Original Article

Antibacterial Effects of Gold Nanoparticles in Different Shapes With and Without Laser

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Abstract

Background: Gold nanoparticles (Au-NPs) possess excellent antibacterial properties and are considered an alternative material for treating antibiotic-resistant bacteria due to their unique physical, chemical, and biological properties. Au-NPs and gold nanorods (Au-NRs) have an inhibitory effect on coliform bacteria growth and mitosis. **Objectives:** To study the effect of Au-NPs (rods, spherical) antibacterial efficiency using with and without laser beam against *Escherichia coli*. **Materials and Methods:** Au-NPs and Au-NRs were fabricated by pulsed laser ablation method, where UV-Vis spectroscopy, transmission electron microscopy, and dynamic light scattering investigated the optical and structural properties. The bactericidal effect of these nanoparticles was then assessed with and without exposure to a 532 nm laser, using the counting method (CFU/mL). **Results:** This effect increases with increasing Au-NP concentration. However, upon exposure to the laser beam, a less significant decrease in the number of bacterial cells was observed compared to the control group. The bactericidal effect of the Au-NPs is enhanced when activated by the 532 nm laser light. Interestingly, the lower concentration of Au-NRs (0.1 mL) had a stronger inhibitory effect on bacteria than the higher concentration (1 mL), possibly due to the accumulation of Au-NR at higher concentrations, which reduces its effective surface area to interact with bacteria and thus reduces its antibacterial activity. **Conclusions:** The study concluded that the antibacterial effect of Au-NPs and Au-NRs was enhanced when they were exposed to 532 nm laser light. This effect may be due to the high temperature caused by the absorption of laser light and nanoparticles, leading to thermal damage and cell death. The largest decrease in bacterial survival was observed with 1 mL of the photoactivated Au-NRs. However, Au-NR at higher concentrations showed reduced effectiveness due to accumulation, which suggests the need for a balance in concentration for optimum antibacterial effect.

Keywords: *Escherichia coli*, gold nanorods, gold nanoparticles, laser, surface plasmon resonance

Introduction

Nanomaterials possess discrete electronic states with distinct electronic, optical, magnetic, and mechanical properties at the nanoscale. They have high surface-to-volume ratios and have been investigated for their medical applications in the control of drug-resistant microorganisms.[1] Nanoparticle toxicity is proportional to the quantity of nanoparticles internalized by cells, with moderately toxic cationic gold nanoparticles (Au-NPs) being internalized by cells, while nontoxic anionic Au-NPs are not.[2] Light is scattered and absorbed by Au-NPs, resulting in a surface Plasmon excitation, which is dependent on the size, shape, composition, and environment of the nanoparticles.[3] The absorption of multi-shaped Au-NPs should be studied to understand the relationship between particle shape and toxicity. Nanospheres were more efficiently absorbed than nanorods in the 10–100 nm range.[4] Antibacterial photodynamic therapy (APDT) involves irradiating photosensitizers to generate reactive oxygen species (ROS) and eradicate microorganisms. It is less effective against Gram-negative bacteria than Gram-positive bacteria, and combining it with other antibacterial strategies is the most effective way to boost its efficacy.[5] Due to their strong localized electromagnetic field and intense plasmatic

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properties, anisotropic metal nanoparticles such as gold nanorods (Au-NRs) have garnered considerable interest for medical applications.[6] Under laser irradiation, one of the most remarkable characteristics of gold nanoparticles (GNPs) is their ability to convert light into heat.[7] Demonstrate that the proportion of positively and negatively charged ligands attached to nanoparticles governs Gram selectivity. This effect is a result of the equilibrium between polyvalent electrostatic and non-covalent interactions, which work together to destabilize the bacterial cell wall.[8] Due to the photothermal effect created by Au-NPs surface plasmon resonance oscillations, laser light increases the antimicrobial activity of Au-NPs by at least one-fold. This can be used for the swift and efficient eradication of bacterial cells.[9] Au-NPs at large concentrations may have bactericidal properties due to the coexisting chemicals (gold ions, surface coating agents, and synthesis chemicals) coexisting in them.[10] Au-NPs have been utilized for the photothermal destruction of microorganisms. The surface coating that is, added to activate the interaction of Au-NPs with the bacterial cell wall can render them mildly toxic. The negatively charged bacterial cell wall does not adhere to negatively charged Au-NPs.[11] Gold nanoparticle internalization, accumulation, and toxicity are all influenced by size, with spherically shaped particles having the highest levels of cellular internalization.[12] Au-NRs are a popular choice for plasmatic applications due to their unique characteristics, high-quality synthesis, tunable Plasmon resonance, tenability, and biocompatibility.[13] PG Au-NPs have a greater antibacterial effect against gram-negative microorganisms due to their narrow cell wall, which can absorb positively charged ions released from metal nanoparticles.[14,15]

