Original Article

Antimicrobial Activity of Some Nanoparticles Synthesized by Laser Ablation Technique against Some Bacteria Isolated from Oral Cavity

Huda Abbas Mohammed, Nebras Mohammed Sahi, Rafea T. Ahmed[1], Abeer fauzi Al-Rubaye

Departments of Biology, [1]Laser Physics, College of Science for Women, Babylon University, Babylon, Iraq

Abstract

Background: Tooth decay is an infectious disease that affects many people around the world and results in tooth pain due to dental cavities, which leads to tooth loss. Nanomaterials were used in the manufacture of fillings to reduce the activity of bacteria that cause tooth decay. **Objectives:** The current study aims to determine the efficiency of the antimicrobial activity of some nanoparticles (Go + Ag + poly, Zn + poly, Zn + poly, Ag + Zn + poly) manufactured by laser ablation technique to determine their ability to disrupt the biofilm formation produced by microorganisms and thus prevent tooth decay. **Materials and Methods:** Samples were collected from people who suffer from gingivitis and tooth decay and identification of bacteria by VITEK 2 Densi screening device. Nanoparticles were manufactured by laser ablation technique at the wavelength 1064 nm and then measuring the ability of bacterial isolates to form biofilm before and after the addition of nanoparticles using an enzyme-linked immunosorbent assay (ELISA) device at wavelength 490 nm. **Results:** The results showed the efficiency of the nanocomposite (Ag + poly) in inhibiting the growth of bacteria, followed by (Go + Ag + poly), (Zn + poly), while (Ag + Zn + poly) did not show any effect in inhibiting the growth of *Sphingomonas paucimobilis*, *Streptococcus pneumonia*, *Serratia plymuthica*, *Staphylococcus hyicus* on the culture media. It was noticed that the average value for formation biofilms that was represented through optical density at 490 nm were noticeably higher before adding nanomaterials than the mean value of biofilm formation amount after adding nanomaterials at $P \leq 0.05$ which is considered as a differential sign. **Conclusion:** Laser ablation technology was used to manufacturing nanoparticles for using as an inhibitor of bacterial species which isolated from patients with dental caries, where it was found that most of the nanoparticles manufactured by this technique have a role for inhibiting of *Sphingomonas paucimobilis Serratia plymuthica*, *Staphylococcus hyicus* that were isolated from people with dental caries. The nanoparticle (Ag + poly) showed a remarkable efficiency in inhibiting the growth of *S. paucimobilis*, *S. pneumonia*, and *S. hyicus* except *S. plymuthica* was not affected by this nanoparticle, while the nanoparticle (Ag + Zn + poly) did not show any effect in inhibiting the growth of *S. paucimobilis*, *S. pneumonia*, *S. plymuthica*, and *S. hyicus* on the culture media. Mostly, laser-ablated nanoparticles showed antimicrobial action in vitro. Accordingly, there is a need for additional research to describe in detail the mechanism of the toxicity of these nanoparticles.

Keywords: Nanoparticles, laser ablation technique, dental caries, biofilm

Introduction

Dental caries is the collapse of teeth because of acids yielded by bacteria.[1] The cause of dental caries is due to the formation of bacteria in the biofilm. Specific bacteria, which make acid in the cause of tooth decay, in the formation of biofilm is due to the formation of bacteria in the biofilm of fermentable carbohydrates such as sucrose, fructose, and glucose.[2] One of the most important bacterial species that cause tooth decay is *Sphingomonas paucimobilis*. It is found in soil, drinking water, and plants. *Sphingomonas paucimobilis* is a

Access this article online

Quick Response Code:

Website: www.medjbabylon.org

DOI: 10.4103/MJBL.MJBL_147_22

Address for correspondence: Dr. Huda Abbas Mohammed, Department of Biology, College of Science for Women, University of Babylon, Babylon, Iraq.
E-mail: hoda.jerawi@uobabylon.edu.iq

Submission: 01-Aug-2022 **Accepted:** 24-Aug-2022 **Published:** 09-Jan-2023

How to cite this article: Mohammed HA, Sahi NM, Ahmed RT, Al-Rubaye Af. Antimicrobial activity of some nanoparticles synthesized by laser ablation technique against some bacteria isolated from oral cavity. Med J Babylon 2022;19:601-8.

Gram-negative bacillus that is naturally found in water and soil, but it is considered an opportunistic bacterium, infecting hospitalized patients with chronic diseases or immunodeficiency.[3] *Streptococcus pneumonia*, a global bacteria, alpha-hemolytic, remains without symptoms in good health transporters usually establishing the respiratory tract, cavities, in addition to nasal cavity.[4] *Serratia plymuthica* rarely causes human infections in hospitals, but this type of bacteria has nevertheless been isolated from hospitalized patients with lymphocytic leukemia or lymph node cancer and from people who have had a stroke, patients who have had knee surgery, or patients who suffer from cholecystitis.[5] *Staphylococcus hyicus* causes sepsis in immunocompetent people; it has several virulence agents, for example, coagulase, lipase, and a homolog of the immunoglobulin G-binding protein.[6]

