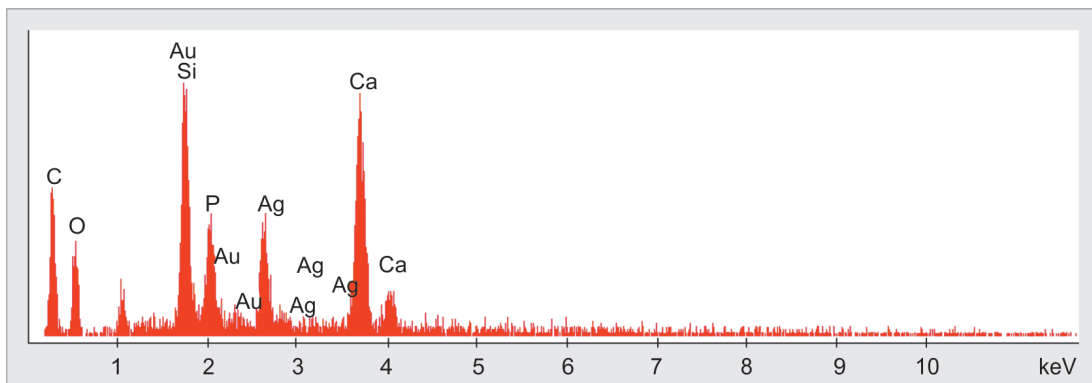
recent times, the survival rate of resin composite restoration was quite satisfactory due to the improvement in material composition and techniques of application. However, the failure of such restorations could be concise in the form of secondary caries, marginal discrepancies, postoperative sensitivity, and fracture in some cases if the restorations were not properly planned.<sup>20-25</sup> In this study, multimode universal adhesive was selected owing to the reported excellent bond performance in etching mode and self-etch method.<sup>20</sup> Also, it allows the clinicians to select the desired mode for usage rendering to the clinical situation. It is not recommended to etch the dentin to minimize collagen degradation and hence compromising the bond strength, however, etching of dentin may be sometimes inevitable, which might lead to decrease in bond strength stability and postoperative sensitivity. The used universal self-etch adhesive in this study has ultramild acidity (hydrogen potential pH = 2.7) comprising MDP that had been reported to maintain the hybrid layer consistent and prevent hydrolytic degradation of the collagen matrix.<sup>26-28</sup> The bond degradation and microleakage overtime pave the path for bacterial ingress into the interface and henceforth recurrent caries. The concept of cavity disinfection had been raised in recent times to prevent the incidence of secondary caries under resin composite restorations. However, the use of cavity disinfectants with resin composite restorations appears to be material specific regarding their interactions with various dentin bonding systems.<sup>13</sup> Chlorohexidine was first used being an antibacterial and matrix-metalloproteinase (MMP) inhibitor, thus it would be able to



**Fig. 3:** Ultramorphological evaluation of Ag–Au NPs dispersion in the hybrid layer with self-etch adhesive mode using ESEM at 2000x

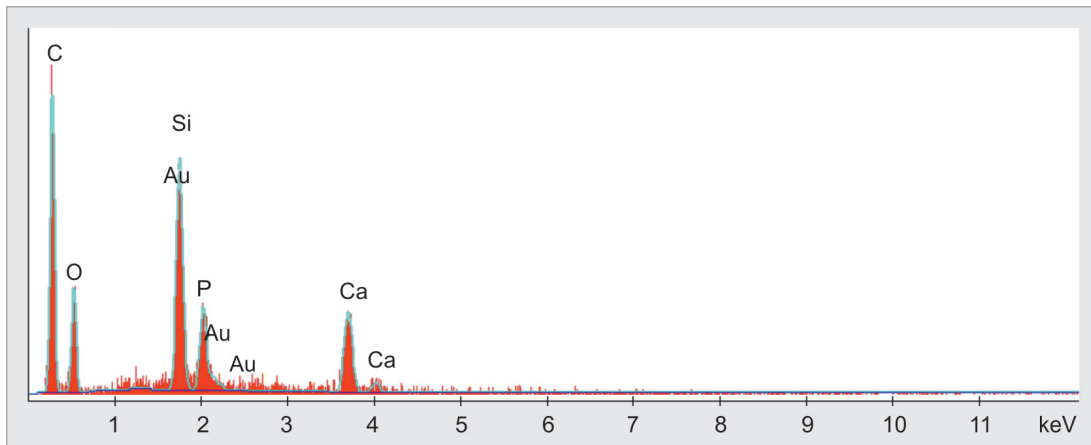


**Fig. 4:** Elemental analysis using EDEX for different quantitative assessment of Ag–Au NPs wt% within tested specimens of self-etch adhesive mode



**Fig. 5:** Elemental analysis using EDEX for different quantitative assessment of Ag–Au NPs wt% within tested specimens of self-etch adhesive mode





**Fig. 6:** Elemental analysis using EDEX for different quantitative assessment of Ag–Au NPs wt% within tested specimens of etch-and-rinse adhesive mode

