RESEARCH ARTICLE

The effect of silver nanoparticles incorporation in the self-etch adhesive system on its antibacterial activity and degree of conversion: an *in-vitro* study [version 1; peer review: 1 approved, 1 not approved]

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Abstract

Introduction: Despite of the recent advances in the adhesive dentistry, high possibility of microbial biofilm development at the resin restoration surfaces may lead to marginal gaps and recurrent caries. Degree of conversion of the dental adhesive represents a relative assessment to its quality, and a direct correlation with its mechanical behavior. This *in vitro* study was carried out to investigate the minimum inhibitory concentration of antimicrobial silver nanoparticles incorporated in two forms into the self-etch adhesive system and the effect of their incorporation on the degree of conversion of the self-etch adhesive.

Methods: Minimum Inhibitory Concentration of the self-etch adhesive system incorporated with nanosilver powder and solution against *Streptococcus mutans* was tested using an agar diffusion test. The effect of nanosilver incorporation (powder and ethanol-based solution) in the self-etch adhesive system on its degree of conversion was assessed using Attenuated Total Reflectance/ Fourier Transform Infra-Red spectrometer (ATR/ FTIR).

Results: The results showed that silver nanoparticles incorporation (powder or ethanol based at 12.5 µg/ml concentration) significantly increased the antibacterial efficacy of the self-etch adhesive against *Streptococcus mutans* ($P < 0.05$). Nanosilver powder possessed higher significant antibacterial effect when compared to silver ethanol based solution ($P < 0.05$). Degree of conversion of self-etch adhesive containing nanosilver powder showed non-significant difference from the control group ($p > 0.05$). In contrast, self-etch adhesive with nanosilver solution recorded significantly lower values when compared to the control or nanosilver powder group ($P < 0.05$).

Conclusion: The antibacterial efficacy of the adhesive system can be greatly potentiated with the addition of silver nanoparticles (12µg/mL

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Approval Status

	1	2
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1. Arivalagan Pugazhendhi , Ton Duc Thang University, Ho Chi Minh City, Vietnam

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Any reports and responses or comments on the article can be found at the end of the article.

concentration) especially the nanosilver powder. Incorporation of the antibacterial nanosilver powder in the adhesive system didn't compromise the degree of conversion of the adhesive resin.

Keywords

Silver nanoparticles, Antibacterial, Self-etch adhesive, Streptococcus mutans, Minimum inhibitory concentration, Degree of conversion.

This article is included in the **Nanoscience & Nanotechnology** gateway.

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Introduction

Dental resin composite is a widely used restorative material that has superior aesthetic properties and strong bonding ability to the tooth structure in comparison to other restorative materials like amalgam¹. In spite of the recent advances in the dental adhesives, there is a high possibility of microbial biofilm development at the resin restoration surface, which may lead to marginal gap and recurrent caries².

Silver is an antimicrobial agent that has broad spectrum activity against gram positive and gram negative bacteria². Nanoparticles are insoluble particles smaller than 100 nm. Their unique size provides higher surface area, thus much more potent antimicrobial activity in comparison to the usual particles size³. Silver nanoparticles can cause cell membrane disruption and damage of the bacterial DNA¹. Degree of conversion of the dental adhesive represents a relative assessment to its quality and directly correlates with its mechanical behavior. Proper polymerization of the dental adhesive can increase the longevity of the bonded restoration⁴. Therefore, it seems valuable to investigate the minimum inhibitory concentration of silver nanoparticles incorporated in two forms into the self-etch adhesive system and their effect on the degree of conversion.

Methods

The materials, preparations, manufacturers, composition and batch numbers are listed in Table 1.

Incorporation of silver nanoparticles into the self-etch adhesive system

1ml of ethanolic solution of silver nanoparticles was added to 5ml of self-etch adhesive system and serially diluted. To produce the adhesive system with the nano-silver powder incorporated, it was accurately weighed first using a scale (AE Adam, Bradford, UK). The previously measured powder (1000 µg) was added to 1 ml of adhesive solution, sonicated in an ultrasonic mixer (Eumax model: UD100SH-3LQ, China) and serially diluted using a micropipette (The final concentration of

nanosilver in the adhesive resin in the two forms is 100µg/ml). The procedure was completely performed in a dark environment using X-ray processing box.

