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Original Article

Evaluation of the Antibacterial Activity, Solubility and Ions Release of Compomer Before and After the Addition of 0.025 ml Silver Nanoparticles: An In-Vitro Study

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Abstract

Aim: Evaluate the effect of silver nanoparticles addition on antibacterial activity, solubility and ions release of compomer at different time intervals. **Subjects and methods:** 0.025 ml nanosilver alcohol solution (5000ppm) was mixed with 2 g of Composan glass (compomer material). Composan glass without nanosilver was used as the control group. Total of 48 disc-shaped samples (15x1mm) were prepared and immersed in distilled water for 24 hours, 1 and 3months. After each time interval, samples were assessed for antibacterial activity by Agar Diffusion Test using *Streptococcus mutans*. Water sorption and solubility were evaluated by weighting the samples before and after immersion. Fluoride Ion-Selective Electrode determined fluoride release while Inductively Coupled Plasma-Mass Spectrometer measured silver ion release. **Results:** The experimental group showed significantly higher inhibition zones for all follow-up results. For water sorption test, the higher values were in the experimental group after 1 and 3months. While for the solubility the higher values were in the experimental group after all follow-ups. Regarding fluoride ion release, comparison between both groups showed no significant difference after all follow-ups. For the silver ion release, results showed significant difference regarding all follow-ups with higher values in the experimental group. **Conclusion:** Compomers incorporated with 0.025ml silver nanoparticles is promising antibacterial restoration against *Streptococcus mutans.*

Keywords: Compomer, Silver Nanoparticles, Antibacterial Activity, Solubility, Ions Release.

I. INTRODUCTION

Secondary caries around and under dental restorations are considered as the most common causes of failure and replacement. Thus development of dental materials possessing anticariogenic properties such as fluoride containing restorative materials have been made to prevent secondary caries and increase restorations longevity (Bürgers et al., 2009).

Polyacid-modified composite resin (compomer) was presented in 1993. They are actually resin composite modified to release fluoride over an extended period (Tiwari et al., 2016). Compomers contain conventional monomers (Bis-GMA or UDMA) and carboxylic acid functional groups. They contain some ion-leachable glass particles similar to that in glass-ionomer cements. The fluoride release, polymerization shrinkage and marginal adaptation remain less than optimal leading to secondary caries formation (Matalon et al., 2006).

Two important physical properties that influence the clinical durability of a resin based restorative material are the water sorption and

solubility. Both leads to dimensional changes of the material in oral environment contributing in loss of marginal integrity, microleakage, secondary caries formation leading to failure of the restoration (Dinakaran, 2014; Jamel, 2020).

Several studies have been made focusing on slow-release of various incorporated low molecular weight antibacterial agents such as antibiotics, iodine, chlorhexidine, zinc ions and silver ions to reduce bacterial adhesion to dental restorations (Surabhilakshan et al., 2018).

Silver has been used against different microorganisms for many years. Silver nanoparticles (<100nm) showed a stronger antibacterial activity owing to their higher surface area to volume ratio. They have been effective against fungi, protozoa, several viruses and bacteria such as *Streptococcus mutans*, *Lactobacillus* and *Staphylococcus aureus* (Kim et al., 2007).

Therefore, the aim of this study was to evaluate the effect of silver nanoparticles addition on antibacterial activity, solubility and ions release of compomer restorative material.

II. SUBJECTS AND METHODS

Materials used in the current study were Compomer: Composan glass (Promedica, Germany, 1814117) and Nanosilver in 5000ppm alcohol solution: Produced by Chemical Reduction (NanoTech Egypt for Photo-Electronics, Al Giza, Egypt). This chemical reduction method includes reduction of silver salt; silver nitrate (AgNO3) with reducing agent; sodium borohydride (NaBH4) in the presence of colloidal stabilizer.

The dispersion of silver was prepared as follows: $AgNO_3$ was dissolved in methanol solution (CH_3OH) containing 5% of polyvinylpyrrolidone (PVP) to obtain the final concentration of silver of about5000 ppm. The mixture was stirred using magnetic stir bar until the ingredients were dissolved. Then, the $NaBH_4$ dissolved in5% PVP was added to the mixture, and the mixture was stirred again with magnetic stir bar for 15 minutes. By mixing both solutions ($NaBH_4$ and $AgNO_3$), Ag ions were reduced and clustered to form monodispersed nanoparticles as a transparent sol in the methanol medium. After this, the whole system was cooled to slow down the reaction and to give better control over final particle size/shape. The test sample was prepared by complete evaporation of solvent at room temperature (Sokołowski et al., 2014).

