

Study the Exposure of Low-Level Laser on rGO/Ag NPs to Improve the Antibacterial Activity of Different Types of Bacteria

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Abstract

The rGO-Ag NPs nanocomposite was synthesized by an easy, low-cost, one-step chemical method. The nanocomposite was analyzed by UV-Vis spectroscopy and atomic force microscope (AFM) to verify the properties of the nanoparticles. The study of the optical properties showed that there are two peaks, one of which belongs to silver and is 400 nm, and the other is related to graphene oxide at 268 nm. The AFM examination of the rGO-Ag NPs revealed that the average grain size is 71.26 nm, and this indicates that the material is within the nanoscale. The rGO-Ag NPs nanocomposite was applied as an antagonist against various types of bacteria that cause gum and tongue infections, and the results showed that with the increase in the nanocomposite concentration, the killing rate of bacteria increases. This means that the killing increases with increasing volumes of rGO-Ag NPs, and the best one is 40 μ L. The result shows that the rGO-Ag NPs show a relationship between adding volumes of the nanomaterial and the percentage killing of bacteria, which is directly proportional. Additionally, through UV-Vis spectroscopy, the absorbance of the rGO-Ag NPs was determined, so that the laser with a wavelength of 405 nm was chosen to be applied to the same kinds of bacteria and for different periods of time with the use of a 40 μ L volume of the rGO-Ag NPs, and it was found that with increasing the irradiation period, the percentage of bacteria killed increases. It showed that increasing the irradiation period with volume stability of 40 μ L causes a higher killing rate. The study indicated that using nanocomposite and laser is a method to remove the infection from the gums and tongue. These results are promising and can be applied in the treatment of gum and tongue infections after clinical studies.

Keywords: Nanocomposite; Optical properties; rGO-Ag NPs; Laser; Antibacterial applications

Introduction

Harmful bacteria cause many diseases, including infections in various parts of the human body, such as acute and chronic infections, blood poisoning, food poisoning, infections of the gums, tongue, caries, infections of burns and wounds, pneumonia and many other diseases that can lead to death in the injured [1].

On the other hand, beneficial bacteria help produce many essential vitamins for the body, help digest complex molecules found in some types of food, stimulate microorganisms inside the body to form immunity, improve metabolism, and reduce excessive weight gain. In fact, some bacteria have developed resistance to antibiotics that were once commonly used to treat them, and these bacteria are able to cause

serious disease, so this is a major public health problem [2, 3].

On the other hand, nanoscience and nanotechnology have attracted great interest over the last decade due to their potential impact on many scientific areas, such as energy, medicine, pharmaceutical industries, electronics, and space industries. One of these nanomaterials is nanoparticles (NPs) that show unique and considerably changed chemical, physical, and biological properties due to their high surface-to-volume ratio, compared with the bulk of the same chemical composition. Thus, nanomaterial-based therapies are promising tools to combat bacterial infections that are difficult to treat, featuring the capacity to evade existing mechanisms associated with acquired drug resistance. In addition, the unique size and physical properties of nanomaterials give them the capability to target biofilms, overcoming recalcitrant infections.

Graphene is an amazing nanomaterial, and detailed information on their structures and properties can be obtained from several studies. The graphene nanomaterials have attracted wide interest due to their excellent properties that arise from a flat monolayer of carbon atoms packed in a two-dimensional honeycomb lattice. High chemical stability, extreme mechanical strength, and exceptional electronic and thermal conductivity are just a few examples of unique properties. As a result, it has a wide range of applications, including supercapacitors [4–6], solar cells [7], sensors [8], and catalysts [9]. It is still a brilliant star on the horizon of materials science [10]. To take advantage of the remarkable properties and unique structure of graphene nanomaterials, great efforts have been made to develop hybrid graphene nanocomposites. So far, graphene sheets have been developed as nanostructures to disperse and stabilize various metallic and metal oxide nanoparticles (Ag, Au, SnO₂, TiO₂, Pt, Pd, Cu, and ZnO) [11]. More importantly, some new properties of the nanocomposites can be obtained after hybridization [12]. Therefore, graphene sheets as support for nanoparticles open a new avenue for material development. Silver is among those nanoparticles. Due to its optical properties depending on the size of the nanoparticles of the noble metals in liquids, silver is a noble metal of great importance. Silver nanoparticles (Ag NPs) have attracted increased interest due to their optical and antibacterial properties [13, 14]. Decorating