Nanoparticles (NPs) have an active function in the expansion of new antibacterial materials to eliminate a numeral of pathogenic bacteria.[7] The laser ablation mechanism can make rising pureness NPs; it is an upper–lower position physical process depending on the base of separator metal ion size ancestors for mineral atoms. This method was used in this work to synthesize NPs.[8] When the laser pulse reaches the surface of the sample, some of the energy is reflected by the surface. It is noted that the reflectivity depends on the material and the wavelength of the laser.[9] The energy absorbed by the sample is transmitted from the optical photons to the electrons and then to the lattice that diffuses the energy into the material.[10] The color of the nanosolutions depends on two important factors: the first is the laser energy and the second is the number of laser pulses. When the laser energy is more than 100 mJ, the NP concentration will increase, and this leads to an increase in the absorbance.[11] The resulting NP is of high concentration, and large nanosize, and this is due to the decrease in the width of the surface plasmon resonance (SPR) beam with an increase in energy, and this agrees with Veeradate *et al*.[12] The current study aims to manufacture some nanoparticles using laser ablation technology to use these nanoparticles as an alternative to antibiotics to eliminate bacteria that cause tooth decay.

Materials and Methods

Samples collection

Seventy-three samples were collected from people who suffer from gingivitis and tooth decay in Specialized Dental Center at Hilla Hospital. Swabs were taken from the infected areas of the patients and cultured on the nutrient agar for identification of bacteria.[13]

Diagnosis of doubtful bacterial strains

The VITEK 2 DensiCheck instrument, fluorescence system (bioMe´rieux) includes 43 nonenterobacterial Gram-negative taxa, using to diagnose bacterial species. The experiment was implemented depending on the guidance of the manufacturing company.[14]

Antibiotic susceptibility

The susceptibility of bacterial isolates to some antibiotics which includes piperacillin/tazobactam, cefepime, imipenem, gentamicin, ciprofloxacin, minocycline, timethoprim/sulfamethoxazole, benzylpenicillin, oxacillin, oxifloxacin, erythromycin, fusidic acid, rifampicin was identified through VITEK 2 DensiCheck device.[14]

Laser ablation and particles formation of nanoparticles

The work was done on the silver wafer in distill and deionized water (DDDW), where the wavelength of the (Nd-YAG) pulsed laser (1064 nm) was used after the laser focused by a light lens and according to the threshold limit for the captured silver substance, which was 80 mJ and frequency 5 Hz. When the laser pulses hit the surface of the metal immersed in 4 mL of polymer dissolved in DDDW, a cloud with a strong vibration wave will be generated that spreads in all directions within the impact area. This cloud emits light and noise. The same steps were repeated when preparing graphene oxide (Go) with silver (Ag) and polymer (poly). The process was repeated again for zinc (Zn) and also in 4 mL of polymer dissolved in water with the same wavelength, frequency, and number of pulses, but with a laser energy different from silver as each material has its own threshold limit. The laser energy for zinc was taken (100 mJ). After that, NPs were synthesized from the two materials (silver and zinc) with the same polymer dissolved in water, with a laser energy of 100 mJ, frequency (6 Hz), wavelength (1064 nm), and the number of pulses (200 pulses)[8,9] [Figure 1].

Antagonistic activity test of nanomaterial

Suspected isolates were cultivated on Mueller Hinton Agar for antimicrobial susceptibility testing. A 0.1 mL of bacterial suspension was added to culture media and spreading it by using spreader, and making three replicates from Petri dishes. Thereafter, made four holes in the culture media by using the cork borer. A 1 mL of the previously prepared nanomaterials were added to the four holes, the plates were incubated at 37°C for 24 h, and then the diameter of the inhibition zone was measured.

Biofilm formation prior to putting nanoparticles

Luria broth (LB) is a nutrient-rich media commonly used to culture previously isolated bacteria in the laboratory. At 48 h bacterial isolates are incubated. Then at 80ºC isolates were fixated later staining together with 0.5% of crystal violet. Four isolates were put into a well of a microtiter plate with about three replicates per isolate. Next, unadhered cells were extracted through an invert of a microtiter plate. After invert of a microtiter plate onto absorbent paper