Materials and Methods

Bacteria

Escherichia coli (Gram-negative) bacteria were used to evaluate the antibacterial effect of Au-NPs and Au-NRs. Au-NPs, Au-NRs, and lasers together. It should be noted that in order to ensure that the bacteria survive, they must be kept at a temperature of 0 to 5°C, and they will remain alive for a week in the environment outside the incubator.

Synthesis of Au-NPs

To verify the effect of Au-NPs, it had been fabricated these structures by laser ablation in a liquid manner. For this purpose, it had been used an Nd:YAG laser of 80-mJ per pulse energy, 4 ns pulse width, and 1 Hz repetition rate. A Q-switch Nd:YAG laser was irradiated on a pure gold plate to produce NPs from it. The laser wavelength, repetition rate, and ablation time were modified at 1064 nm, 10 Hz, and 4, 3, and 2 min, respectively. The laser beam was focused on the golden objective after being reflected from the mirror and passing through a convex lens (f = 100 mm). The objective was immersed in 15 mL of double ionized water solution and stirred during the excision of the objectives at room temperature. Three different concentrations, 50%, 25%, and 12.5%, were obtained as a result of different irradiation times of 4, 3, and 2 min, respectively.

Nanorods were fabricated using laser ablation, applying 10 V electrical voltage to a sample with a first harmonic power of 1064 nm. Three concentrations of 50%, 25%, and 12.5% were obtained with irradiation times of 3, 4, and 2 min.

Laser

Use a semiconductor laser continuous wavelength 532 nm at 8-mW output power to radiate bacteria samples.

Addition of Au-NPs

The antibacterial potential of synthesized Au-NPs with and without irradiation was evaluated against *E. coli*. To divide the bacteria, we use two mediums of 10 cells to grow the bacteria and here we use two methods to kill the bacteria. In the first method, bacteria are first instilled in a volume of 0.1 mL in five cells, and in the next step, Au-NPs are successively added in concentrations of 0, 0.05, 0.1, 0.5, and 1 mL. Then we keep these solutions in the incubator for 24 h. It should be noted that the presence of a concentration of zero means that there are pure bacteria, which is to control the test conditions. In the second method, we use a laser based on the absorption peak of the nanostructures to obtain the killing efficiency. For this purpose, due to the absorption peak of gold NPs at 532 nm as in the first method, bacteria were added to a volume of 0.1 mL in 5 cells, and in the next step, as in the first method, Au-NPs were added to the cells in concentrations of 0, 0.05, 0.1, 0.5, 1 mL. Then each cell irradiation by laser for 4 min, the laser has a wavelength of 532 nm and a power of 8-mW then we keep these solutions in the incubator for 24 h and after that, we start counting the bacteria.

Addition of Au-NRs

First, bacteria are introduced to a volume of 0.1 mL in 6 cells, and in the next step, gold nanosticks are successively added in 3 cells at concentrations of 0, 0.1, and 1 mL. It should be noted that the presence of a concentration of 0 means that there are pure bacteria controlling the test conditions, has been added 0, 0.1, and 1 milliliters of gold nano sticks to each colony cell, and then each cell is hit with a wavelength of 532 nm and a power of 8-mW for 4 min. Then we keep these solutions in the incubator for 24 h, after which we start counting the bacteria.