reduced graphene oxide rGO with Ag NPs in different methods will obtain a nanocomposite which is rGO-Ag NPs [15].

The effectiveness of antibiotics against different types of bacteria can also be increased through the use of a laser whose wavelength is proportional to the absorption of the nanocomposite used, which is obtained after conducting UV-Vis spectroscopy for the nanocomposite. On the other hand, the diode laser is important and has a distinct role in systems such as systems with high penetration for processing because it is characterized by unique and important characteristics, including its small size, narrow beam width, high efficiency, and low-power and high-power quality [16, 17].

In fact, the bacteria have shown resistance whether they used laser only or nanomaterials only, so we will use a nanocomposite with a laser as an antibacterial for different types of bacteria that cause gum and tongue infections.

There are several types of bacteria that have been identified, including *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Porphomonas*, *Streptococcus mutans*, *Enterococcus faecalis*, *Lactobacillus*, *Streptococcus pyogenes*, and *Staphylococcus epidermidis*. There are a wide number of nanomaterials that can be used as antibacterial for different types of bacteria [18].

In this study, we will synthesize rGO-Ag NPs nanocomposite by an easy, low-cost, one-step chemical method, and study their optical and structural properties. They will be utilized as an antibacterial for four different types of bacteria that cause gum and tongue infections, and we will study the improvement of the effectiveness of the antibacterial by exposure to a low-level laser to the rGO-Ag NPs nanocomposite that absorbs the laser wavelength.

Materials and Methods

Silver nitrate (AgNO₃), Sodium Borohydride (NaBH₄) was purchased from market commercial, and Graphene Oxide (GO) has been synthesized by the hummer method [19]. The four types of bacteria were obtained from the microbiology laboratory and were activated and then put in dishes. Different concentrations of the nanocomposite were added and then irradiated [20, 21].

Method of synthesis of silver nano-particles with graphene oxide

The rGO-Ag NPs were synthesized using the same chemical method as in our previous work [22]. In detail, the concentration of GO was 1 mg/mL by adding 10 mg of GO to 10 mL of distilled water, which was gradually added to 0.3 mmol/L of AgNO₃ aqueous solution under vigorous stirring at room temperature, followed by the slow gradual addition of 0.1 mol/L of NaBH₄ to the mixture solution. The color of the mixture turns from light yellow to dark yellow, then purple, and then dark brown. Then the rGO-Ag NPs nanocomposites were obtained by centrifugation at 8000 r/min and washed with distilled water to get rid of impurities and reduce toxicity. Finally, the rGO-Ag NPs are ready for analysis.

On the other hand, silver nanoparticles were prepared without graphene oxide by adding gradually 0.1 mol/L of NaBH₄ to 0.3 mmol/L of AgNO₃, in the same way.

Antibacterial activity assay of rGO-Ag NPs

The antibacterial activity of rGO-Ag NPs has been examined against four clinical isolates: *Lactobacillus*, *Escherichia coli* (*E. coli*) (gram-negative), *Porphyromonas*, and *Streptococcus mutans* (gram-positive). The stock cultures for these four bacterial isolates were transferred into Mueller Hinton agar medium, incubated overnight at 37 °C, and stored in the refrigerator at 4 °C until used. The antibacterial activity of rGO-Ag NPs was inspected by utilizing a healthy dissemination procedure. Wells with diameters of about 6 mm was made at the agar media surface by micropipette tips, then NPs suspensions with different concentrations were added to the wells. These plates were incubated for 24 h. The antibacterial effectiveness of rGO-Ag NPs was recorded by

measuring inhibition zone diameters from different directions using a ruler more than once. All tests were duplicates, and refined water was utilized as a control treatment.