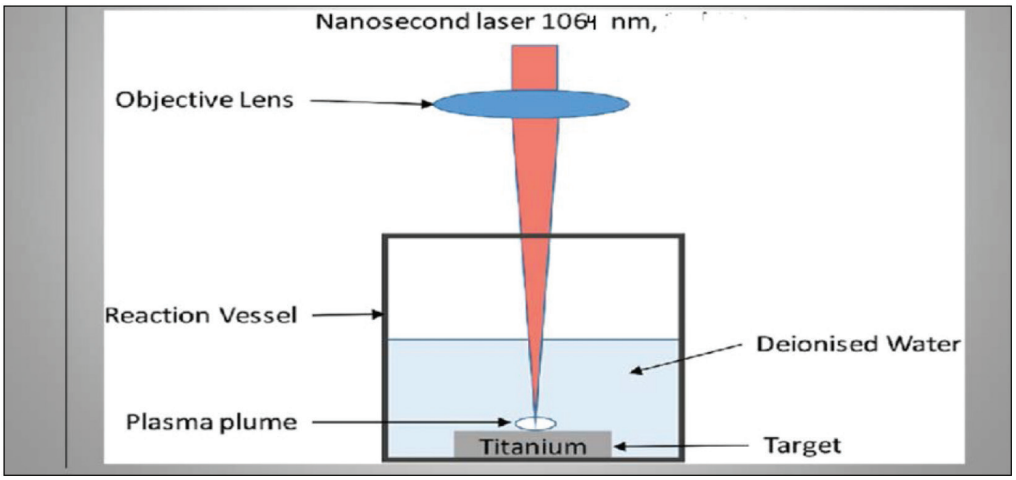

Figure 1: Goal of precipitation in liquids to obtain solutions nanoparticles

Table 1: Number and percentage of *S. paucimobilis*, *S. pneumonia*, *S. plymuthica*, and *S. hyicus* isolated from patients with dental caries

No. of samples	Isolated sample	Isolated diagnosis by VITEK 2	%
73	*Sphingomonas paucimobilis*	2	2.73
	Streptococcus pneumonia	13	17.8
	Serratia plymuthica	4	5.47
	Staphylococcus hyicus	7	9.58

and lightly beating on the surface of the paper to remove non-adherent cells, the plates are washed with water to remove excess stains, and the plates are dried for 30 min at 37°C. By distilled water, the excess dye was extracted through rinsing. The extent of biofilm was determined by measuring the absorbance of stained adherent film upon treatment with acetone : ethanol (20 : 80) by using ELISA apparatus at a wavelength of 490 nm.[15] At 490 nm, the absorption of the removed dye was measured. Depending on the optical density, explanations of biofilm formation for *S. paucimobilis, S. pneumonia, S. plymuthica,* and *S. hyicus* bacterial species were ranked into the following divisions: weak biofilm creators, moderate, and strong biofilm creators, as formerly mentioned.[16]

Biofilm formation after addition nanoparticles

A 1 µL from each (Go+Ag+poly), (Zn+poly),(Ag+poly), (Ag+Zn+poly) of NPs was added to each bacterial strains, reiterated the same reiterates up in fashioning of biofilm prior putting NPs by using ELISA apparatus at weave length 490 nm,[17] for easier interpretation of the results, strains may be divided into the following categories: no biofilm producer, weak biofilm producer, moderate biofilm producer, and strong biofilm producer.[16]

Statistical analysis

Differences between four groups were analyzed by IBM SPSS Statistics 23 and used to extract at least one standard deviation value and for three identical replicates from independent experiments. The mean and standard deviation were calculated, where the biofilm production was significantly decreased by the bacterial isolates after treatment with nanoparticles ($P \leq 0.05$). A value of $P = 0.05$ was considered statistically significant.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 1034 (including the number and the date in 07/11/2021) to get this approval.

Results

A total of 73 isolated samples were collected from patients who visited the dental center specialized in Al-Hilla Hospital with dental caries. The samples were collected by swab transport media. The isolates were identified by VITEK 2 device, the results were two isolates (2.73%) of *Sphingomonas paucimobilis*, thirteen isolates (17.8%) of *Streptococcus pneumonia*, four isolates (5.47%) of *Serratia plymuthica* and seven isolates (9.58%) of *Staphylococcus hyicus* [Table 1].

In this study, *S. plymuthica* resisted to the highest numbers of antibiotics (five antibiotics), which includes

piperacillin/tazobactamm, imipenem, oxacillin, fusidic acid, rifampicin, and susceptible to seven antibiotics, and it was showed intermediate susceptible to one antibiotic (ciprofloxacin). *S. paucimobilis* was resisted to benzylpenicillin, oxacillin fusidic acid, and rifampicin, whereas *S. pneumonia* was resisted to four antibiotics which includes ciprofloxacin, minocycline, erythromycin, and rifampicin. *S. paucimobilis* and *S. pneumonia* were susceptible to the highest numbers of antibiotics (nine antibiotics), finally *Staphylococcus hyicus* sensitive to eight antibiotics, resisted to four antibiotic that includes minocycline, benzylpenicillin, oxacillin, fusidic acid, and intermediate susceptible to one antibiotic (erythromycin) [Table 2].

In this study, (Ag+poly) used as nanomaterials were used to find out their ability to inhibit bacterial species. The results showed that the nanomaterial was more efficient in inhibiting *S. pneumonia*, as the diameter of inhibition was 30 mm; however, when treating the same material with *S. paucimobilis, S. hyicus*, and *S. plymuthica* the diameter of inhibition was 25, 18, and 0 mm, respectively. Other NP (Go+Ag+poly) was affected on *S. pneumonia*, where the diameter of the inhibition was 17 mm followed by *S. paucimobilis* and *S. hyicus*, which have the same inhibition diameter (13 mm). There was no effect of this nanomaterial on *S. plymuthica*, whereas the nanomaterial (Zn+poly) was affected only on *Streptococcus pneumonia,* which has inhibition zone of 14 mm [Figure 2].