Determination of minimum inhibitory concentration (MIC) of self-etch adhesive system with and without nano-silver incorporation

The minimum inhibitory concentration (MIC) defined as the lowest concentration of nanosilver in micrograms per milliliter (µg/ml) that inhibits the growth of an organism⁵. Tryptic soy agar medium; culture nutrient media for *Streptococcus mutans* (ATCC 25175) was poured in 10 petridish plates in a laminar flow (Telstar BIO- II-A, VWR Company, UK.). *Streptococcus mutans* strains; ATCC 25175 (Cairo MIRCEN, Faculty of agriculture, Ain Shams University, Egypt.) were cultured on Tryptic soy agar medium at 37°C for 24 hours⁶.

10 petridish plates were punched by a cork-borer with a 6 mm diameter to produce rounded holes in each plate and the bacterial strain was applied equally on the agar plates^{7,8}. The specimens were grouped as N_1 : Self-etch adhesive system + nanosilver solution and N_2 : Self-etch adhesive system+ nanosilver powder. Each agar plate contained 5 holes representing the five different concentrations of the nanosilver. For N_1 and N_2 groups; the self-etch adhesive system was serially diluted to produce 5 subgroups with different concentrations, C_1 : 100 µg/ml, C_2 : 50 µg/ml, C_3 : 25 µg/ml, C_4 : 12.5 µg/ml and C_5 : 6.25 µg/ml. The adhesive system without nanosilver incorporation was used as a control group (N_0).

200 µl of the adhesive agent was injected in each hole by micropipette and polymerized for 20 seconds using light emitting diode unit (Woodpecker Medical Instrument Company, Model LED. F; Model No. L14A0116F, China.) (Figure 1). The plates were incubated for 48 hours in the incubator at 37°C under completely anaerobic conditions (Shell lab Company, SMI6, Canada). The diameter of bacterial inhibition zone halo of each adhesive was measured in millimeters using a ruler⁷.

Table 1. Materials and preparations' composition, manufacturers and batch numbers.

Material	Specifications	Composition	Manufacturer	Batch number
Universal Bond	One-step, Self-etch adhesive system (Ethanol based)	Phosphorylated Meth-acryloxydecyl phosphate (MDP)- 2-Hydroxyethyl methacrylate HEMA - Ethanol Photoinitiators-Silane Nanofillers (10% by weight 5 nm spherical silica particles)	3M ESPE, St. Paul MN, USA.	19970303
Silver Nanoparticles Solution	Nanosilver in ethanolic solution (Antibacterial agent)	10 ml ethanolic solution of Polyvinyl pyrrolidone + 0.2 ml Silver nitrate powder.	Nanotech Company, 6th October City, Egypt	
Silver Nanoparticles powder	Nanosilver powder form (Antibacterial agent)	25 ml Silver nitrate+ Sodium Hypoboride+ Polyvinyl pyrrolidone.	Nanostream Company, 6th October City, Egypt	
Tryptic Soy Agar Medium	Culture Media for <i>Streptococcus mutans</i> (ATCC 25175)	15 gm Tryptone + 15 gm agar+ 5gm Soytone + 5gm Sodium Chloride.	Becton and Dickinson (BD) Company	236950

Figure 1. Agar plate with polymerized adhesive system of different nanosilver concentrations.

Degree of conversion measurement of the self-etch adhesive system with and without nanosilver incorporation

The specimens were prepared using a cylindrical Teflon mold surrounded by metallic ring (3mm diameter and 2mm height)⁹ (Figure 2). The mold was placed on a Mylar strip (Universal strips of acetate foil, Germany) that was placed on a clean flat glass slab. 200 μ l of self-etch adhesive resin was injected in the hole of the mold using a micropipette then covered with the Mylar strip (to avoid the presence of oxygen inhibiting layer and pressed to obtain a uniform smooth specimen surface)^{4,9}. The adhesive resin was cured in the presence of the top Mylar strip by LED device for 20 seconds according to manufacturer instructions. The light curing tip was applied perpendicular and with intimate contact with the top surface of the Mylar strip (Zero distance)⁴.