Results and Discussion

The optical properties of rGO-Ag NPs and Ag NPs were obtained by using a UV-Vis spectroscopy and the result is shown in Fig. 1.

Figure 1(a) showed a curve for Ag NPs only and appeared with one peak at (392 nm) referring to the Ag NPs, and Fig. 1(b) for rGO-Ag NPs showed two peaks, one at 400 nm referring to Ag NPs and the second at 268 nm referring to reduced graphene oxide. rGO represented the transitions between $\pi = \pi^*$ in C=C bond. The two peaks have a redshift that indicates the nanoparticles of silver seemed to be getting smaller and the graphene oxide has been reduced. Also it indicates the removal of the oxygen groups and the close proximity to graphene. The curve in Fig. 1(b) indicates that the silver nanoparticles have an anchor on the reduced graphene sheets. This result is compatible with other reports [23].

From the wide absorbance at 400 nm, the diode laser (GaN) with a wavelength of 405 nm was chosen for radiation of the nanomaterials and study of the effect of the laser on the killing rate of different types of bacteria.

The atomic force microscopy (AFM) images show the structure of the sample in 2D and 3D and the statistical distribution as shown in Fig. 2. Figures 2(a) and 2(b) are images of the rGO-Ag NPs nanocomposite, and in Fig. 2(c), we note that the average particle size is 71.26 nm, which indicates that the material is within the nanoscale.

