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ASSESSMENT OF ANTIMICROBIAL CHARACTERISTICS OF NOVEL ENAMEL TREATMENT PROTOCOL USING SILVER GOLD NANOPARTICLES WITH ORTHODONTIC BRACKETS FOR CARIES PREVENTION: IN VITRO STUDY

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ABSTRACT

Objectives: The aim of this study is to evaluate the antimicrobial activity of novel enamel pretreatment protocol using silver gold nanoparticles to enamel bonded orthodontic brackets on *Streptococcus mutans* and *lactobacillus* strains.

Methodology: Twenty sound upper anterior teeth were used. They were equally divided into two groups. In Group A (comparator group): The NanoCare gold was applied after enamel etching, followed by application of the bonding agent then the composite adhesive. As for Group B (control group): the etching procedure was followed by applying the bonding agent directly then by the composite adhesive. All teeth were cut off from the cured acrylic resin below the crown portion by 2 mm. The specimens were prepared. Bacterial testing was carried out using the agar well diffusion method. The diameter of the halo formed around the agar well (inhibition zone) was measured after 24 h, 48 h and 72 h. TEM assessment of the mode of action was performed data were collected and statistically analyzed. The significance level was set at $p<0.05$ within all tests.

Results: Results showed that regardless of the type of bacteria and measurement time, inhibition zones formed with the NanoCare gold group were significantly higher than those of the control group. TEM results revealed rupture of the bacterial cell membrane and penetration of nanoparticles inside the cells causing their death.

Conclusions: the treatment protocol using Silver Gold nanoparticles was successful in caries prevention by inhibiting several types of microbial strains such as *S. mutans* and *Lactobacilli* around orthodontic brackets.

Clinical significance: Enamel treatment with silver gold nanoparticles prior to the cementation of orthodontic brackets can decrease the chance of the likelihood demineralization during treatment than uncoated brackets.

KEYWORDS: Antimicrobial activity, silver gold nanoparticles, orthodontic brackets, caries prevention, *Streptococcus mutans*, *lactobacillus*

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INTRODUCTION

The incorporation of new materials into the orthodontic practice seems to be mandatory, in an attempt to overcome some of the most lagging problems in this field such as bracket debondings, prolonged treatment durations and in particular enamel demineralization which has been obviously increased with associated microbial resistance to available usual antimicrobial treatments[1],[2]. Fixed orthodontic appliances have a role in affecting oral microbiota in terms of amounts and type, resulting in decreased pH and increased plaque formation with higher affinity of stainless steel materials for microbial adhesion when compared to ceramic ones[3].Yet, Orthodontic adhesives are reported to have a higher retaining ability of cariogenic bacteria than the orthodontic brackets themselves[4] , with the most common sites for demineralization at the junctions of the bonding adhesives[5].

White spot lesions (WSLs), also known as "white opacity," develop as a result of underlying enamel demineralization on teeth's smooth surfaces. The decalcified enamel's altered optical characteristics that scatter light are what give it its white appearance. The development of these early lesions is further aided by a number of host variables, including inadequate salivary volume, tooth decay, and a diet high in sugar, as well as a number of risk factors, including fermentable carbohydrates and acid-producing bacteria[3]. The fixed orthodontic appliances—brackets, bands, wires, and other applications—account for one of the major risk factors. This equipment increase the number of plaque retention sites and complicate oral hygiene procedures[4]. Within a month of the orthodontic brackets being placed, enamel demineralization has been found to occur around them[6]. This, in turn, has been linked to the emergence of white spot lesions, which afflict 55 to 75 percent of patients receiving orthodontic treatment[2, 7].

The classical way for prevention of the initiation of the demineralization process depends mainly on patient's compliance through control of dietary habits, adequate oral hygiene procedures and topical fluoride applications, however all those procedures have not been effective enough and were considered of low reliability[8, 9]. Not to mention, several studies reported that on adding antimicrobial agents to orthodontic adhesives, reduces bond strength and increases bond failure[10, 11].

As one of the fields that truly needs to reduce microbial colonization on orthodontic appliances and auxiliaries during the lengthy treatment process in an effort to lessen the possibility of initiation of white spot lesions, dental caries, and consequently periodontal breakdown, the role of advanced nanoparticles technology in orthodontics appears promising[12].

One of the most prominent instances of how nanoscience has significantly improved dentistry is the manufacture and application of nanoparticles in dental care. Despite being in their early stages of research and development, Nano biomaterials have a wide range of potential clinical uses because of their special qualities and capacity to work either alone or in combination with other biomaterials. It is difficult or impossible to achieve the practical application of nanoparticles in conservative dentistry because the particles must first be manufactured and analyzed to ensure that they are the right size, shape, and type before they can be loaded into a material. Recently, a new disinfectant for dental procedures based on nanoparticles was introduced to the market. The product has undergone characterization and is completely defined. Dental Nanotechnology, located in Katowice, Poland, manufactures Nano Care Plus Silver Gold® (Nano Care), which contains silver nanoparticles together with a trace quantity of gold nanoparticles. With an average size of 29.07 nm, AgNPs made up 99% of the particles in the Nano Care solution, while the remaining 1% were gold nanoparticles (AuNPs), which had an average size of 136.7 nm and were suspended in 70% isopropyl