In Figures 3–6, the optical density of biofilm formation by *S. paucimobilis*, *S. pneumonia*, *S. plymuthica,* and *S. hyicus* at a wavelength 490 nm of absorbance by using ELISA device, bacterial isolates were cultured in a 96-well plate to allow them to form biofilm, and the experiment was repeated again, but by treatment Isolates with four agents of Nano composites (Go + Ag + poly, Zn + poly, Ag + poly, Ag + Zn + poly) and compared with the control (well without NPs). Then the efficiency of these nanocomposites for inhibiting the ability of bacterial species to form biofilm was observed.

Discussion

Approximately 1000 species of bacteria gather in the environment of the oral cavity in the extracellular matrix to form a biofilm, where the biofilm acts as a barrier preventing the entry of antibiotics into the bacterial cell to destroy it.[18] The biofilm formed by microorganisms is considered a station of interest for scientists in previous research. Biofilm is formed from the collection of bacterial cells that are linked for the outer surfaces of the material. Biofilms are mainly composed of polysaccharide substance.[19] The bacterial species that have the ability to form biofilms are more resistant to antibiotics and disinfectants and work to weaken the immune system of the host.[19,20] Therefore, previous studies showed that NPs can be used as an alternative to antibiotics that bacterial species have shown resistance to due to their ability to form biofilm.[21]

The present study showed, the formation of Biofilm from *S. paucimobilis*, *S. pneumonia*, *S. plymuthica, S. hyicus* isolates by maturing in 96-well sheet, added with four agent(Go+Ag+poly, Zn+poly, Ag+poly, Ag+Zn+poly), using these tests, the efficiency of agents can be distinguished by their ability to eliminate or stop biofilm formation, Where previous research showed the possibility of using Nano composites as an alternative to antibiotics.[22] A significant difference was observed in the optical density of biofilm formation compared to the control (well in microtiter without nanoparticles) in the weave length 490 nm. A value of zero indicates no biofilm formation, and if the values are 1 and 2, then the biofilm formation is

Table 2: Minimum inhibition concentrations (MIC) of different antibiotics tested against environmental isolates of *S. paucimobilis*, *Streptococcus pneumonia*, *S. plymuthica*, and *S. hyicus*

Isolates antibiotics	*Sphingomonas paucimobilis*	*Streptococcus pneumoniae*	*Serratia plymuthica*	*Staphylococcus hyicus*
Piperacillin/tazobactam	<= 20(s)	<=0.12(S)	>=128(R)	<=0.5(S)
Cefepime	<= 1(S)	<=0. 5(S)	< = 1(S)	<=0.5(S)
Imipenem	<= 0.25(S)	<=0. 5(S)	>=120(R)	<=0.5(S)
Gentamicin	<= 1(S)	<=0.25(S)	<=1(S)	<=0.5(S)
Ciprofloxacin	<=1(S)	>=4(R)	2(I)	<=0.5(S)
Minocycline	<=1(S)	>=3(R)	<4(S)	>=0.5(R)
Timethoprim/sulfamethoxazole	<=1(S)	<=0.5(S)	<=2(S)	<=10(S)
Benzylpenicillin	>= 1(R)	<=0.5(S)	<= 1(S)	>=0.5(R)
Oxacillin	>= 0.5(R)	<=1(S)	>=87(R)	>=4(R)
Moxifloxacin	<=1(S)	<=1(S)	<=1(S)	<=0.25(S)
Erythromycin	< =0.5(S)	>=125(R)	<=1(S)	4(I)
Fusidic acid	>=32(R)	<=0.03(S)	>=1(R)	>=8(R)
Rifampicin	>=64(R)	>=4(R)	>=128(R)	1(S)

S = sensitive to antibiotic, R = resistant to antibiotic, I = intermediate
Antibiotic MIC was calculated through VITEK 2 device (bioMe´rieux)