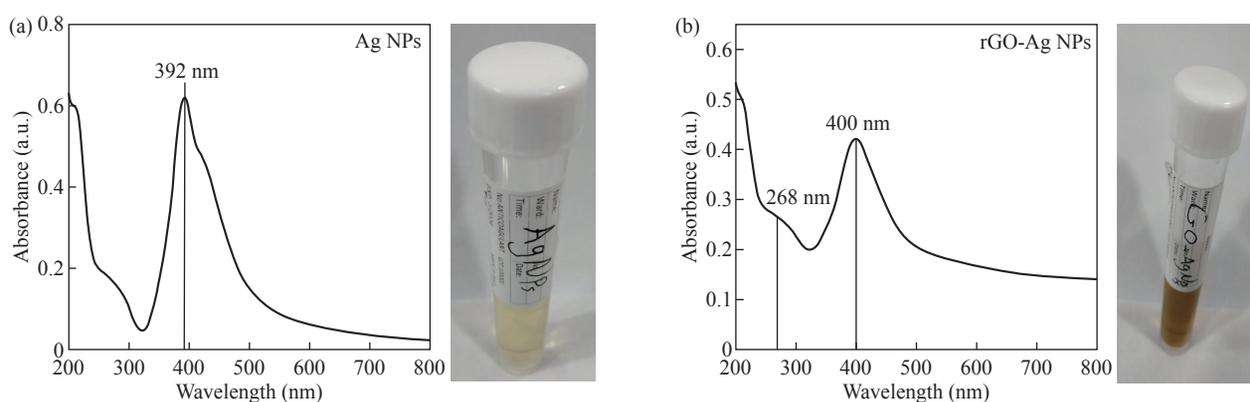


Fig. 1 UV-Vis absorption for (a) Ag NPs and (b) rGO-Ag NPs

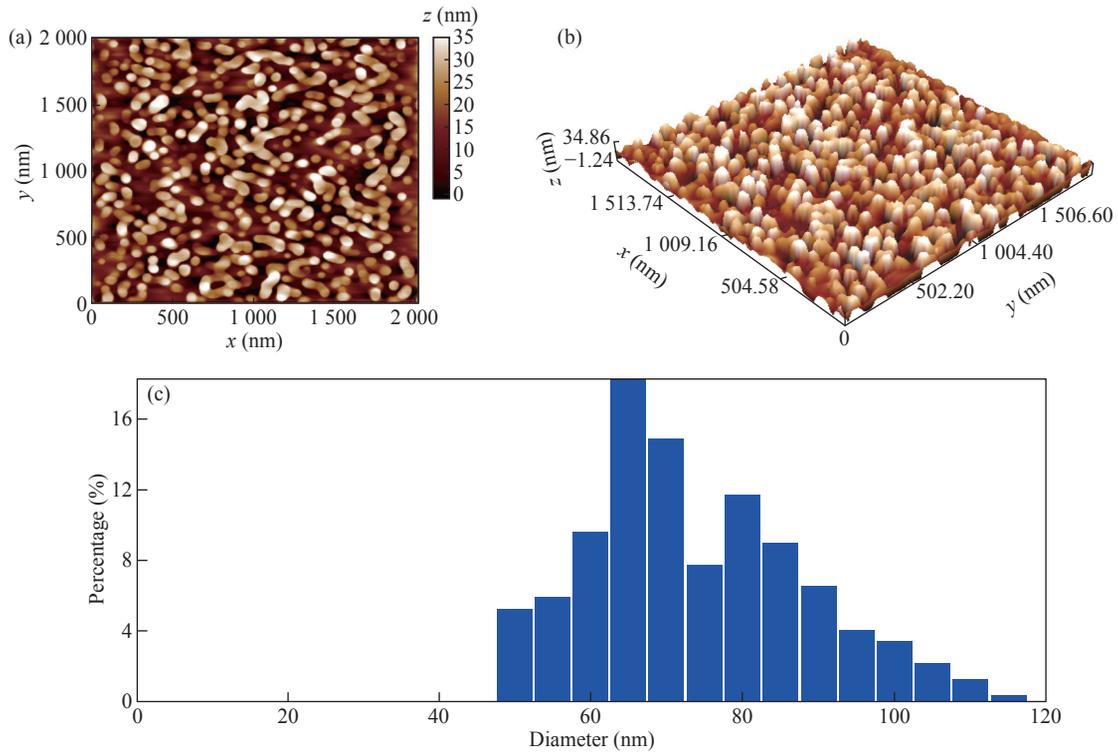


Fig. 2 The AFM images show the structure of the sample in (a) 2D, (b) 3D and (c) the statistical distribution

Effect of rGO-Ag NPs nanocomposite on bacteria

Figures 3–6 show the percentage of bacterial killing after being injected individually with different volumes of 10, 20, 30, and 40 μL of distilled water, graphene oxide, silver nanoparticles, and the rGO-Ag NPs nanocomposite. Furthermore, a 24 h incubation period at 37 $^{\circ}\text{C}$ with increasing volumes of nanomaterials killed a large percentage of bacteria for bacterial species (*E. coli*) (*Lactobacillus-Porphomnas* and *Streptococcus*).

We note that there is a relationship between adding volumes of the nanomaterial and the percentage killing

of bacteria, which is directly proportional. This means that the killing increases with increasing volumes of rGO-Ag NPs and the best one is 40 μL . This result is compatible with other reports [24, 25].

Effect of nanocomposite and laser in killing bacteria

When irradiating samples with a diode laser of wavelength 405 nm at a distance of 20 cm for different time periods (1, 2, 3, 4 min), for both types of bacteria (gram-negative and positive), using the same volume for all samples, which is 40 μL of each of silver Ag, GO, and nanocomposite rGO-Ag NPs, after an incubation period that lasted 24 h at a temperature of