Counting method

A growth inhibition experiment was conducted to evaluate the antibacterial effectiveness of Au-NPs against

E. coli. After 24 h, count the number of live bacteria and their colony-forming units (CFU/mL). In the first stage of serial dilution preparation, a liquid sample was diluted with a dilution factor of 0.1 (1 mL of the first tube was added to 9 mL of solution in the second tube). About 0.1 mL was taken from each prepared tube (serial dilution) and transferred to the Mueller Hinton Agar solid culture center. The cultures were kept in an incubator at 37°C. Then the number of colonies in each plate was counted and by multiplying by the dilution factor, the number of colonies was obtained.

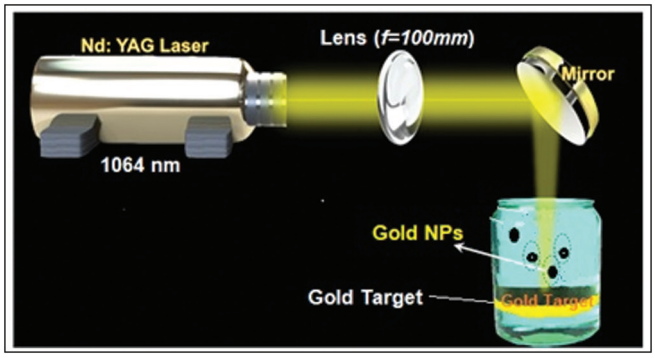

Figure 1: Laser ablation liquid experimental setup

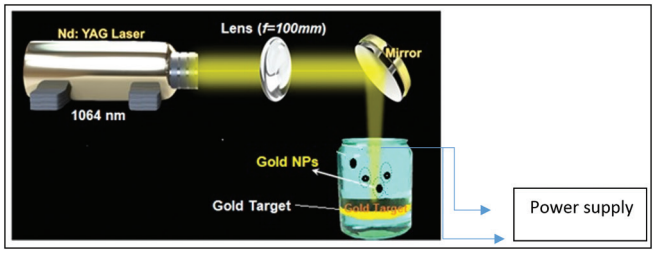

Figure 2: Laser ablation liquid experimental setup to produce nanorods

Results

UV–visible spectroscopy

Absorption spectra of Au-NPs prepared by laser ablation show the first evidence of a synthetic reaction [Figure 3A]. The surface resonance peak of the plasmon is observed at 520 nm in the spectra. With varying irradiation times of 2, 3, and 4 min, three concentrations of nanoparticles of 12.5%, 25%, and 50% were reached [Figure 3B]. The reason that the spectrum of nanorods is broader than that of nanoparticles is due to their different shapes and sizes, which affect their plasmonic properties The absorption spectrum of nanorods is broader than that of spherical nanoparticles. The main reason for this difference lies in the shape and aspect ratio of these nanostructures [Figure 3C]. Nanorods have two exact resonant absorption peaks, one for the transverse mode and one for the longitudinal mode. The transverse mode remains at a fixed wavelength, while the longitudinal mode can be tuned across a wide range of spectrum. This results in a broader absorption spectrum than spherical nanoparticles.

Transmission electron microscopy (TEM)

Figure 4A shows the TEM analysis of the size and shape of the obtained particles at 80-mJ laser energy with an ablation time of 4 min. It was found that the Au-NPs