Characterization of the Prepared Nanosilver

To evaluate the size and shape of the nanosilver, Transmission Electron Microscope (TEM) [JEM-2100, JEOL Ltd., Japan] of the nanosilver solution was done by the manufacturer, on high resolution transmission electron microscope at an accelerating voltage of 200 kV.

A. Sample Size Calculation

For antibacterial activity: Based on (Vermeersch et al., 2005) the difference in inhibition zone between the 2 groups is3±1.3mm. Using power80% and5% significance level 4 agar plates in each group (at each immersion time) was needed. While for Solubility, Based on (Yantcheva & Vasileva, 2016) the difference in water solubility between the 2 groups is 0.7±0.2. Using power80% and5% significance level, at least 3 discs in each group was needed. Sample size calculation was achieved using PS: Power and Sample Size Calculation software Version3.1.2 (Vanderbilt University, Nashville, Tennessee, USA). A total of 48 disc-shaped samples were divided into two groups according to composition:

Group I: 24 discs of commercially available compomer (control).

Group II: prepared 24 discs of compomer mixed with nanosilver alcohol solution (5000 ppm) in concentration of 0.025ml per 2g of compomer (experimental).

B. Sample Preparation

For group I, compomer (composan glass) was dispensed into a split Teflon mold **(**15±1mm in diameter and 1±0.1mm in thickness). For group II, 0.025ml of nanosilver alcohol solution (5000ppm) was taken by micro pipette and incorporated into 2g of compomer which was placed over a glass slab. They were manually mixed using a plastic spatula in a x-ray developer box to provide a dark room (Farahani et al., 2018). The mixed material was then packed into the split Teflon mold.

For both groups, a conventional length of fluoride free dental floss was embedded into the compomer to allow suspension of the samples inside the glass bottles. The compomer were covered by a celluloid strip and pressed using a glass slab with a 500g load for 30sec to extrude excess material, ensure uniform and flat surface. Samples were photoactivated by visible light-emitting diode (LED) curing unit [3M ESPE Elipar^{TM}, Germany] with light intensity of 600-800mW/cm^2 for 40seconds from top and bottom with 90^o angulation.

C. Antibacterial Test

The antibacterial activity was assessed by the Agar Diffusion Test using *Streptococcus mutans.* Plates of mitis-salivarius agar were inoculated with freshly grown *Streptococcus mutans* which was evenly spread over the blood agar with a sterile swab. A total of 24 discs (4 per group) were individually suspended with the floss into a sealed numbered glass bottle containing 60ml distilled water for 24 hours, 1 and 3months.

After each time interval, samples were removed from the solution, dried and placed onto the agar medium in petri dish. The plates were incubated in an anaerobic container at 37˚C, 5% CO_2 for 24 hours. The diameter of inhibition zones in millimeters (mm) formed around the discs was measured using a digital caliper (Tavassoli Hojati et al., 2013).

D. Sorption and Solubility Tests

A total of 24 discs samples (12 per group) were placed in a desiccator at 37ºC, after 22 hours they were removed and stored in another desiccator at 23ºC for 2 hours. Samples were weighed on a digital analytical balance [SCALTEC, Heiligenstadt-Germany] accurate to 0.0001g to obtain the initial dry weight (W). Samples were immersed in glass bottles containing 60ml distilled water and held at 37ºC in an incubator. After 24 hours, they were removed from the water, washed, dried using tissue paper and weighed to obtain the wet weight (W_1). Samples were dehydrated in a desiccator at 37ºC for 24 hours and weighed again to record the final dry weight (W_2) (Bhatia et al., 2017). Samples were then transferred into new glass bottles containing fresh distilled water repeating the same procedure after 1 and 3 months. The volume of each sample in $mm^3$was calculated as follows (Bhatia et al., 2017):

$$\mathbf{V = \pi \times r^2 \times h}$$

where,
π: 3.14,
r: radius of the sample (diameter/2) in mm,
h: thickness of the sample in mm.

The water sorption (W_{sp}) and solubility (W_{sol}) in $\mu g/mm^3$ were obtained as follows (Bhatia et al., 2017):

$$\mathbf{W_{sp} = W_1 - W_2 / V}$$
$$\mathbf{W_{sol} = W - W_2 / V}$$

where,
W: weight in µg before immersion,
$\mathbf{W_1}$: weight in µg after immersion,
$\mathbf{W_2}$: weight in µg after immersion and desiccation,
V: volume of the sample.