15 disc shaped specimens were prepared of self-etch adhesive system. The specimens were divided into 3 equal groups (n=5) according to the form of incorporated nanosilver (N_0 : Adhesive system without nanosilver, N_1 : Adhesive system+ nanosilver solution, N_2 : Adhesive system+ nanosilver powder). The concentration of the incorporated nanosilver was set according to the minimum inhibitory concentration (MIC) that was tested before.

Degree of conversion was measured using an Attenuated Total Reflectance/ Fourier Transform Infra-Red spectrometer (ATR/FTIR) (Vertex 70, Bruker Company, Germany)^{4,10}. All the data were recorded and plotted on a special computer software (OPUS Bruker Spectroscopy Software, version 7, Germany) to draw the linear graphs from which the degree of conversion of each specimen was calculated.

Data statistically was described in terms of mean values and standard deviation (SD) using ANOVA test (IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA, Statistics Version 22 for Windows).

Results

Agar Diffusion test

Mean $\pm$ SD measures of the diameter of inhibition zone (DIZ) were summarized in Table 2 and Table 3 and graphically drawn in

Figure 2. Teflon mold with surrounding metallic ring.

Table 2. Mean and standard deviation (SD) for diameter of inhibition zone (DIZ) (mm) of self-etch adhesive as a function of nanoparticles incorporation at different concentrations.

Concentration of nanosilver in adhesive system	Nanosilver solution (N_1) Mean $\pm$ SD	Nanosilver Powder (N_2) Mean $\pm$ SD
C_1 : 100 μ g/ml	20.00 $\pm$ 1	22.00 $\pm$ 0.7
C_2 : 50 μ g/ml	15.20 $\pm$ 0.84	18.80 $\pm$ 0.45
C_3 : 25 μ g/ml	14.80 $\pm$ 0.45	17.20 $\pm$ 0.84
C_4 : 12.5 μ g/ml	13.80 $\pm$ 0.45	15.60 $\pm$ 0.55
C_5 : 6.25 μ g/ml	10.40 $\pm$ 0.55	12.00 $\pm$ 0.55
Control (N_0): 0 μ g/ml	11.60 $\pm$ 1.35	11.60 $\pm$ 1.35
P value	$\leq 0.001^*$	$\leq 0.001^*$

NS; non-significant ($p > 0.05$), *; significant ($p < 0.05$).

Table 3. Comparison of diameter of inhibition zone (DIZ) values in mm (Mean $\pm$ SD) as a function of nanosilver incorporation in two different forms in the adhesive system at different concentrations.

Concentration of nanosilver in adhesive system	Nanosilver solution (N_1) Mean $\pm$ SD	Nanosilver Powder (N_2) Mean $\pm$ SD	P value
C_1 : 100 μ g/ml	20.00 $\pm$ 1	22.00 $\pm$ 0.7	$\leq 0.001^*$
C_2 : 50 μ g/ml	15.20 $\pm$ 0.84	18.80 $\pm$ 0.45	$\leq 0.001^*$
C_3 : 25 μ g/ml	14.80 $\pm$ 0.45	17.20 $\pm$ 0.84	$\leq 0.001^*$
C_4 : 12.5 μ g/ml	13.80 $\pm$ 0.45	15.60 $\pm$ 0.55	$\leq 0.001^*$
C_5 : 6.25 μ g/ml	10.40 $\pm$ 0.55	12.00 $\pm$ 0.55	0.006*

NS; non-significant ($p > 0.05$), *; significant ($p < 0.05$).

Figure 3 (underlying data available from OSF¹¹). The largest zone of inhibition was at 100 μ g/ml concentration of nanosilver powder while the smallest was at 6.25 μ g/ml concentration of nanosilver solution.

All the concentrations recorded significantly higher DIZ when compared to the control group ($P \leq 0.05$) except C_s ; 6.25 $\mu\text{g}/\text{ml}$. The MIC of adhesive containing nanosilver (powder and solution form) was determined at 12.5 $\mu\text{g}/\text{ml}$ concentration. All the concentrations of nanosilver powder recorded significantly higher mean values of DIZ when compared to the different concentrations of nanosilver solution.