alcohol. Silver and gold nanoparticles' varied sizes, shapes, and surface energies ensure that they have antibacterial qualities against a range of bacterial types.[13] It consists of a large number of 48 nm-sized, round, and discoid spherical nanoparticles. Because there is just one site of interaction, the spherical shape of the nanoparticles lessens the possibility of agglomeration. Because they only have one point of contact, the spherical shape of the nanoparticles lowers the possibility of agglomeration. Additionally, the business claims that the metal nanoparticles are dispersed in a liquid carrier, such as isopropanol, which offers an additional benefit by reducing NP agglomeration by dissolving in a liquid carrier, such as isopropanol and methanol.[13]

The use of nanoparticles including Ag and Au as antimicrobial agents has gained much popularity. Silver nanoparticles specifically have proven efficacy as antimicrobial agents[14, 15] with selective toxic properties for prokaryotic microorganisms, minimum effect on eukaryotic cells[15] and bacterial strains that are antibiotic resistant[16]. In this sense, nanoparticles could provide a good alternative.

The goal of this work is to evaluate the microbial activity of *Streptococcus mutans* and *lactobacillus* strains formed around agar well after adding silver gold nanoparticles as a novel treatment to enamel bonded orthodontic brackets as this treatment protocol was not used before with orthodontic treatment however it was assessed in restorative dentistry[13]. This treatment protocol, if validated and adopted, might enable orthodontic clinicians to provide their patients with safe orthodontic treatment without development of post-orthodontic white lesion development. The null hypothesis was that "NanoCare gold" application to treated enamel before orthodontic bracket bonding would result in bacterial inhibition around orthodontic brackets which in turn results in decreasing the possibility of enamel demineralization.

MATERIAL AND METHODS

An in-vitro study was carried out to determine the bacterial inhibition zone in millimeters of *streptococcus mutans* and *lactobacilli* strain formed around agar well after 24 hours, 48 hours and 72 hours after adding silver gold nanoparticles to the enamel surface before applying orthodontic adhesive for bracket bonding.

The material tested is called "NanoCare gold" (Dental Nanotechnology, Poland), this material is based on Ag and Au nanoparticles with their known bacteriostatic effects. This specifically developed pharmaceutical formula is claimed to seal micro-fissures created between the tooth tissue to be treated and the adhesive material, thus preventing bacterial infiltration into these fissures.

The brackets of choice were the Forestadent Mini Sprint orthodontic brackets (Forestadent, Germany) and was used on all teeth involved in the study. Ortho Solo (Ormco Corporation, An Envista Company) Universal sealant and bonding primer and Brace Paste (American Orthodontics Corporation, USA) adhesive were used for the bonding procedure. The materials used in the study, their description, manufacturer and lot number were listed in table 1.

Study setting

The study protocol of this experimental laboratory preclinical study was approved by the research ethical committee at the Faculty of Dentistry, Ahram Canadian University, Egypt (IRB00012891#88) at its meeting held on December 3. 2023, that is in accordance with the declaration of Helsinki and its later modification.

Specimen Preparation

This study involved collecting 20 sound upper anterior teeth that were extracted for periodontal purposes, the teeth were collected according to previous study [8]. The teeth were obtained from

TABLE (1) Materials used in the study and its composition

Materials	Specification	Composition	Manufacturer	Lot No
Nano Care	Silver Gold Naoparticles	-99% Silver nanoparticles (AgNPs) with average size of 29.07 nm. -1%Gold nanoparticles (AuNPs) with average size 136.7 nm. -70% isopropyl alcohol.	Dental Nanotechnology, Katowice, Poland	270213
Ortho Solo	Universal Bonding Primer	-2.5-12% Ethoxylated Bisphenol A Dimethacrylate -≤ 3 % Tetramethylene Dimethacrylate -≤ 3 % Diphenyl (2,4,6 trimethylbenzoyl) phosphine oxide	Ormco	9856528
Brace paste	Orthodontic Light-Curing Adhesive	-≤ 2.5% Tetramethylene Dimethacrylate	American Orthodontics	202000440

patients between the ages of 40 and 60 visiting the clinics at the Faculty of Dentistry, Oral Surgery Department, Ahram Canadian University in December 2023 according to ethical approval and infection control protocols. Teeth were subsequently immersed in a 1% chloramine-T solution for duration of 72 hours, serving as a disinfectant. The teeth were examined using magnification lens of ×7 to exclude any tooth with cracks or structural defects. The chosen teeth were carefully cleared of calculus and tissue buildup, polished with pumice, and rotated with a brush at a standard speed. Teeth were then stored in distilled water at 37^{o}C± 1^{0}C, using and incubator (BTC, model: BT1020, Cairo, Egypt). This isolation was replaced every five days. Teeth were distributed equally between 2 groups. The teeth were mounted vertically in cold curing acrylic resin 2 mm below cemento-enamel junction, using plastic circular molds.