E. Ions Release Test

The stored distilled water solutions used in the sorption and solubility tests after 24 hours, 1 and 3months were used to determine silver and fluoride ion release.

- **Silver Ion Release**

After each predetermined time intervals, the water solutions from each container were filtered through disposable 0.2µm PTFE (Polytetrafluoroethylene) syringe filters. The silver ion concentrations in these extracts were determined by means of Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) (iCAP, Thermo, Germany). Silver ion release values were obtained and recorded in ppm.

- **Fluoride Release**

The fluoride ion release was measured using Fluoride Ion-Selective Electrode (WTW, pH/ION 7320-Germany). After the same predetermined time intervals, 20ml of the storage water was taken from each container and analyzed after 1:1 dilution with 0.1ml of Total Ionic Strength Adjustment Buffer (TISAB II). The fluoride electrode was dipped into the solution and waited for 10-15 minutes until a steady result. The fluoride electrode was gently rinsed before and between each measurement with de-ionized water to prevent crossover contamination between samples. Fluoride release values was obtained and recorded in ppm (Makkai et al., 2019).

F. Statistical Analysis

Statistical analysis was performed using SPSS 20®, Graph Pad Prism®, Microsoft Excel 2016. Data was represented as mean and standard deviation; significance level set at p-value <0.05. Comparison between the two groups at different follow up periods was performed by Independent T-test. On the other hand, comparison between two follow up periods was performed by Paired t-test, while comparison between three groups was performed by One Way Analysis of Variance ANOVA followed by Tukey`s post hoc test.

III. RESULTS

- **Particle Size of Nanosilver**

The TEM showed the observed particle with a spherical like shape with an average size of 40±5 nm (**Figure 1**).

- **Antibacterial Test**

The largest inhibition zones against *Streptococcus mutans* were observed with group

Although, there was no inhibition zone observed with group I (**Figure 2**) and (**Table 1**).

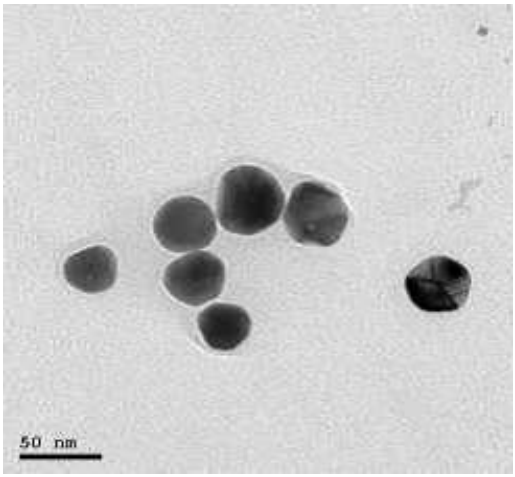

Figure 1: TEM micrograph of the prepared Ag nanoparticles. Bar: 50nm

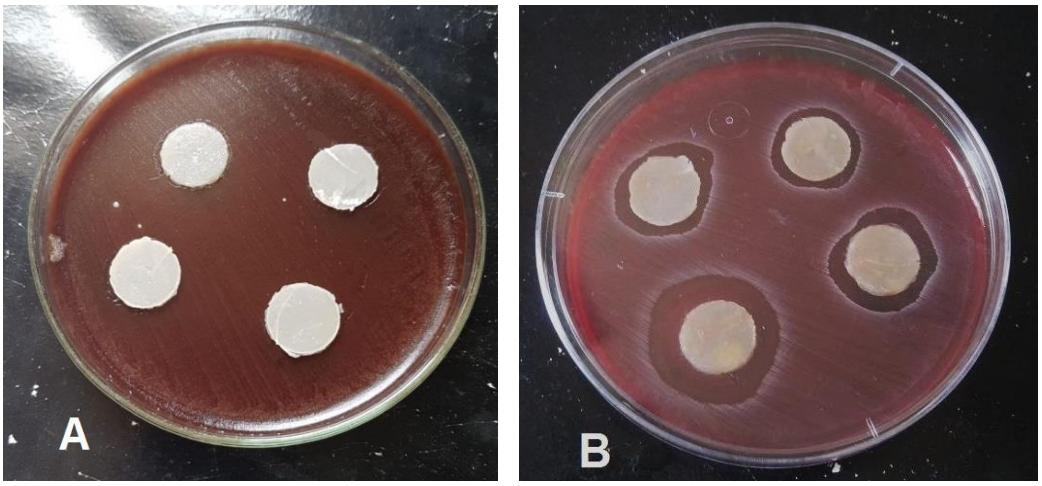

Figure 2: Inhibition zones around the prepared discs (A) control group and (B) experimental group.