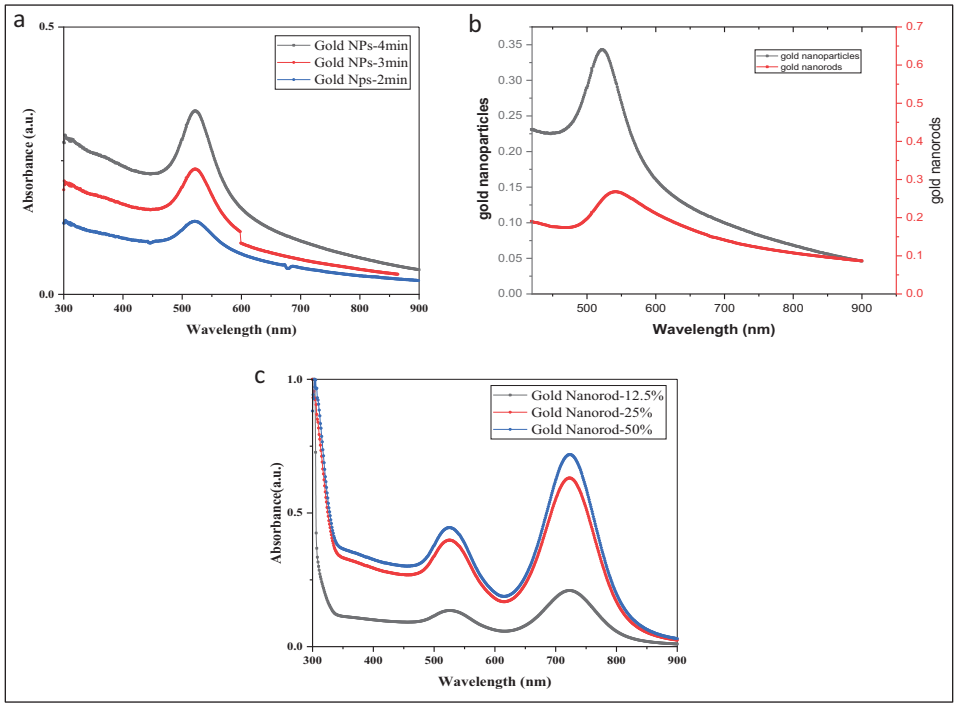

Figure 3: Absorption spectrum of Au-NPs. (A) Gold NPs with 50%, 25%, and 12.5% concentrations. (B) Au-NRs with 50%, 25%, and 12.5% concentrations. (C) comparison of the absorption spectrum of NPs and nanorods

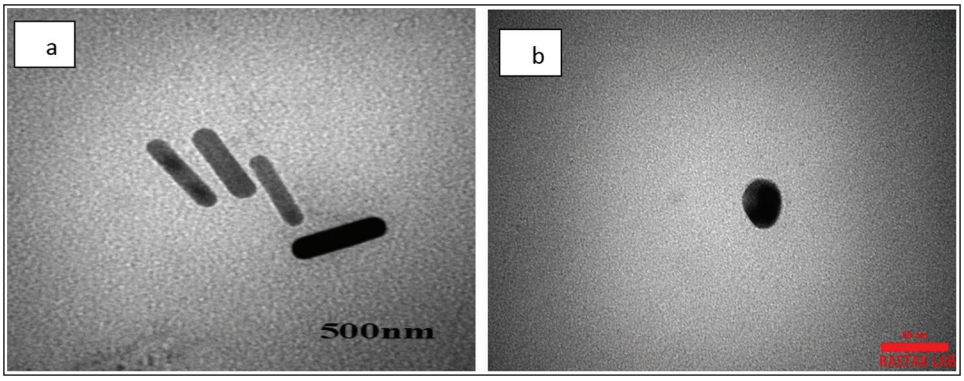

Figure 4: The TEM image of the (A) nanorods (B) nanoparticles

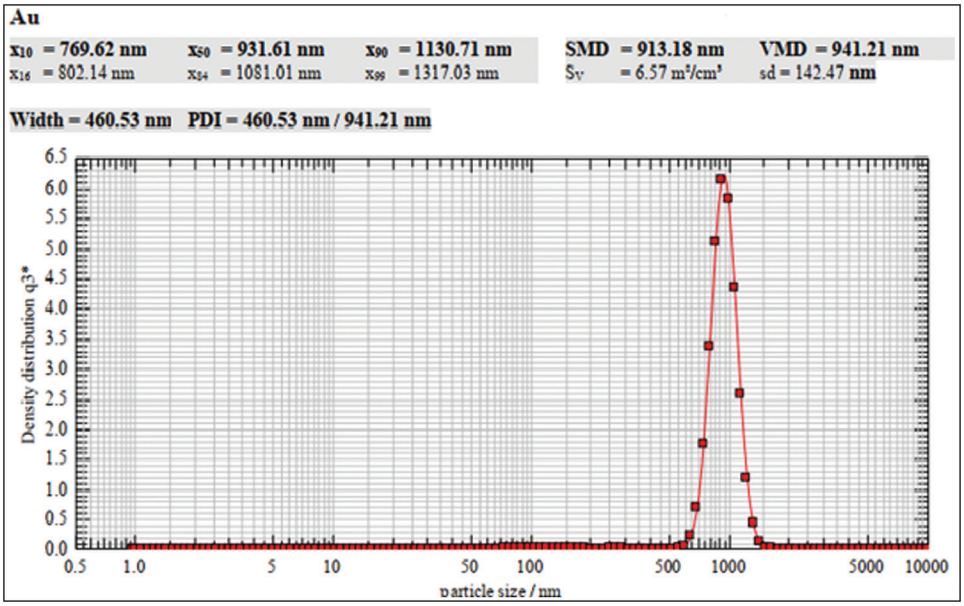

Figure 5: DLS spectrum of Au-NRs solution

produced by laser ablation were spherical in shape. Figure 4B shows the size and shape of the obtained nanorods with 100-mJ laser energy.