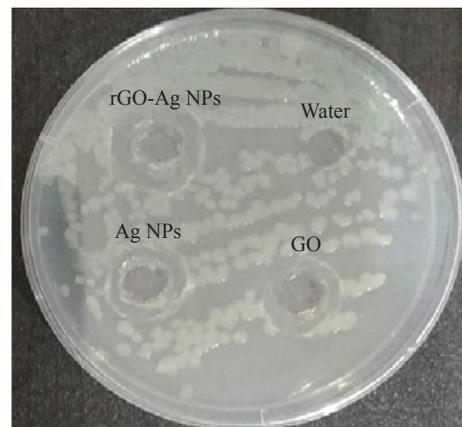
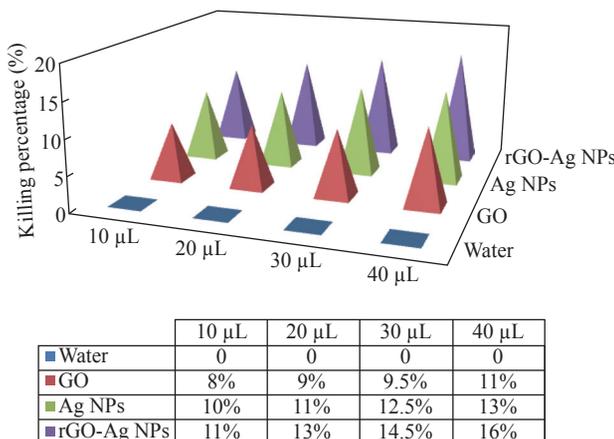


Fig. 3 The killing of percentage of Lactobacillus bacteria with different amounts of nanomaterials (10, 20, 30, 40 μL)

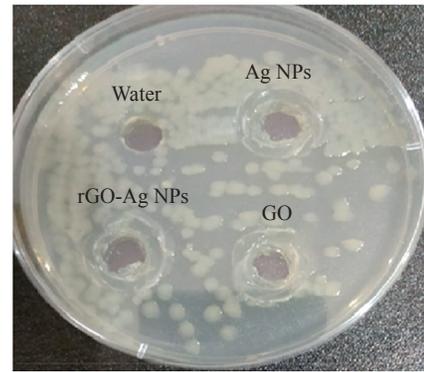
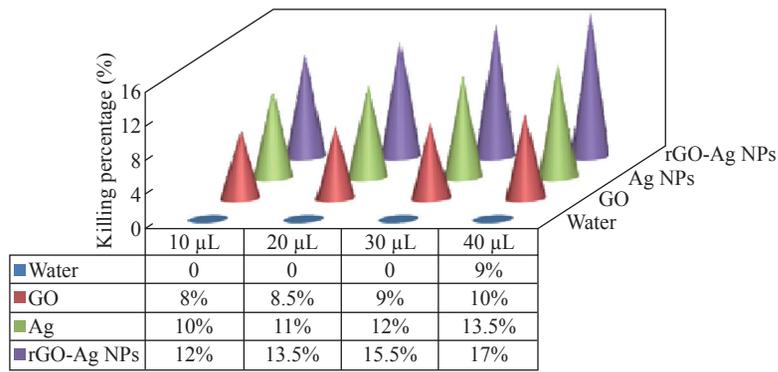


Fig. 4 The killing percentage of *E. coli* bacteria with different amounts of nanomaterials (10, 20, 30, 40 µL)

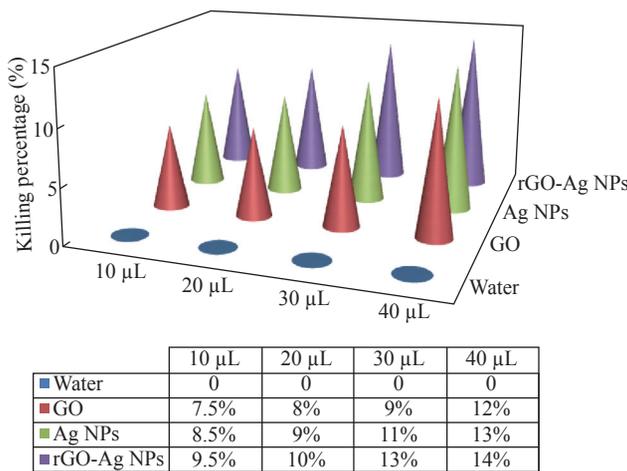


Fig. 5 The killing percentage of *Porphomnas* bacteria with different amounts of nanomaterials (10, 20, 30, 40 µL)