Table 1: Mean values and standard deviation of inhibition zone in (mm) of control and experimental groups after 24 hours, 1 and 3 months.

Group	After 24 hours		After 1 month		After 3 months		P value
	M	SD	M	SD	M	SD	
Control	15.25 [a]	0.51	14.5 [a]	1.01	16.5 [a]	1.29	0.056 ns
Experimental	16.5 [a]	0.57	19.5 [b]	0.57	21.5 [a]	2.38	0.002*
P value	0.01*		0.0001*		0.008*		

M; mean SD; standard deviation
*significant difference ns; non-significant difference

- **Water Sorption Test**

On comparing the two groups, results showed significantly higher water sorption in group II after 1 and 3 months (P<0.05) compared to group I. In group I, a significant difference was revealed between 24 hours and 3 months. While, for group II there was significant increase in water sorption between all follow-ups (**Table 2**).

- **Solubility Test**

In group I and group II, results showed a significant increase in water solubility between all follow-ups. In addition, Comparison between both groups revealed higher water solubility values were in group II in comparison to group I (P<0.05) (**Table 3**).

- **Silver Ion Release Test:**

Results showed significantly higher silver ion release for group II (0.8015±0.001, 0.8337±0.0398 and 0.8667±0.0296 after 24 hours, 1 and 3 months respectively) between all follow-ups in comparison to group I (0.00 after 24 hours, 1 and 3 months) (P<0.05) (**Figure 3**).

- **Fluoride Ion Release Test**

For both group I and group II, results showed statistically no difference in fluoride release between all follow-ups (P<0.05) (**Figure 4**).

Table 2: Mean values and standard deviation of water sorption in (µg/mm^3) of control and experimental groups after 24 hours, 1 and 3 months.

Group	After 24 hours		After 1 month		After 3 months		P value
	M	SD	M	SD	M	SD	
Control	6.65 ^{a}	1.56	8.35 ^{ab}	0.97	10.33^{b}	1.25	0.009*
Experimental	7.64 ^{a}	1.18	13.02 ^{b}	1.67	15.99 ^{c}	0.85	0.001*
P value	0.32 ns		0.001*		0.0002*		

M; mean SD; standard deviation
*significant difference ns; non-significant difference

Table 3: Mean values and standard deviation of solubility in (µg/mm^3) of control and experimental groups after 24 hours, 1 and 3 months.

Group	After 24 hours		After 1 month		After 3 months		P value
	M	SD	M	SD	M	SD	
Control	2.41 ^{a}	1.17	13.02 ^{b}	2.22	32.84 ^{c}	1.67	0.001*
Experimental	10.33 ^{a}	1.56	18.26 ^{b}	1.49	37.22 ^{c}	0.97	0.001*
P value	0.001*		0.0027*		0.003*		

M; mean SD; standard deviation
*significant difference ns; non-significant difference

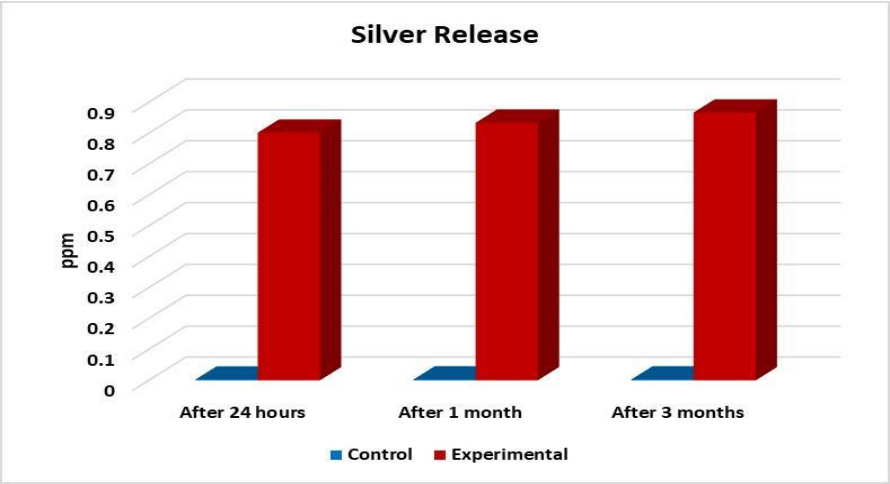

Figure 3: Mean values in (ppm) for the silver release of control and experimental groups.