Dynamic light scattering (DLS)

The particle size and distribution were also determined by DLS measurements. Au-NRs are shown in Figure 5, respectively, which confirms the size distribution of these nanorods around 500 and 1000 nm for rod distribution. In addition, the DLS technique measures the hydrodynamic diameter of the particles, which is strongly augmented by water molecules.

Effect of Au nanoparticles with and without laser beam

The results of adding different quantities of manufactured Au-NPs at a concentration of 50% with an irradiation period of 4 min, as well as the effect of Au-NPs exposed to 532 nm laser beam, are shown in Table 1. Where we notice a slight decrease in the number of viable bacteria when increasing the amount of nanoparticles at concentrations of 0.5 and 1 mL, the number of live bacteria was 2.3×10^8 CFU/mL, indicating that there was no noticeable difference in the bactericidal effect. Au-NPs can inhibit bacterial growth and division, and this effect tends to increase with increasing amounts of Au-NPs. When the cultures were treated with Au-NPs at concentrations of (0.5, 1) mL followed by photo irradiation for 4 min compared to the control group, a less significant decrease in the number

Table 1: CFU/mL of *E. coli* exposed to varying amounts of Au-NPs, with and without irradiation, and the percentage of growth inhibition after 24 h of incubation

Without laser		With laser
Au-NPs amount	*E. coli* (CFU/mL)	*E. coli* (CFU/mL)
0 mL	2.9×10^8	2.55×10^8
0.05 mL	2.8×10^8	2.35×10^8
0.1 mL	2.75×10^8	2.3×10^8
0.5 mL	2.3×10^8	2.2×10^8
1 mL	2.3×10^8	1.8×10^8

Table 2: CFU/mL of *E. coli* exposed to varying amounts of Au-NRs, with and without irradiation, and the percentage of growth inhibition after 24 h of incubation

Au-NRs amount (mL)	Without laser	With laser
	E. coli (CFU/mL)	*E. coli* (CFU/mL)
0	2.51×10^8	2.55×10^8
0.1	2.45×10^8	2.35×10^8
1	2.5×10^8	2.3×10^8

of bacterial cells was observed. The results show that the antibacterial activity of the photoactivated Au-NPs increases with increasing NP amount in the presence of laser light because the correlation between the amount of Au-NPs and the rate of bacteria destruction is dose-dependent, which leads to an increase in the concentration of the nanoparticles. The highest decrease in bacterial cell survival was observed upon treatment with 1 mL of photoactivated Au-NPs.

Effect of Au-NRs with and without laser beam

Table 2 shows the addition of Au-NRs in different quantities, as well as the effect of laser light (532 nm) in the presence of Au-NRs on the antibacterial efficiency of *E. coli*. Only the effect of Au-NRs with different amounts (0.1, 1 mL) was investigated. Some cells of bacteria are not treated with Au-NRs were used as controls. After 24 h treatment with 0.1 mL Au-NRs, the number of live bacteria was lower compared to treatment with 1 mL Au-NRs. The reason behind this collection of particles, which might pertain to Au-NRs, could lead to the clustering of these particles, subsequently diminishing their active surface area in relation to their interaction with bacteria. On the other hand, the large amount of Au-NRs may not be evenly distributed throughout the medium. While the results showed that Au-NRs that were exposed to laser light had a stronger antibacterial effect than Au-NRs that were not exposed to laser light, the antibacterial effect of Au-NRs that were not exposed to laser light was comparable. Compared to the control group, exposure to green laser light significantly reduced the survival of *E. coli*. With 1 mL of photoactivated Au-NRs, the greatest reduction in bacterial cell survival was observed.