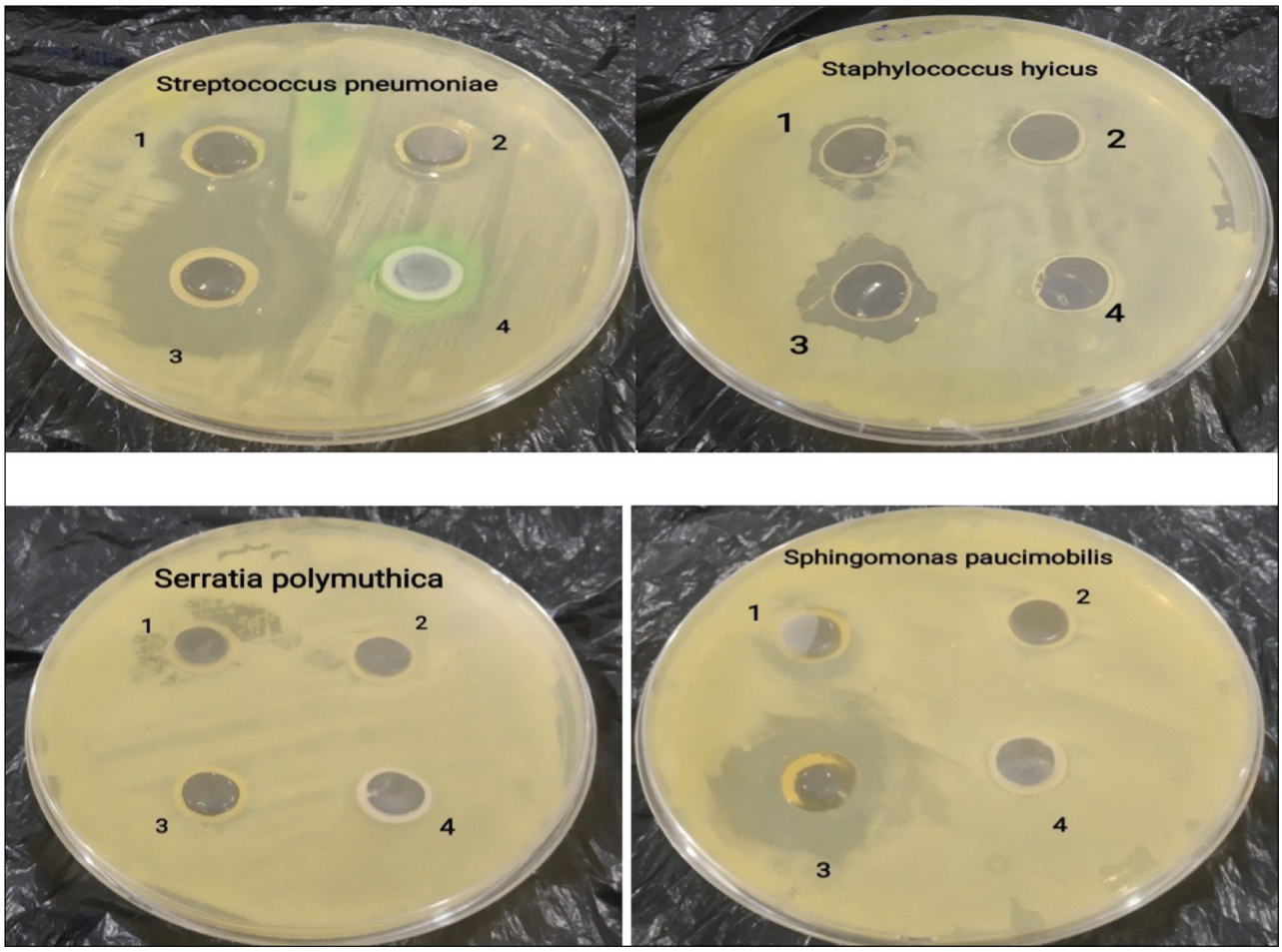

Figure 2: Effect of nanoparticles (Go+Ag+poly, Zn+poly, Ag+poly, and Ag+Zn+poly) to inhibit the growth of *S. paucimobilis*, *S. pneumonia*, *S. plymuthica*, and *S. hyicus* *(1 = Go+ Ag+poly, 2 = Zn+poly, 3 = Ag+poly, and 4 = Ag+Zn+poly)

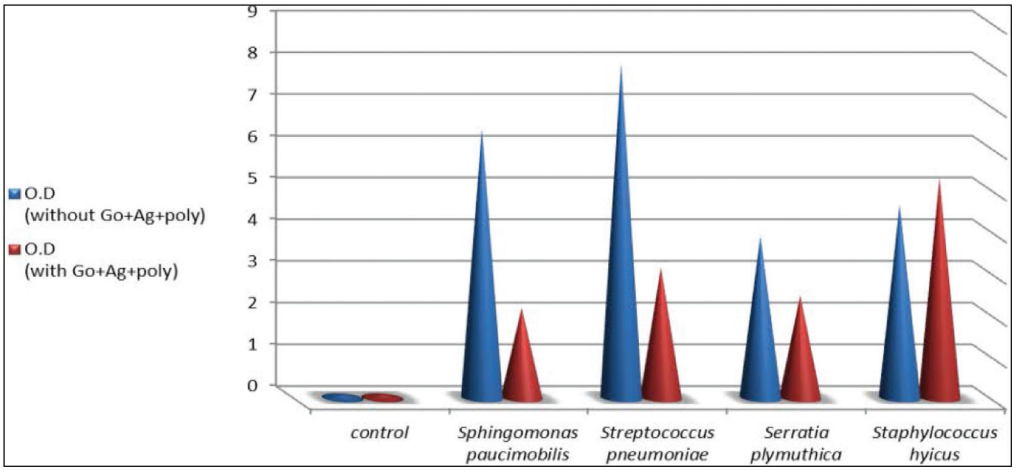

Figure 3: Biofilm formation (optical density) of *S. paucimobilis*, *S. pneumonia*, *S. plymuthica*, and *S. hyicus* isolates compared with control (without bacteria), before and after adding nanoparticles (Go+Ag+poly)

weak, medium, or strong, respectively.[16] *S. paucimobilis, S. pneumonia,* and *S. plymuthica* produced the highest level of biofilm with absorbance value and standard deviation of 6.46±1.24, 8.04±1.54, 3.89±0.74, respectively, than after adding (Go+Ag+poly), the biofilm formation of with an absorbance value about (2.156±0.01, 3.13±0.13, 2.46, 2.46±0.03), respectively, whereas this NPs not decreased the biofilm formation of *S. hyicus*. Graphene oxide inhibits the ability of bacteria to form biofilms by preventing the accumulation of polysaccharides which