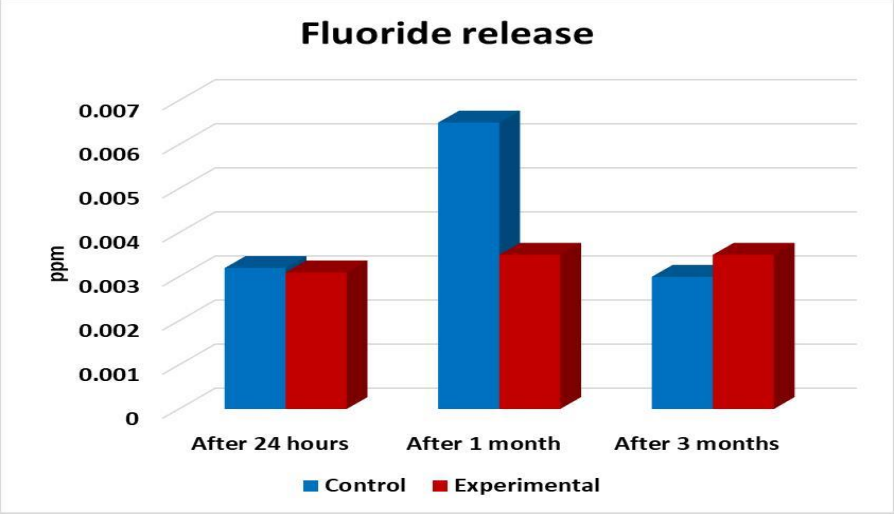

Figure 4: Mean values in (ppm) for the fluoride release of control and experimental groups.

IV. DISCUSSION

Compomers possess advantages superior to glass-ionomer cements or composites. However, they lack the ability to bond chemically to tooth tissues and antibacterial activity thus decreasing the longevity of the restoration (Hugar et al., 2017).

In the past few years, nanosilver became one of the most investigated nanotechnology-derived nanostructures as silver has a strong proven and a long-term antibacterial activity due to sustained silver ion release compared to other metals (Bapat et al., 2018). The nanosilver in this study was prepared by Chemical Reduction method (Sokołowski et al., 2014) dispersed in alcohol solution (5000ppm) in concentration as a linear relationship between silver concentration in the alcoholic dispersion (from 125 up to 5000ppm) and antibacterial activity was reported (Dutra-Correa et al., 2018). The prepared nanosilver was characterized by the manufacturer using TEM to provide conclusive information about morphology and particle size (**Figure 1**). The observed particle size (40±5nm) lied within the range normally reported for exhibiting higher antibacterial activity than larger sizes tested up to 450 nm, as the antibacterial activity is inversely correlated with the nanoparticles size (Dutra-Correa et al., 2018; Panacek et al., 2006).

The antibacterial test was evaluated by Agar Diffusion Test, as it allows bacteria to be screened

in a routine, economical and easy way for detecting its resistance (Pietrokovski et al., 2016). Streptococcus mutans was used in this study as it is the primary etiological factor of caries lesions (Tartici et al., 2020).

Results showed higher antibacterial activity of group II (**Figure 2**) and (**Table 1**). This may be attributed to the nanosilver release resulting in a direct interaction with the Streptococcus mutans present in the media. Several mechanisims can explain the antibacterial activity of nanosilver: they can attach and damage bacterial cell membrane making it porous, leading to bacterial death (Liao et al., 2019; Yin et al., 2020). Silver ions can act on the bacteria's DNA (sulfur and phosphorus) destroying it, leading to cell death. They could also inactivate the vital enzymes of bacteria causing the loss of DNA's replication ability (Burdușel et al., 2018; Ibrahim et al., 2017). These results were in accordance with Farahani et al. 2018 who reported that all the tested composites containing nanosilver had antibacterial effect which increased by increasing the amount of nanosilver to 0.03% by weight (Farahani et al., 2018).

In the current study, water sorption and solubility were conducted according to ISO-4049 specification. For water sorption, results showed that group II had higher sorption values after 1 and 3months compared to group I (**Table 2**). One explanation can be that the addition of nanosilver act as a filler within the compomer, decreasing the cross-linking and rate of polymerization separating the polymer chains from each other making it more susceptible to water sorption (Hasab et al., 2018).