Since the study involved 2 groups. the prepared specimens were randomly divided into these main groups and all the teeth were prepared using phosphoric acid which was applied for 30 seconds to initiate enamel etching, afterwards It was washed with copious amount of water. In Group A (comparator group): Enamel etching with 37% phosphoric acid etching gel (Meta Biomed Europe GmbH, Germany) was applied for 30 seconds, then washed for 30 seconds, and dried for 20 seconds, then five consecutive coats of the NanoCare gold was applied using a disposable microbrush after and left for 30 seconds until being completely dry, followed by application of the bonding agent that was light cured for 20 seconds at zero distance using an LED curing unit (800 mW/cm^2). Then the composite adhesive was applied according to the manufacturer instruction and light cured for 40 seconds. As related to Group B (control group): the etching procedure was followed by applying the bonding agent directly then by the composite adhesive. After completing the bonding procedure, all teeth were cut off from the cured acrylic resin below the crown portion by 2 mm, then the crown was sectioned to separate the specimen slabs with the bonded brackets, which were then ready for microbial testing.

Bacterial strain activation and preparation of culture media

Bacterial testing was carried out using agar well diffusion method by preparing six agar plates. The six plates were prepared in the form of three plates for each of the *streptococcus* and the *lactobacilli* strains and each plate contained specimens for both the control and intervention groups (Figure 2).

Strict adherence to the manufacturer's specifications was maintained during the bacterial preparation process. The reference strains Streptococcus mutans (ATCC 25175) and Lactobacillus (ATCC 4356) from the Microbiological Resources Centre, Cairo MIRCEN department (Faculty of Agriculture, Ain Shams University, Egypt) were used as bacterial isolates. They were kept in brain heart infusion broth (BHI) (Merck KGaA 64271 Darmstadt, Germany) that was prepared and sterilized in accordance with the manufacturer's instructions and then poured into sterile 90 mm^2 Petri plates to form an 8 mL base layer. While Lactobacillus was cultivated on MRS Lactobacillus agar, Streptococcus mutans was cultivated on blood agar (Oxoid, Rodano, Milano, Italy).

Diffusion technique in Agar wells

Agar well diffusion method was used to evaluate the antimicrobial activity of the NanoCare gold material. In this method, the agar plate surface was inoculated by spreading 1 x 107 cells/ml of an overnight culture of each strain over the entire agar surface at 37°C. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the material was introduced into the well. Then, agar plates were incubated under aerobic condition with 5% CO2 for *Streptococcus mutans* and under anaerobic condition for *Lactobacillus* for 24-72 hours. To prevent contamination throughout the cultivation process, all the procedures were performed in a laminar flow environment (Class II Telstar BIO II, CSC Ltd, Ireland), with a minimum distance of 10 cm from a blazing torch. The plates were completely anaerobically incubated for 24 hours at 37° C in the incubator. Following incubation, a spotless halo, or limited bacterial growth, developed around the cavity, signifying both bacterial growth and antibiotic action. The inhibition zones of the bacterial growth were measured in three distinct locations and represented in millimeters using a millimeter ruler. The samples were measured once again after being incubated for 48 and 72 hours under the identical circumstances. The diameter of the halo formed around the agar well (inhibition zone) was measured after 24 h, 48 h and 72 h[17]. (Fig. 1)

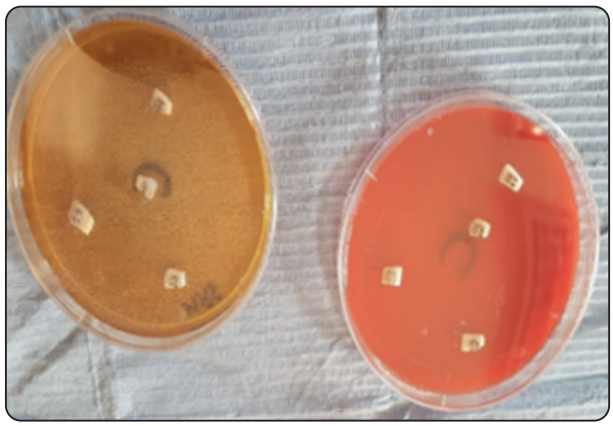

Fig. (1) Insertion of prepared tooth slabs into the Agar plates.

Evaluation of silver-gold nanoparticles' mechanism of action using the MTT test and HPLC

The antibacterial mechanism of silver-gold nanoparticles was examined using transmission electron microscopy (TEM) to determine their effects on microorganisms and examine the morphological changes of Lactobacillus and Streptococcus mutans after treatment.

A JEOL JEM-1010 Transmission Electron Microscope (JEOL Ltd, Japan) was used to analyze electron micrographs of the bacteria treated with silver gold nanoparticles that were taken from a stained slice. After being collected by centrifugation at 10,000 rpm for five minutes, the bacteria were ready for TEM investigation. The specimens were preserved for five minutes at room temperature by submerging them in a 2% glutaraldehyde solution. After that, fixed specimens were cleaned three times for fifteen minutes each in distilled water. After being fixed and cleaned, the specimens were immersed in a series of ethanol grades—30%, 50%,

70%, 90%, and 100% ethanol—to dehydrate them. Specimens were kept at room temperature for fifteen minutes in each concentration. Through a gradual series of ethanol, propylene oxide was substituted for absolute alcohol: propylene oxide 2:1, 1:1, and 1:2; pure propylene oxide was then maintained.