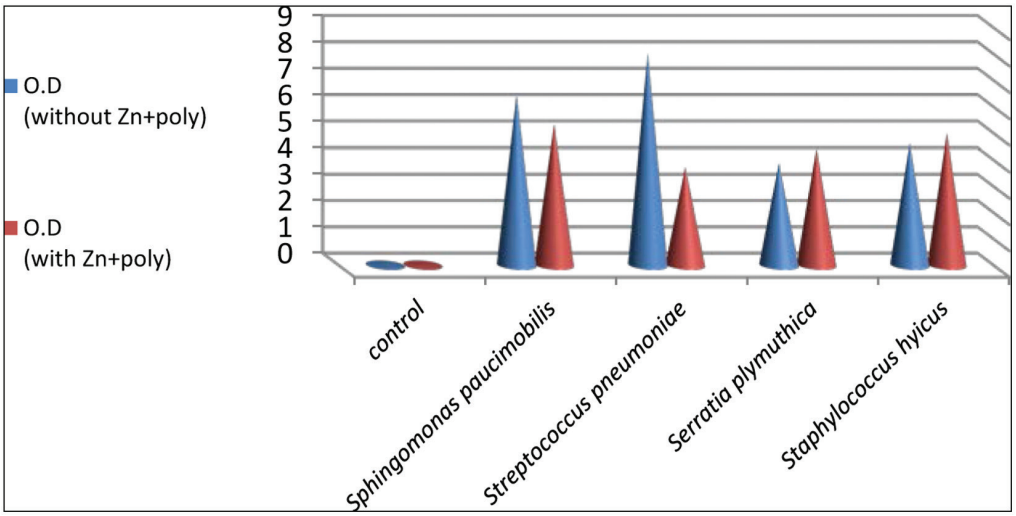

Figure 4: Biofilm formation (optical density) of *S. paucimobilis*, *S. pneumonia*, *S. plymuthica*, and *S. hyicus* isolates compared with control (without bacteria), before and after adding nanoparticles (Zn+poly)

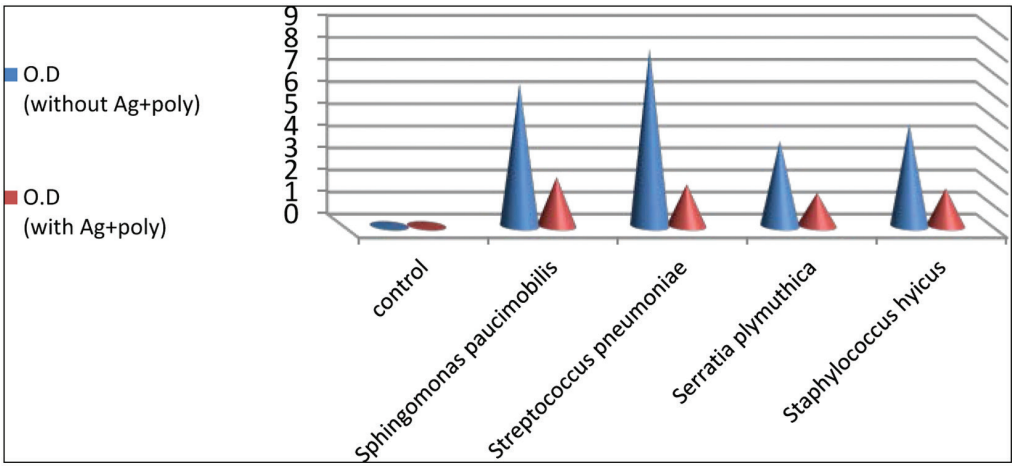

Figure 5: Biofilm formation (optical density) of *S. paucimobilis*, *S. pneumonia*, *S. plymuthica*, and *S. hyicus* isolates compared with control (without bacteria), before and after adding nanoparticles (Ag+poly)