Degree of conversion results

(Mean $\pm$ SD) measures of degree of conversion in % were summarized in Table 4 and graphically presented in Figure 4. Groups of adhesive system with incorporated nanosilver solution (26.14 ± 4.47 %) recorded a statistically significant lower degree of conversion when compared to control (50.31 ± 4.04 %) and nanosilver powder (47.72 ± 4.47 %) ($P \leq 0.001$). There was no significant difference in the degree of conversion between the control group and the group of the adhesive system containing nanosilver powder ($p \geq 0.05$).

Discussion

Self-etch adhesives exhibit limited antibacterial activity against *Streptococcus mutans* due to the presence of a low molecular weight monomer that possesses bacteriostatic action against *Streptococcus mutans*¹².

The MIC for adhesive containing nanosilver in ethanol solution and powder was 12.5 $\mu\text{g}/\text{ml}$ concentration which was significantly different when compared to the control group ($P \leq 0.05$). These results confirmed the potential antibacterial effect of low concentrations of nanosilver. Adhesive resin with nanosilver powder at different concentrations showed significantly higher inhibition rates than that with nanosilver/ ethanol dispersion.

The higher significant efficiency of nanosilver powder may be attributed to the presence of the ethanol as a dispersion medium for nanosilver solution that can act as a diluting agent of the adhesive system. That was observed at concentration 6.25 mg/ml in nanosilver solution as it showed lower significant value than the control group despite the presence of silver nanoparticles.

The nanosilver powder group recorded a statistically non-significant difference in the degree of conversion when compared to the control group. Presence of nanofillers in the adhesive system had no harmful effect on the degree of conversion¹³.

Besides reducing of the amount of residual monomer, the nanoparticles size is less than the wavelength of the blue light of the curing units that allow the passage of the light without scattering; thus doesn't affect the degree of conversion and the depth of cure of the adhesive system¹⁴.

Figure 3. Bar chart showing the mean values of diameter of inhibition zone (DIZ) (mm) of self-etch adhesive as a function of nanoparticles incorporation in different forms and concentrations.

Table 4. Mean and SD for the degree of conversion (%) of self-etch adhesive as a function of nanoparticles incorporation.

Variables	Mean $\pm$ SD	Rank	P value
Nano-particles incorporation in the adhesive system	Control (N_0)	50.31 $\pm$ 4.04	A
	Nanosilver Solution (N_1)	26.14 $\pm$ 4.16	B
	Nanosilver Powder (N_2)	47.72 $\pm$ 4.47	A

Means with the same letter within each row are not significantly different at $p=0.05$. NS= Non-significant, *=Significant

Figure 4. Bar chart showing the mean values of degree of conversion (%) of self-etch adhesive as a function of nanoparticles incorporation.

Groups of adhesive system with incorporated nanosilver solution recorded statistically significant lower degree of conversion when compared to the control and nanosilver powder ($p \leq 0.05$). Presence of excessive amount of ethanol (Over 10% of the neat resin blend) lead to dilution of the adhesive resin, (decrease the percent of polymerized resin). Moreover it can cause physical separation of some reactive components of the adhesive resin with subsequent reduction of the degree of conversion². Previous research has attributed the negative effect of excess ethanol on the degree of conversion to its cooling effect (polymerization reaction is exothermic and the liberated heat increase the rate of conversion). Ethanol can absorb the liberated heat thus decrease the rate of polymerization and the degree of conversion of the adhesive¹⁵.

There were no recorded studies evaluated the effect of nanosilver (powder form or ethanol based solution) incorporation in the self-etch adhesive system on the degree of conversion.

Conclusion

The antibacterial efficacy of the adhesive system can be greatly potentiated with the addition of silver nanoparticles (12µg/mL concentration) especially the nanosilver powder. Incorporation of the antibacterial nanosilver powder in the adhesive system didn't compromise the degree of conversion of the adhesive resin.