In glass vials with polypropylene lids, dehydrated specimens were infiltrated with the resin mixture using a graded sequence of resin: propylene oxide (1:2) for one hour and (1:1) for six hours, followed by resin: propylene oxide (2:1) for twenty-four hours at room temperature with shaking. After being embedded in pure resin for an hour at 40°C to remove any remaining propylene oxide, the specimens were moved to an oven. After 48 hours of polymerization at 60˚C, the resin was allowed to cool to ambient temperature.

Leica Ultracut UCT Ultramicrotome (Leica Microsystems GmbH, Germany) was used to section the material. Glass knives were used to cut extremely thin portions that were between 50 and 80 nm thick. The resulting parts floated on the water's surface like a ribbon. With a cutting speed of 0.4 mm/sec, the knife's clearance angle was 3°. High-quality pieces cut to a silver-gray or gold color. After that, pieces were compressed using chloroform vapor, and before they were picked up on grids, they were moved to the middle of the boat using an eyelash.

Glass Petri dish with filter paper containing 2.05-mm hexagonal copper mesh grids that have been cleaned with chloroform. Uranyl acetate and lead citrate were used as duplicate stains on the sections. With portions facing the stain, each grid was floated onto a drop of the stain. Parts were stained for fifteen minutes. Before being examined under a TEM, stained areas on grids were cleaned with 0.02 N NaOH and then distilled water. They were then placed on filter paper in a Petri dish.

Statistical analysis

The mean, standard deviation (SD), median, and interquartile range (IQR) values were used to represent numerical data. By examining the data distribution and using Shapiro-Wilk's, they were examined for normality and variance homogeneity. Kruskal-Wallis's test and Dunn's post hoc test were used to assess the non-parametric inhibition zone data. Bonferroni adjustment was applied to P-values to account for multiple comparisons. Spearman's rank-order correlation coefficient was used for correlation analysis. For all tests, the significance threshold was set at $p<0.05$. R statistical analysis software, version 4.3.3 for Windows, was used to conduct the statistical study.[18].

RESULTS

Summary statistics and results of inter and intragroup comparisons for bacterial inhibition zones are presented in Tables and Figures from (2) to (5). Results showed that regardless of the type of bacteria, inhibition zones formed with the nanogold group were higher than those of the control group, yet the difference was not statistically significant ($p<0.05$). Additionally, inhibition zones formed in the nanogold group increased in size over time. However, the increase was similarly not statistically significant ($p>0.05$). Results presented in Table (4) and Figure (6) showed that there was a strong significant correlation between values of inhibition zones formed against both types of bacteria that was statistically significant ($rs= 0.916$, $p<0.001$). The inhibition zones formed with different bacterial strain tested at different time intervals were presented in figure 7.

Results showed that regardless of the type of bacteria and measurement time, inhibition zones formed with the NanoCare gold group were significantly higher than those of the control group. For the NanoCare gold group, there was a significant increase in the size of inhibition zones formed

against *Streptococcus mutans*, with zones formed after 72 hours being significantly larger than those formed at earlier intervals. However, for the control group, the change was not statistically significant.

Similarly, for NanoCare gold zones formed against *Lactobacillus*, there was a significant increase by time but with values measured after 72 hours being significantly higher than the 24-hour value only. The change measured in the control group was also statistically significant, but with the value measured after 48 hours being significantly higher than other intervals.

S. mutans treated and untreated exterior morphological traits were displayed by TEM investigation. The S. mutans cells that were left untreated had normal cell properties, a homogenous electron density in the cytoplasm, and a coccal shape with a cell wall and cytoplasmic membrane that were very well preserved. But *S.mutans* cells treated with the silver nanoparticle solution showed notable morphological alterations, including the development of "pits" in their cell walls and subsequent lysis of the cells with damaged walls and cytoplasmic membranes (figure 8a).

The exterior morphological characteristics of both treated and untreated *L. acidophilus* were revealed by TEM examination. The untreated *L. acidophilus* cells maintained their rod-like shape with a well-preserved cell wall and cytoplasmic membrane, as well as normal cell properties and a homogenous electron density in the cytoplasm. Nevertheless, *l. acidophilus* cells treated with the silver nanoparticle solution showed notable morphological alterations, including the development of "pits" in their cell walls and subsequent lysis of the cells with damaged walls and cytoplasmic membranes (figure 8b).

TABLE (2) Summary statistics, inter and intragroup comparisons of bacterial inhibition zones (mm) (Streptococcus mutans).