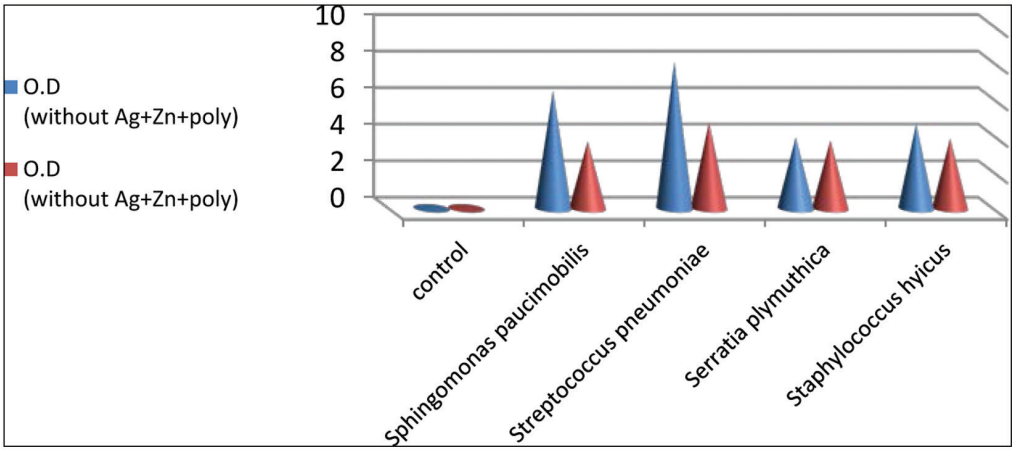

Figure 6: Biofilm formation (optical density) of *S. paucimobilis*, *S. pneumonia*, *S. plymuthica*, and *S. hyicus* isolates compared with control (without bacteria), before and after adding nanoparticles (Ag+Zn+poly)

Table 3: Formation of biofilms (optical density) of *S. paucimobilis*, *S. pneumonia*, *S. plymuthica*, and *S. hyicus* in comparison to control (without nanoparticles) at $P \leq 0.05$

Isolates	Optical density and standard deviation				
	Without nanoparticles	Go+Ag+poly)	Zn+poly	Ag+poly	Ag+Zn+poly
Sphingomonas paucimobilis	6.46±1.24	2.156±0.01	5.35±0.108	2.23±0.006	3.68±0.03
Streptococcus pneumonia	8.04±1.54	3.13±0.13	3.75±0.106	1.901±0.002	4.71±0.02
Serratia plymuthica	3.89±0.74	2.46±0.03	4.45±0.07	1.505±0.0118	3.73±0.02
Staphylococcus hyicus	4.64±0.89	5.28±0.15	5.02±0.066	1.71±0.0101	3.84±0.05

produced by bacteria to form biofilms..[23] The results of this study agree with other recent studies that showed the role of graphene in inhibiting bacteria by inhibiting the strength of the biofilm formed by bacteria to cause disease. Recent studies also showed that graphene has other uses in dentistry, which is its use in stimulating bone differentiation of stem cells,[23] and thus it is used in the installation of missing teeth.[24]

When adding (Zn+poly) decreased biofilm formation of *S. paucimobilis, S. pneumonia* (5.35±0.108, 3.75±0.106), respectively, but *S. plymuthica* and *S. hyicus* also increased their ability to form biofilm(4.45±0.07, 5.02±0.066), respectively, with compared with control. Zinc nanocomposite is one of the most widely used nanomaterials in dentistry, where recent studies have shown that zinc nanoparticles have toxic and lethal effects on bacterial cells while being at the same concentration non-toxic to human cells.[21] It also has a higher toxic effect on bacterial cells compared with other metals due to its ability to get rid of ions and because of that it is used in the field of nanodentistry.[21] As for the treatment with (Ag+poly) and (Ag+Zn+poly), all the four bacterial isolates had a decrease in the optical density (2.23±0.006, 1.901±0.002, 1.505±0.0118, 1.71±0.0101) and (3.68±0.03, 4.71±0.02, 3.73±0.02, 3.84±0.05), respectively, as compared with before adding the nanomaterial. This study showed that (Ag+poly) was more inhibiting than the other three treatments (Go+Ag+poly, Zn+poly, Ag+Zn+poly) for biofilm formation, thus weakening the ability of bacteria to cause disease and thus stopping tooth decay resulting from bacterial species at $P \leq 0.05$, which is considered as a differential sign [Table 3].

These results of this study are in agreement with previous studies, which showed that silver nanoparticles (AgNPs) can release silver ions. The liberated silver ions work to break down the bacterial envelope, in addition, they disrupt respiratory enzymes, disrupt ATP production, inhibit deoxyribonucleic acid (DNA) replication and protein synthesis.[25] In addition, the release of silver ions from silver nanoparticles leads to their gathering in the pits formed on the bacterial cell wall, leading to the death of bacteria on their own or denaturation of the bacterial cell membrane.[26] This is consistent with what recent studies have shown of the effectiveness of silver nanoparticles in reducing lactic acid formation during biofilm formation, in addition to their use in treating tooth enamel layer, which in turn prevents the loss of dental minerals during biofilm formation by bacterial species.[27] Consequently, hence, more research is required to investigate the optimal concentration of nanoparticles for the prevention of tooth decay.

Conclusion

In general, we found that most of the nanocomposites are manufactured by laser ablation technique; it has a role in inhibiting biofilm formation of *Sphingomonas paucimobilis*, *Streptococcus pneumonia*, *Serratia plymuthica*, and *Staphylococcus hyicus* isolated from people suffering from dental caries. NPs are encouraging materials for protecting teeth from caries. As most of the studies are *in vitro* studies, more clinical studies are needed before using them on patients.

Financial support and sponsorship

Not applicable.

Conflicts of interest

The authors declare that there is no competing interest.