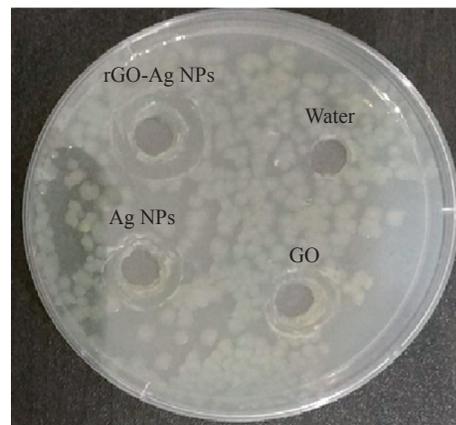
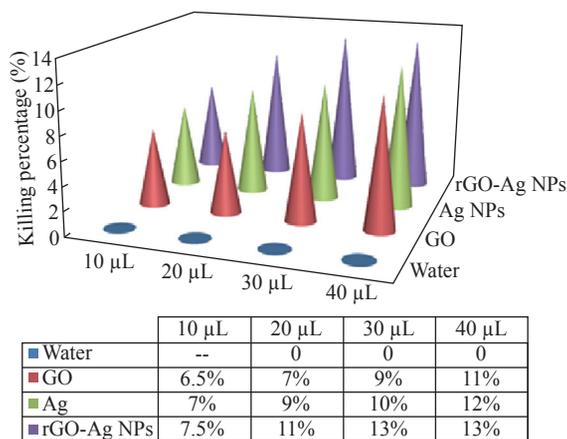


Fig. 6 The killing percentage of *Streptococcus* bacteria with different amounts of nanomaterials (10, 20, 30, 40 µL)

37 °C, we found that the killing percentage in bacteria increased with the increase in the time irradiation periods, and the highest killing percentage obtained was an irradiation period of 4 min.

In addition, it was found that the killing percentage increases when using the laser with the nanocomposite; the reason is that the nanocomposite has a high absorption capacity for the laser, and the laser used will be absorbed by the nanocomposite and thus become a movement of the nanoparticles in a state of irritation,

which helps in their penetration into the cell wall to contribute to the increasing bacteria killing percentage, as shown in Figs. 7–10.

Disruption of bacterial cells is the main mechanism against gram-negative bacteria, while inhibition of cell division can explain the lysis of gram-positive cells. The nanocomposites cause stronger damage towards the gram-negative membrane compared to gram-positive bacteria, and the antimicrobial potential of this nanocomposite is affected by the thickness of the cell

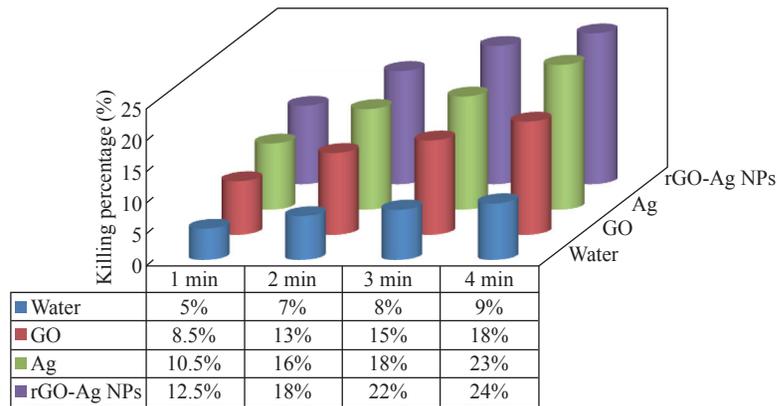


Fig. 7 The killing percentage of *Streptococcus* bacteria when adding 40 μ L of nanomaterials with diode laser irradiation with a wavelength of 405 nm (1, 2, 3, 4 min)