Interval	*Measurement*	*NanoCare gold*	*Control*	*Test statistic*	*p-value*
24 hours	***Mean± SD***	18.60±2.88^{B}	12.40±0.89^{A}	**25.00**	**0.011***
	Median (IQR)	17.00 (3.00)^{B}	13.00 (1.00)^{A}		
48 hours	***Mean± SD***	20.00±3.67^{B}	12.40±0.89^{A}	**25.00**	**0.011***
	Median (IQR)	20.00 (3.00)^{B}	13.00 (1.00)^{A}		
72 hours	***Mean± SD***	30.20±3.96^{A}	11.80±0.45^{A}	**25.00**	**0.011***
	Median (IQR)	30.00 (5.00)^{A}	12.00 (0.00)^{A}		
Test statistic		**9.19**	**2.49**		
p-value		**0.020***	**0.288**		

SD Standard deviation, IQR Interquartile range.

TABLE (3) Summary statistics, inter and intragroup comparisons of bacterial inhibition zones (mm) (Lactobacillus).

Interval	*Measurement*	*NanoCare gold*	*Control*	*Test statistic*	*p-value*
24 hours	***Mean± SD***	18.40±2.07^{B}	12.00±0.71^{B}	**25.00**	**0.011***
	Median (IQR)	18.00 (3.00)^{B}	12.00 (0.00)^{B}		
48 hours	***Mean± SD***	22.20±3.83^{AB}	14.80±0.45^{A}	**25.00**	**0.011***
	Median (IQR)	23.00 (4.00)^{AB}	15.00 (0.00)^{A}		
72 hours	***Mean± SD***	25.40±2.07^{A}	12.00±0.71^{B}	**25.00**	**0.011***
	Median (IQR)	25.00 (3.00)^{A}	12.00 (0.00)^{B}		
Test statistic		**7.88**	**10.23**		
p-value		**0.020***	**0.012***		

SD Standard deviation, IQR Interquartile range.

TABLE (4) Correlation between inhibition zones formed against both bacteria.

Variables	*Correlation coefficient (95% CI)*	*p-value*
SM and LA inhibition zones	0.919 (0.835:0.961)	**<0.001***

CI Confidence interval, * Significant (p<0.05).

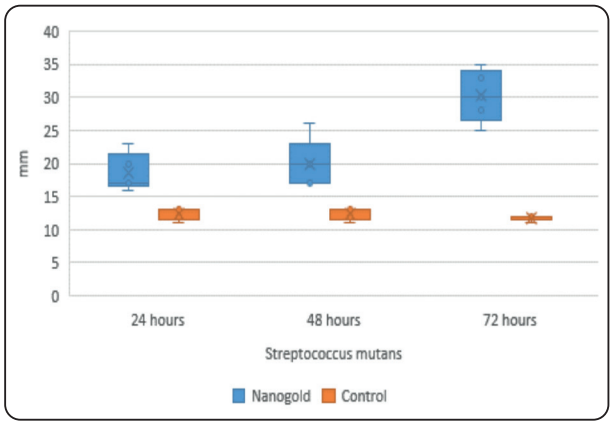

Fig. (2) Box plot for bacterial inhibition zones (mm) (Streptococcus mutans).

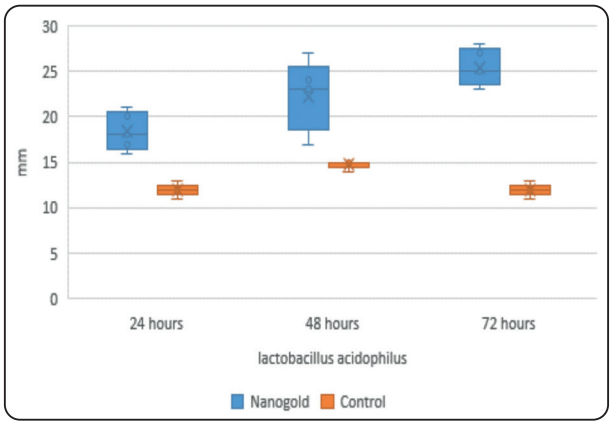

Fig. (3) Box plot for bacterial inhibition zones (mm) (Lactobacillus).

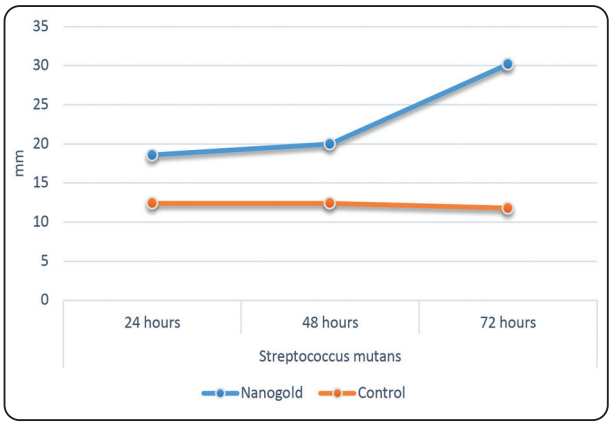

Fig. (4) Line chart showing average bacterial inhibition zones (Streptococcus mutans).