Discussion

This study aimed to evaluate the effect of the light beam (532 nm) on the antibacterial efficiency of Au-NPs and Au-NRs against *E. coli* cells. The results showed that Au-NPs exposed to laser light showed a stronger antibacterial effect compared to unexposed Au-NPs. Also, the Au-NRs exposed to laser irradiation were higher than the Au-NRs that were not exposed. It is interesting to compare the observations of this research with other studies that have reported phototherapy applications based on metal NPs. Annesi *et al.*[16] demonstrated that SEM analysis of the dried culture of *E. coli*/GNRs clearly interpreted the morphology of the bacteria, whereas backscattered electron microscopy (BSE) imaging demonstrated that the GNRs were not in contact with the bacteria. Photothermal experiments using laser light (810 nm) demonstrated that by irradiating GNRs in *E. coli*, the temperature could be increased to 50°C in about 5 min, and cell viability experiments demonstrated that under this laser photoirradiation (I = 6.3 W/cm), the presence of GNR in the *E. coli* culture was able to induce a 2 log CFU reduction in live bacteria. Castillo-Martínez *et al.*[17] studied the bactericidal and bioprotective activities of gold nanofilms (Au-NRs) against microorganisms using plasmonic photothermal therapy (PPTT). They used the seed-and-growth solution method, to fabricate Au-NRs and measure them with a length of 33.2 nm and a width of 7.33 nm. *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus oralis*, *Streptococcus salivarius*, and *E. coli* were used to ascertain the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of Au-NRs and PPTT Au-NRs. Fluorescent microscopy revealed that Au-NRs PPTT was more effective than Au-NRs alone on MICs and MBCs. The capacity of GNPs to convert light into heat when exposed to laser radiation is one of their most impressive characteristics. This characteristic is important because it may be used to create nano-photothermal vectors that can kill bacteria at the molecular level. The most recent developments in the realm of GNP-mediated, nano-targeted laser hyperthermia of multidrug resistance (MDR) bacteria are summarized in the current work.[7] Photodynamic therapy employs photosensitizers (PS) in conjunction with either visible or ultraviolet light in order to generate cytotoxic singlet oxygen and free radicals, which induce deleterious effects on microorganisms. Photosensitizers possess a stable electronic configuration that is situated at the lowest or ground state level. Following irradiation at a specific wavelength, the photosensitizer is elevated from the ground state to an excited state. In simpler terms, electrons relocate to higher energy orbitals.[18] As we explained in the previous paragraph, there is a mechanism for laser interaction with bacteria when nanoparticles are present, as the metal surface absorbs light and converts this energy into a photochemical effect, resulting in the formation of cytotoxic ROS and the biochemical composition, all of these factors are contributing for enhancing antibacterial effect. The combination of Au-NPs and a 532 nm laser can significantly cause bacterial cell death. The Au-NPs are able to bind to the bacterial cell wall or penetrate the cell, The reason for the increase in bacterial death, when using the laser with the presence of Au-NPs is the possibility of one of these reasons, first, which is the increase in temperature due to the absorption of these particles of the laser beam produced by the occurrence of the

Plasmon phenomenon, and then the absorbed laser light is converted into heat, which leads to thermal damage and cell death. Second, the possibility of the formation of active free radicals has a great ability to kill microorganisms. In addition to alterations in biochemical composition, particularly the relative quantities and distribution of various cellular components such as DNA, proteins, fatty acids, and polysaccharides. Significantly contribute to the inhibition and destruction of microorganisms. Found that cellular and tissue toxicity is significantly influenced by nanoparticle-induced ROS.

Conclusion

Both Au-NPs and Au-NRs exhibit antibacterial effects, which can be further enhanced when activated by 532 nm laser light. In addition to observing the effect of nanoparticle shape, when exposed to laser light, Au-NPs that exhibit Plasmon resonance phenomena can cause large temperature rises that are largely proportional to their size. From the results, we note that the effect of nanoparticles and nanorods without irradiation was minimal compared to the effect of nanoparticles and laser light, as the number of living bacteria decreased by a large percentage. When comparing the effect of shape, Au-NPs had a greater effect than Au-NRs.

Ethical Approval

Not Applicable.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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