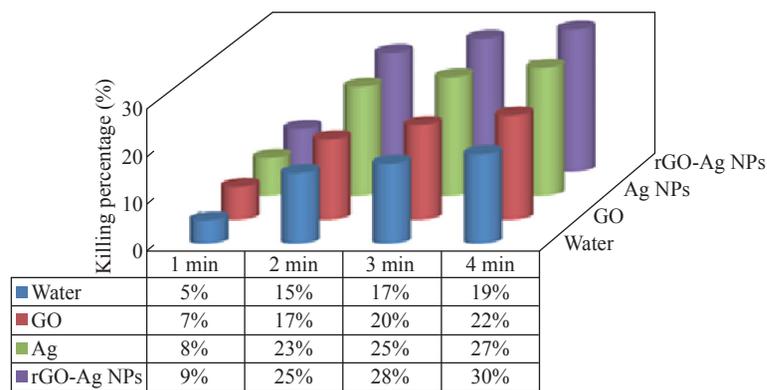


Fig. 8 The killing percentage of *Porphomnas* bacteria when adding 40 μ L of baking materials with diode laser irradiation of 405 nm wavelength (1, 2, 3, 4 min)

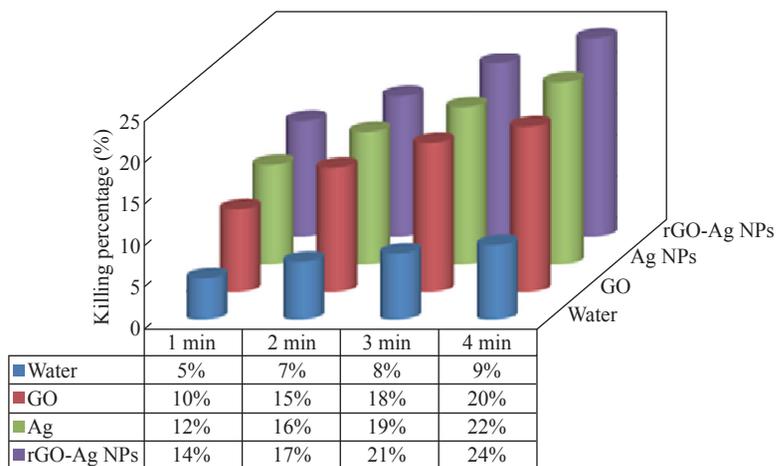


Fig. 9 The killing percentage of *E. coli* bacteria when adding 40 μ L of nanomaterials with diode laser irradiation with 405 nm wavelength (1, 2, 3, 4 min)

wall of the microorganisms. The gram positive contains a thick layer (20–80 nm) of peptidoglycan bound to teichoic acid. Whereas in gram-negative bacteria, the cell wall is of a thin (7–8 nm) peptidoglycan layer. Their bactericidal action can be portrayed as releasing metal ions, penetrating through the cell membrane, and generating reactive oxygen species (ROS), DNA, protein, mitochondria, lipids and membrane damage,

leading to cell death.

Through a comparison with the results of researchers on the use of nanomaterials in killing and inhibiting bacteria as shown in Table 1, it was found that the rGO-Ag NPs nanocomposite with laser caused a high rate of killing for different types of gram-negative and gram-positive bacteria when adding a small volume (40 μ L) of the rGO-Ag NPs nanocomposite, which is quick