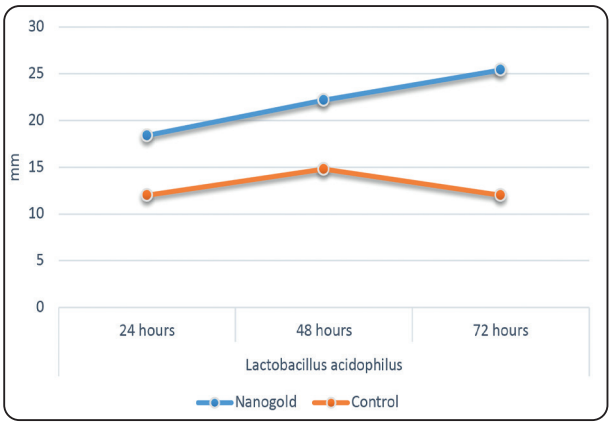

Fig. (5) Line chart showing average bacterial inhibition zones (Lactobacillus).

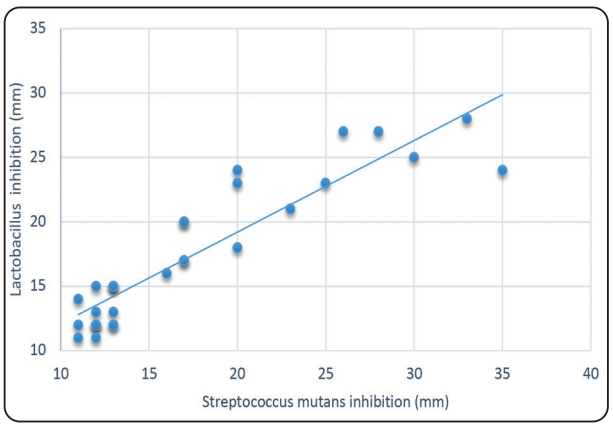

Fig. (6) Scatter plot showing the correlation between inhibition zones formed against both bacteria.

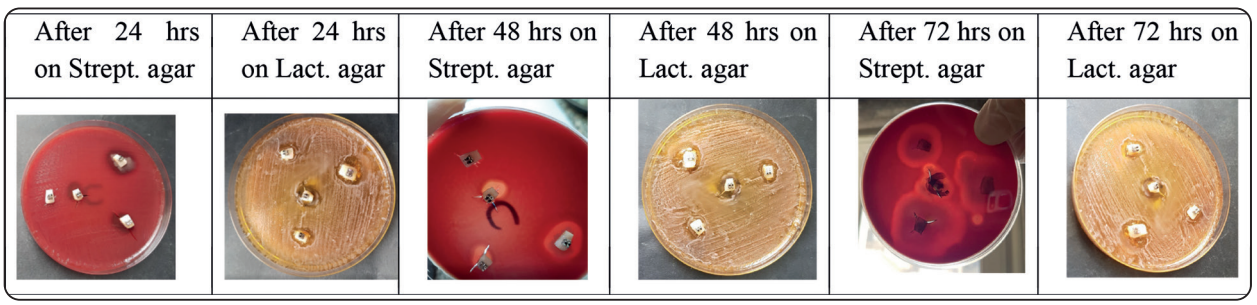

Fig. (7) Revealed the formation of inhibition zone with both control and intervention groups at different time intervals.

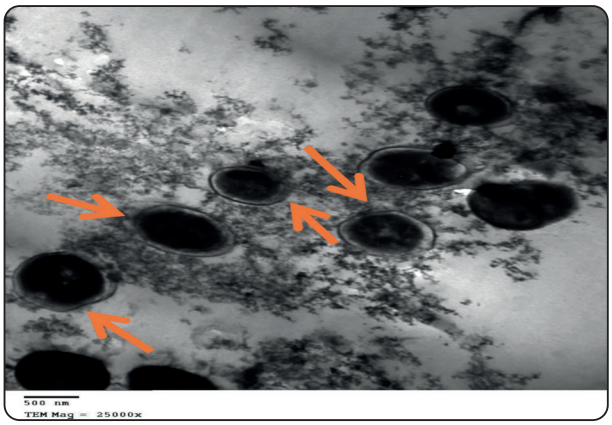

Fig. (8a) TEM images (2500X) of treated S. Mutans after nanocaregold treatment the arrow show pitted area

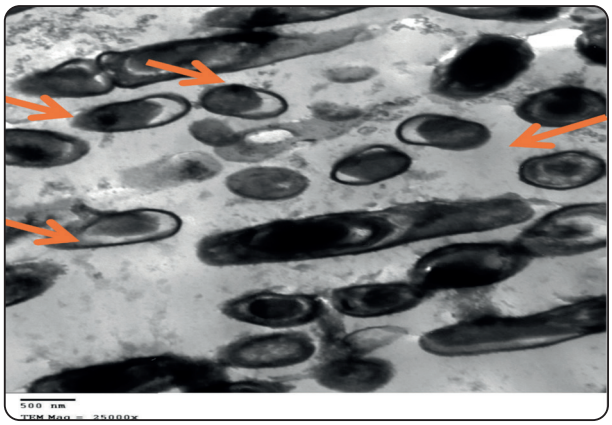

Fig. (8b) TEM images (2500X) of treated lactobacilli after nanocare gold treatment the arrow show pitted area