**Table 7:** Intergroup comparisons for elements' weight percentage (%)

| Element | Mean $\pm$ SD   |                 | p-value |
|---------|-----------------|-----------------|---------|
|         | Group B         | Group D         |         |
| Gold    | 2.82 $\pm$ 0.26 | 2.46 $\pm$ 0.57 | 0.373   |
| Silver  | 1.77 $\pm$ 0.47 | 1.31 $\pm$ 0.29 | 0.225   |

prevent collagen degradation and improve bond performance.<sup>29</sup> Recently, a new cavity disinfectant (NanoCare gold) was released into the dental market formulated from silver and gold nanoparticles gaining the advantage of having unique characteristics of its ingredients.<sup>30,31</sup> It was reported that its application as dentin-surface pretreatment delivers the required antibacterial properties without any risk of bacterial resistance<sup>30,31</sup> and without interfering with bond strength.<sup>32,33</sup> Moreover, it improves the wetting of the adhesive system.<sup>34</sup> However, by reviewing the literature, limited number of researches were found on this material and actual interpretation of their effect was not clear.

This study was designed to laboratory analyze the effect of silver–gold nanoparticles as dentin-surface pretreatment on bond strength of resin composite restoration, besides determining and correlating the mode of failure to the bond strength of tested specimens. Nevertheless, it was intended to assess the ultramorphology, depth of penetration, and elemental analysis of Ag–Au NPs with different modes of adhesive system.

The results of the current study revealed that different etching had no statistically significant effect on bond strength. This might be attributed to the chemical composition of the bonding agent used containing MDP that is responsible for the long-term bonding stability and antigelatinolytic effect. This was in agreement with Giacomini et al.<sup>27</sup> who found that there is no difference in bonding performance and gelatinolytic activity at the hybrid layer of both systems at initial and after 6 months of storage, regardless of the adhesive strategy. Even though, other researchers have been reporting deterioration of the bond strength with the use of MDP-containing adhesive systems after 6 and 18 months of clinical evaluation, for both adhesive protocol with self-etch and etch-and-rinse modes.<sup>26</sup> Thus, revealing that the bond performance in some cases, might be unpredictable which might increase the chance for secondary caries. Here comes the importance of evaluating the usage of cavity disinfection and

considering its beneficial antibacterial action in caries prevention versus interfering with the bond-strength stability of the adhesive system.

In the present study, dentin-surface pretreatment had no statistically significant effect. The interaction between the two variables (adhesion strategy and surface pretreatment) also had no statistically significant effect. Thus, NanoCare gold has no negative effect on bond strength of resin composite to dentin. By appraising the literature, this was in accordance with other studies, but there are only few published clinical trials confirming these results yet.<sup>18,35</sup>

In the present study, ultramorphological examination of the tested samples treated with silver–gold nanoparticles revealed deep penetration of nanoparticles inside the dentinal tubules and along with resin tags. Consequently, cavity disinfection with NanoCare gold did not disturb hybridization, resin tag formation, and the sealing ability of the bonding system used to dentin. These findings were in agreement with Porenczuk et al.<sup>14,15</sup> and Ramasetty et al.<sup>35</sup> who revealed that NanoCare gold had no effect on the bond strength of different adhesive systems. This might be attributed to the nanoparticles characterization in terms of size and shape. NanoCare gold is being formed of 48 nm (average size) and abundant spherical nanoparticles (round, discoid). Lohbauer et al.<sup>36</sup> advocated that Ag–Au nanoparticles with spherical shape decrease the tendency of agglomeration as it provides only one point of contact. As well, the manufacturer demands that the liquid carriers, such as methanol and isopropanol in which metal nanoparticles are dispersed, provide an excellent benefit by preventing agglomeration of nanoparticles. Furthermore, the presence of different shapes and sizes of nanoparticles may simulate hybrid composites acting as inorganic fillers enabling Ag–Au nanoparticles to preserve the physical properties of restorative material.<sup>11</sup> This was confirmed by the ultramorphological examination and elemental analysis of the current laboratory study.

Appraising the literature and associating the suggestions with the findings of the current study revealed that when applying silver–gold nanoparticles after etching dentin surface by phosphoric acid gel, many authors have reported the advantage of good bond strength of resin–dentin interface that was gained because of formation of positively charged ionic bond that is very strong to bind to tooth structure and phosphate groups, in addition, to increase in surface free energy of enamel.<sup>18,22,23</sup>

Although this is a laboratory study that might have some inherent limitations to have a clinical relevance such as oral environmental conditions in terms of thermal and acidic fluctuation as well as forces of mastication, however, it is still compulsory to deliver important interpretations and deeper analysis of scientific findings that could not be assessed in clinical trials. Thus, the null hypothesis was accepted and the use of NanoCare gold did not interfere with the bond strength and sealing ability of resin composite restoration in different adhesion modes.

## RECOMMENDATIONS

- Further, *in vivo* studies should be done to evaluate the impact of application of silver–gold nanoparticles in long duration with other adhesive systems.
- Further, studies are required to assess the antibacterial efficacy of silver–gold nanoparticles in preventing the incidence of secondary caries.

## CONCLUSION

Within the limitations of this study, the following assumptions could be drawn that the new formulation of silver–gold nanoparticles did not interfere with the bond strength and sealing ability of resin composite restoration in different adhesion modes.

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