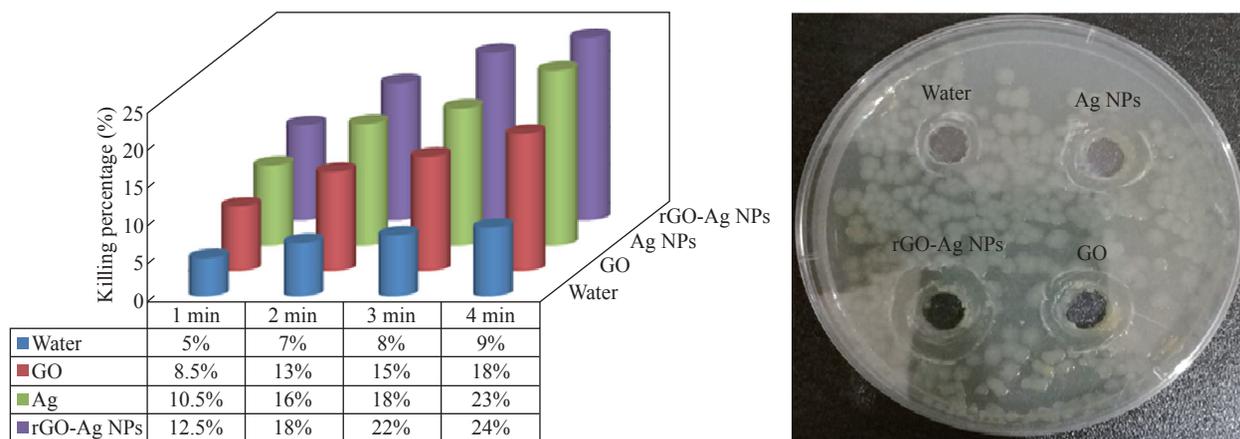


Fig. 10 The killing percentage of Lactobacillus bacteria when adding 40 μL of nanomaterials and 405 nm diode laser radiation (1, 2, 3, 4 min)

Table 1 Comparison of the previous works with this study

Compound	Nanomaterials	Type of bacteria	Volumes/ concentrations	Ref.
rGO-AgNPs	GO, AgNO ₃ , sodium citrate, 1 h, 50 °C	<i>E. coli</i>	40 μL	[26]
rGO-AgNPs	AgNO ₃ , rGO, L.AA, NaNO ₃ , H ₂ O ₂ , KMnO ₄ , HCL 60 °C, 1 h	<i>E. coli</i> <i>S. aureus</i> <i>P. aeruginosa</i> <i>C. albicans</i>	80–85 $\mu\text{L}/\text{mg}$	[27]
rGO-AgNPs	H ₂ SO ₄ , H ₃ PO ₄ , graphite flakes, HCL, KMnO ₄ , pure silver plate 99.9%	<i>E. coli</i> <i>S. aureus</i>	10 ⁴ –10 ⁵ CFU/mL	[28]
rGO-AgNPs	GO, AgNO ₃ , KMnO ₄ , H ₂ SO ₄ , NaBH ₄ , Hydrochloric acid 4 h, 50 °C	<i>E. coli</i> <i>S. aureus</i>	10 μL	[29]
rGO-Ag NPs	AgNO ₃ , NaBH ₄ , rGO, Diode laser 405 nm 3 min, room temperature	<i>E. coli</i> <i>S. aureus</i> <i>Lactobacillus</i> <i>Porphomonas</i>	10–40 μL	This study

to prepare, is not financially expensive and does not require the availability of special laboratory conditions.

Conclusion

It turns out that the proposed method for preparing the reduced graphene oxide compound with silver nanoparticles, rGO-Ag NPs, was an easy and inexpensive method. The study of the optical and structural properties of the rGO-Ag NPs nanocomposite showed that there are two peaks at 400 nm and 268 nm, and from the AFM examination, it was shown from the knowledge of the surface properties that the average particle size is 71.26 nm.

Also, the result shows that the rGO-Ag NPs show a relationship between adding volumes of the nanomaterial and the percentage killing of bacteria, which is directly proportional. This means that the

killing increases with increasing volumes of rGO-Ag NPs, and the best one is 40 μL . Through the absorbance, a diode laser of wavelength 405 nm was selected and applied with the rGO-Ag NPs nanocomposite for the same kinds of bacteria and for different periods of time, and it showed that increasing the irradiation period with volume stability of 40 μL causes a higher killing percentage.

These results are promising and can be applied in the treatment of gum and tongue infections after clinical studies.

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