DISCUSSION

Nanotechnology implementation is flourishing in the field of medicinal diagnosis, prevention and treatment of diseases. In dentistry, specifically in the field of orthodontics there remains a problematic issue related to fixed orthodontic appliances which act as a reservoir for plaque which is the best environment for colonization of oral microbiota, knowing that dental plaque contains 32 microbial strains some of which are prokaryotic, and others are eukaryotic. *Streptococcus* and *Lactobacillus* strains being examples of prokaryotic unicellular, single membrane bacterium that are considered main residents of dental plaque and the main cause of dental caries[19, 20] . Not only the presence of fixed orthodontic appliances is associated with the increases of plaque accumulation, but also decrease oral pH levels so raise risk for enamel demineralization and the appearance of white spot lesions[21].Lengthy orthodontic treatments demand strict oral hygiene measures throughout the hole treatment duration in the form of meticulous teeth brushing and the use of oral rinses. Since the prevention is better than the cure, we attempted in this study to prevent white spot lesions by adding silver gold nanoparticles to adhesive systems in pursuit of promising results for our practice.

The newly created Silver Gold nanoparticles' strong antibacterial activity is guaranteed by their varied particle sizes and unequal surface energy distribution[22]. This novel combination of mixed silver and gold nanoparticles may be recommended as an enamel surface therapy before orthodontic brackets are cemented, to stop demineralization of the enamel and the formation of white spot lesions. The size, shape, concentration, and ability of the nanoparticles to agglomerate—that is, to join forces with other particles or with other materials—determine their biological features, which include their cytotoxicity, genotoxicity, and bactericidal characteristics. The antibacterial and cytotoxic effects of nanoparticles are dependent on their concentration[23, 24] .

Despite the increased demand of orthodontic treatment and in turn the need of orthodontic bonding of braces yet limited information is available on bacterial adhesion susceptibility of different orthodontic bracket brands. Metallic orthodontic brackets in particular have been related to changes in oral environment Ph, plaque accumulation and elevated count of bacterial accumulation especially Streptococcus mutans strains, therefore metal brackets pose a potential risk for enamel decalcification[25]. Several studies analyzed free surface energy stated that stainless steel materials

had higher potential for microbial attachment[3] with highlights mainly on stainless steel brackets[26] and this also was supported by the findings Eliades et al who claimed that stainless steel presented the highest critical surface tension[27]. This was opposed by other findings that stated adhesion vulnerability of streptococcus mutans to metallic brackets when compared to plastic and ceramic ones[22, 28].

Bacterial adherence to either composites or brackets occurs as a result of Van der Waals forces, electrostatic and hydrophobic interaction adding to that their complex designs.[29]Chemical agents' ability to inhibit bacteria is typically based on their ability to attach specifically to surfaces and how the agents are metabolized by the microbe. According to Sondi and Salopek-Sondi [30]and Kim et al[31] the generation of radicals and direct contact with the nanoparticles are two additional mechanisms that have been deemed significant because the mode of action of silver nanoparticles is not entirely known. Chaloupka et al. [32]stated that the peptidoglycan cell wall and plasma membrane, bacterial DNA, and bacterial proteins, particularly enzymes, were thought to be the three main components of the bacterial cell that silver ions interact with to create the bactericidal action. Therefore, two approaches were used in the current investigation to assess the mode of action of silvergold nanoparticles within the prepared samples. First, inhibition zone measurements and the second approach involves using a transmission electron microscope (TEM) to observe the morphological changes of *Streptococcus mutans* and *Lactobacillus* treated Nano-silver.

According to Mirzajani et al. [33], because silver gold nanoparticles have a particular interaction with certain proteins, phosphate lipids, and amino acids that causes the bacterial cell membrane to collapse, cell decomposition and disruption of the membrane also causes the proton motive force to collapse, inhibits ATP synthesis, and ultimately results in bacterial death. Therefore, after treating the bacteria with silver nanoparticles, TEM images were captured in the current investigation to determine whether the bacteria's cell walls had been damaged or not.

The results of inhibition zone assessment of the current study showed that, in comparison to the control group, there was a statistically significant difference in the size of the inhibitory zones generated against strains of *Lactobacillus* and *Streptococcus mutans*. The three tested periods demonstrated a significantly better rate of bacterial inhibition with Nano Care at 100% concentration, as indicated by the mean values of the inhibition zones result. The values recorded after 48 hours were much higher than those of 24 hours, and those measured after 72 hours were much greater than those of 48 hours; yet, the inhibitory impact increased with time. This could be explained by the fact that the antibacterial activity of silver nanoparticles is greatly influenced by their size and surface coating. AgNPs' antibacterial activity was brought on by ATP-related metabolism rather than the permeability of the outer membrane. AgNPs also produced hydroxyl radicals, a highly reactive oxygen species produced by bactericidal agents. AuNPs demonstrate their antibacterial effect by modifying the membrane potential, inhibiting ATPase, and preventing the ribosome's component from binding tRNA[34] AuNPs have piqued the interest of biotechnology due to their unique properties and multifunctional surface. This multidimensional surface can be used to bind oligonucleotides, proteins, and antibiotics. According to research on the antibacterial qualities of gold nanoparticles, spherical gold nanoparticles with a mean size of 17 to 11 nm and a maximum optical density of 534 nm have shown strong antibacterial activity against bacteria[35]. These nanoparticles emit ions that can alter bacterial phenotypic, decrease antibiotic inhibitory activity, enhance permeability of the outer membrane, and decrease drug resistance. The findings of this study are consistent with earlier research that