Further investigation is required for assessing the mechanical behavior and chemical reactions of adhesive systems containing

silver nanoparticles in short and long-term. It is also recommended to evaluate the antibacterial activity of adhesive system containing silver nanoparticles against dental plaque biofilm rather than single bacterial species.

Data availability

Underlying data

Open Science Framework: The effect of silver nanoparticles incorporation in the self-etch adhesive system on its antibacterial activity and degree of conversion: an In-vitro Study. <https://doi.org/10.17605/OSF.IO/RS4D2>¹¹

This project contains the following underlying data:

- Raw Data DC note pads (folder containing out files from ATR/FTIR)
- raw Data final DC.docx (ATR/FTIR data with explanation of analysis pipeline)
- results heba fathy.docx (inhibition zone measurements)

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Reviewer Report 10 August 2020

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Sashidhar Rao Beedu

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This manuscript details application of nano silver incorporation into a self-etch dental adhesive system. The antibacterial efficacy of the adhesive system was tested, *in vitro*. Even though the experimental approach seems to be fine, the experimental design and technical details appear to be poor. This manuscript requires additional details and clarification as detailed below:

Comments:

1. What is the source of nanosilver powder? If silver nitrate is used, then the calculation needs to be based on elemental Ag.
2. If AgNPs are insoluble, then how can you get solution? It may form an emulsion or suspended particles only.
3. What is the wave length (λ) of LED?
4. Table 1: Silver Nitrate powder is not nano silver. The valency of nano silver is 'zero'. If you have nano-silver powder, why are you reducing it again by sodium borohydride?
5. The authors should provide an additional representative figure showing the zone of inhibition (ZOI), in addition to table 2. Delete fig. 1.
6. The authors need to check the silver nitrate ions as additional control in the agar – antimicrobial assay.
7. If the adhesive solidifies along with nano-silver, then how does it diffuse in agar medium?
8. What happens beyond 6.25 μ g/ml concentration of silver nano? What is the *rationale* to consider 10 mm ZOI as cut-off? It is appropriate to establish the IC₅₀ value for the nano metal.

Recommendation: the manuscript is not acceptable in the present form.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nanotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 03 August 2020

<https://doi.org/10.5256/f1000research.19339.r67096>

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Arivalagan Pugazhendhi

Innovative Green Product Synthesis, Ton Duc Thang University, Ho Chi Minh City, Vietnam

- The manuscript reports the effect of silver nanoparticles incorporation in the self-etch adhesive system on its antibacterial activity and degree of conversion: an in-vitro study. Overall the experiments are performed well and also the manuscript was well written, I recommend this research manuscript is appropriate to be indexed with minor revision mentioned below.
- Avoid using words such as we, he, she, them, their, etc.

- Language is good, but, proof reading by a native speaker would avoid the minor errors.
- Please concrete the keywords and make it formally and academically.
- Regarding the Introduction section, to make the reading more clear and smooth, it should be organized according to the following items: i) present state of the art; ii) literature review; iii) motivation and objective of the study proposed; iv) innovative contribution in terms of methodology developed.
- The authors are suggested to read the following articles and cite them in the appropriate places. The introduction must be revised to enhance readability i.e. I would also like to see following references in the revised version:
 1. Shanmuganathan *et al.* (2019¹).
 2. Jacob *et al.* (2019²).
 3. Saravanan *et al.* (2018³).
 4. Pugazhendhi *et al.* (2018⁴).
 5. Shanmuganathan *et al.* (2018⁵).
 6. Saravanan *et al.* (2018⁶).
 7. Samuel *et al.* (2020⁷).
- Typographical errors are present throughout the manuscript. The authors are required to pay keen attention to this.
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1. *English language throughout the manuscript. Please ensure to have a proof reading to your manuscript.*
2. *Similarity index shall not exceed 15% with no more than 1% from any source.*
3. *Citation of references with latest publications on the topic. Please also cite some relevant papers from F1000Research.*
4. *Manuscript shall have an adequate number of Figures and Tables.*
5. *Figures must be in high quality format. Please ensure also to submit a high quality Graphical Abstract representing a summary of your study.*

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Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nanoparticles, Anticancer, Photocatalytic degradation; Cytotoxicity; Organic and inorganic Nanoparticles, Antibacterial

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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