While for the solubility values, group II showed higher values over time in comparison to group I, where maximum values were reached after 3months of immersion in group II (**Table 3**). A possible explanation could be due to the diffusion of nanosilver from the bulk to the surface in order to form equilibrium between the material and the environment (Hasab et al., 2018). Regarding the effect of storage time, results showed a continuous increase in both water sorption and solubility from 24 hours to 3 months (**Table 2**) and (**Table 3**). This may be due to continuous diffusion of water along the filler matrix interface, requiring a longer time in comparison to the water diffusion at the surface (Jamel, 2020).

The obtained water sorption values were lower than that recommended by ISO specifications, which state that the maximum acceptable value of polymer-based restorative materials must be equal/less than 40µg/mm3. While the obtained water solubility values were higher than that recommended, which state that the maximum acceptable value must be equal/less than 7.5µg/mm3.

To confirm that the obtained antibacterial results were caused by nanosilver release not fluoride, fluoride and silver ion release tests were performed. Silver ion release was measured using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) as it measures a wide range of elements with a concentration down to µg/L(ppt; parts per trillion) and below (Alissawi, 2013; Bajsman et al., 2014).

Results showed higher silver release values in group II at all-time intervals (**Figure 3**). Several possible factors may explain these findings, including; the concentration and the large surface area of nanosilver incorporated into the polymer (Damm et al., 2008; Monteiro et al., 2012). Another possible explanation is that water uptake of the polymer matrix is a crucial factor for silver ion release, as they are released only when the elemental silver particles come to contact with water and dissolved oxygen ($O_{2(aq.)}$) according to the following Equation:

$$O_{2\,(aq.)} + 4H_3O^+ + 4Ag_{(s)} \longrightarrow 4Ag^+{}_{(aq.)} + 6H_2O$$

Hence, the initial release of silver ions occurs from the nanoparticles at the surface of the sample. With increasing immersion time, water diffuses into the bulk, increasing the silver ion release into the aqueous environment (Damm & Münstedt, 2008; Moussa et al., 2018).

Fluoride Ion-Selective Electrode was used in this study as it is inexpensive, does not require using complex laboratory equipment and measures total fluoride concentration containing free fluoride ions and fluoride complexes (Elshweekh et al., 2019). Total ionic strength adjustment buffer (TISAB II) was added to the solutions to provide constant background ion strength, de-complex the fluoride and ensure an acidic pH between 5.0 and 5.5 (Surabhilakshan et al., 2021).

Results showed no difference between group II and group I, where fluoride release levels remain relatively constant over time in both groups with no initial fluoride increase (burst effect) (**Figure 4**). This may be attributed to the complex process of fluoride release from compomer which includes denseness of the polymerized compomer, material's chemistry (setting reaction and formation of hydrogel layer) and content of fluoride

in the composition (Sagmak et al., 2020; Zafar & Ahmed, 2015). The fluoride release is associated with the hydrogel layer thickness, where the greater the thickness, the greater the fluoride release. Since compomers have very thin hydrogel layer, fluoride is released with no burst action (Sen & Natarajan, 2022; Zafar & Ahmed, 2015). These were in agreement with Makkai et al. 2019 who measured the fluoride release of two compomers. They found that both compomers did not show any increases in fluoride release during their study (Makkai et al., 2019). In addition, Bansal et al. 2015 and Garoushi et al. 2018 also reported that fluoride release of compomer was in constant level compared to the other tested materials in their studies (Bansal & Bansal, 2015; Garoushi et al., 2018).

Ion release tests results confirmed that the antibacterial activity from group II was due to the silver ion release, as nanosilver addition to compomer did not influence/interrupt fluoride release. Hence, the antibacterial activity of compomer can be greatly potentiated with the addition of 0.025ml nanosilver. However, further research is necessary on the cytotoxicity and biocompatibility of compomer containing nanosilver before its clinical application.

V. CONCLUSION

Within the study limitations, the following could be concluded:

1. Compomers incorporated with 0.025ml silver nanoparticles seem to be a promising antibacterial restoration against *Streptococcus mutans*.
2. The water sorption values of compomer after the incorporation of 0.025ml silver nanoparticles lied within the acceptable range of ISO-4049 specifications in contrary to the water solubility values.

Conflict of Interest:
The authors declare no conflict of interest.

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Ethics:
This study protocol was approved by the ethical committee of the faculty of dentistry- Cairo university on: 22-5-2018, approval number: 8518

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