References

1. Silk H. Diseases of the mouth. Prim Care 2014;41:75-90.
2. Holloway PJ, Ritson C. Commercial and political influences on dental health. J Dent 1983;11:159-63.
3. Göker T, Zülal Aşık R, Bahadır Yılmaz M, Çelik İ, Tekiner A. *Sphingomonas paucimobilis*: A rare infectious agent found in cerebrospinal fluid. J Korean Neurosurg Soc 2017;60:481-3.
4. Baucells BJ, Hally MM, Álvarez Sánchez AT, Figueras Aloy J. Probiotic associations in the prevention of necrotising enterocolitis and the reduction of late-onset sepsis and neonatal mortality in preterm infants under 1,500g: A systematic review. Anal Pediatr 2015;85:247-55.
5. Carrero P, Garrote JA, Pacheco S, García AI, Gil R, Carbajosa SG. Report of six cases of human infection by *Serratia plymuthica*. J Clin Microbiol 1995;33:275-6.
6. Rosander A, Guss B, Pringle M. An IgG-binding protein A homolog in staphylococcus hyicus. Vet Microbiol 2011;149:273-6.
7. Patra JK, Baek KH. Antibacterial activity and synergistic antibacterial potential of biosynthesized silver nanoparticles against foodborne pathogenic bacteria along with its anticandidal and antioxidant effects. Front Microbiol 2017;8:167.
8. Rashid SN, Aadim KA, Jasim AS. Silver nanoparticles synthesized by Nd:YAG laser ablation technique: Characterization and antibacterial activity. Karbala Int J Mod Sci 2022;8:71-82.

9. Liu C. A Study of Particle Generation during Laser Ablation with Applications. Doctoral thesis. Berkeley, CA: University of California; 2005. p. 1-189.
10. Zhigilei LV, Lin Z, Ivanov DS. Atomistic modeling of short pulse laser ablation of metals: Connections between melting, spallation, and phase explosion. J Phys Chem C 2009;113:11892-1190.
11. Sánchez-Ramírez JF, pal V, Nolasco-Hernández L, Mendoza-Álvarez, J, Pescador-Rojas JA. Synthesis and optical properties of Au-Ag alloy nanoclusters with controlled composition. J Nanomater 2008;110:1155-620412.
12. Piriyawong V, Thongpool V, Asanithi P, Limsuwan P. Preparation and characterization of alumina nanoparticles in deionized water using laser ablation technique. J Nanomater 2012:2012. doi: 10.1155/2012/819403.
13. MacFaddin JF. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Baltimore, MD: Williams and Wilkins; 2000.
14. Funke G, Monnet D, Debernardis C, von Graevenitz A, Freney J. Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. J Clin Microbiol 1998;36:1948-52.
15. Agarwal RK, Singh S, Bhhileganokar KN, Singh VP. Optimization of biofilm formation ability in different *Salmonella serotypes*. Int Food Res J 2011;18:1493-98.
16. Stepanović S, Cirković I, Ranin L, Svabić-Vlahović M. Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett Appl Microbiol 2004;38:428-32.
17. AL-Jaryawi HAMM, Zgair AK. The effect of silver nanoparticles on biofilm formation in environmental isolates of *Pseudomonas aeruginosa*. Indian J Public Health Res Develop 2020;11:1474-9.
18. Donlan RM. Biofilms: Microbial life on surfaces. Emerg Infect Dis 2002;8:881-90.
19. Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167-93.
20. Pitts B, Hamilton MA, Zelver N, Stewart PS. A microtiter-plate screening method for biofilm disinfection and removal. J Microbiol Methods 2003;54:269-76.
21. Moradpoor H, Safaei M, Mozaffari HR, Sharifi R, Imani MM, Golshah A, *et al*. An overview of recent progress in dental applications of zinc oxide nanoparticles. RSC Adv 2021;11:21189-206.
22. Sodagar A, Akhavan A, Hashemi E, Arab S, Pourhajibagher M, Sodagar K, *et al*. Evaluation of the antibacterial activity of a conventional orthodontic composite containing silver/hydroxyapatite nanoparticles. Prog Orthod 2016;17:40.
23. Li X, Liang X, Wang Y, Wang D, Teng M, Xu H, Zhao B, Han L. Graphene-based nanomaterials for dental applications: Principles, current advances, and future outlook. Front Bioeng Biotechnol 2022;10:21.
24. Dias HB, Bernardi MIB, Marangoni VS, de Abreu Bernardi AC, de Souza Rastelli AN, Hernandes AC. Synthesis, characterization and application of Ag doped ZnO nanoparticles in a composite resin. Mater Sci Eng C Mater Biol Appl 2019;96:391-401.
25. Wu R, Zhao Q, Lu S, Fu Y, Yu D, Zhao W. Inhibitory effect of reduced graphene oxide-silver nanocomposite on progression of artificial enamel caries. J Appl Oral Sci 2018;27:e20180042.
26. Mancinelli R, Filippo E, Tumedei M, Marrone M,Fulle S. Human dental pulp stem cell osteogenic differentiation seeded on equine bone block with graphene and melatonin Appl Sci 2021;11:3218.
27. Di Giulio M, Di Lodovico S, Fontana A, Traini T, Di Campli E, Pilato S, *et al*. Graphene oxide affects staphylococcus aureus and pseudomonas aeruginosa dual species biofilm in Lubbock chronic wound biofilm model. Sci Rep 2020;10:18525.