found experimental composite adhesives improved with AgNPs exhibit reduced susceptibility to the formation of bacterial biofilms on their surfaces while maintaining the physical properties of the polymer[22, 36].

The TEM image in this study demonstrates that the creation of irregularly shaped pits in the outer membrane is caused by silvergold nanoparticles. The relationship between these nanoparticles and bacterial cell walls is the subject of numerous studies. Positively charged nanoparticles and negatively charged bacterial cells exhibit electrostatic attraction, as described by Sondi and Salopek-Sondi[30] in Continuous release of silver ions is ensured while the nanoparticles are in suspension. The negatively charged bacterial cell wall may be attached to and ruptured by the silver ions that the nanoparticles release, resulting in protein denaturation and cell death. In 2008, Ruparelia et al[37]. showed that when silver ions and nanoparticles adhered to the bacterial cell wall, envelope protein precursors accumulated. This led to the dissipation of the proton motive force, the destabilization of the outer membrane, and the subsequent reduction of intracellular ATP. The proton translocating F-ATPase enzyme is the primary engine of a bacterial cell. This membrane-associated enzyme creates and sustains apH gradient across the cytoplasmic membrane by means of ATP hydrolysis. F-ATPase's enzymatic activity was reduced by silver nanoparticle. Because of this alteration in membrane permeability and disruption of pH across the cell membrane, the bacterial cells are unable to regulate transport via the plasma membrane, which causes the membrane to rupture and results in cell death.

According to a 2011 study by Mirzajani et al.[33], peptide branching, glycan, and closely packed lipopolysaccharide (LPS) molecules make up most of the bacterial cell's outer membrane and operate as an efficient permeability barrier. Thus, in the current study it was revealed that Silvergold nanoparticles and silver ions that bind to the ß-1 to ß-4 bonds of N-acetylglucosamine and N-acetylmuramic of glycan strands, break their connections, and release them into the media, causing protein denaturation and bacterial cell wall disintegration, may have an impact on the peptide branches. According to Espinosa-Cristobal et al.[38], nanosilver interacts with the bacterial membrane's "building elements," particularly the rhamnose–glucose polymers (RGPs) component, which combines side chains of glucose polymers with rhamnose polymers to form a backbone. These side chains may alter the sensitivity or resistance of the bacterial cell membrane to a particular size of silver nanoparticle, leading to structural alterations, disintegration, and ultimately, cell death. Therefore the null hypothesis tested for the current study was rejected as the findings revealed the potency of silvergold nanoparticles present in Nanocare gold against *S.Mutans* and *Lactobacilli*.

Limitations of the study

There are various factors in the mouth, such as saliva, enzymes, different types of food and beverages with varying pH levels that can impact the clinical outcome. Despite these factors, the findings of this study still offer some valuable information regarding the mode of action and antibacterial activity of the new formulation of nanocare gold.

CONCLUSION

Under the limitations of the current study, the following conclusions could be derived:

1. Silver Gold nanoparticles can inhibit several types of microbial strains as *S. mutans* and *Lactobacilli*.
2. Enamel treatment with silver gold nanoparticles prior to the cementation of orthodontic brackets was successful in bacterial inhibition around brackets.

Clinical relevance

Enamel treatment with silver gold nanoparticles prior to the cementation of orthodontic brackets can decrease the chance of the likelihood demineralization during treatment than uncoated brackets.

Recommendations

It should be noted that there is one concern about nanoparticle addition to the procedure of bracket bonding, is the effect of these particles on the mechanical properties such as bond strength.

Declaration

Ethical approval and Consent to participate

The study protocol of this experimental laboratory study was approved by the research ethical committee at the Faculty of Dentistry, Ahram Canadian University, Egypt (IRB00012891#88) at its meeting held on December 3. 2023 that is in accordance with the declaration of Helsinki and its later modification.

Human Ethics and Consent to Participate declarations:

An informed consent was obtained from all of the participants who's teeth were extracted and used in the study according to ethical approval and infection control protocols.

Consent for publication: Not applicable

All authors read and approved the submission and publication.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare that they have no known competing interests.

Author contribution

N.A.M.: organized study samples, conducted methodology & wrote the first draft.

A.H. I.: organized study samples, conducted methodology & wrote the manuscript.

S. H. I.: conceptualization, conducted methodology & wrote and review the manuscript, approved the final draft.

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