Original Article

Eradicating bacteria with silica and polyethylene glycol-coated gold nanoparticles using photothermal therapy

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Abstract

Various species of bacteria have adapted to man-made chemicals and antibiotics, developing into superbugs posing a threat to humankind, and soon creating a future where antibiotics are futile against various diseases. In recent studies, the gold nanoparticle were tested in photothermal therapy to treat cancerous cells, as gold has conductive properties that make it an excellent medium for electromagnetic dissipation. The silica and polyethylene glycol coating was selected, because its properties that prevents the deformation of gold nanoparticle when exposed to NIR laser at 808 nm with 500mW and green laser 520nm with 100mW. The angle of divergence and radius of light at any given distance equations were incorporated to finding the time and distance for the laser set up. The resonating gold nanoparticles irradiated by the infrared laser produced heat radiation that eradicated the E. coli strain k12 and Staphylococcus Epidermidis within 24mm and 26mm zones of inhibition, respectively. This compared favorably to penicillin as it created a 27mm zone of inhibition. The determination of the heat that the particles produced was calculated with the equation describing the heat of a system that is energized by electromagnetic radiation. The photothermal therapy method demonstrated a potential to treat bacterial infection.

Keywords: photothermal therapy.

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INTRODUCTION

Purpose

The main objective of this experiment will be evaluating and analyzing the spherical silica and polyethylene-glycol (PEG) covered gold nanoparticles' ability to act as a photosensitizer to raze the bacteria that are commonly being exposed antibiotics repeatedly and fostering resistance to those medicine. Lasers with wavelengths of 520nm at 100 mW and 808nm at 500mW will be targeted at discs containing 20 nm silica and PEG covered gold nano sphere in petri dishes cultured with bacteria such as ***Escherichia coli k strain 12 and Staphylococcusepidermidis.*** Mathematical models and equation, such as angle of divergence, Macroscopic Model and Mie Theory, will be applied to analyzed the efficiency of gold nanoparticles wavelength and energy to heat conversion. The zone of inhibition will be used to measured effectiveness of the different test methods ability to eradicate the bacteria.

Problem

Patients are often prescribed higher and unnecessary levels of antibiotics by over 50% of doctors around the world. Furthermore, some countries allowing consumers to obtain antibiotics without prescription, which leads to consumers overdosing have contributed to the rise of superbugs. Deleterious bacterias are developing resistance to hackneyed uses of common and strong antibiotics in long periods of time, such as ***Escherichia coli k strain 12 andStaphylococcus Epidermidis***. Those bacteria have fostered their resistance to antibiotics by blocking the entry of the drugs into the cell, inactivating or eradicating the drugs using enzymes, altering the drug's target site, and causing a rapid effluxing of the drug from cell. Each year these drug-resistant bacteria infect more than 2 million people nationwide and kill at least 23,000 according to the U.S.

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Centers for Disease Control and Prevention. As these bacteria and fungi developed apex resistance, the era of antibiotics will succumb to an end and humans will fear even the smallest cuts.

BACKGROUND INFORMATION

Gold nanoparticles (GNP) are extremely particles of gold ranging from size from 1 nm to 100 nm that irradiated with specific wavelength can convert these photon energy into heat to eradicate the bacteria. GNP were selected to eradicate the bacterias due to it's application in human anatomy as a method to destroy troublesome cells like cancerous cell in the body. Gold is more chemically stable compared to silver and other metal, which is why gold was the best choice of metal. When laser's wavelength comes into contact with the gold, the gold absorbs it and converts it into heat out towards the cells in the particle's vicinity. GNP can be controlled by mid electric current, which allows for greater precision when targeting troublesome cells. Also the mid electric current can be used to prevent damaging other healthy or benign cells. The gold nanoparticles are coated in polyethylene glycol (PEG) and silica. The PEG helps to prevent the GNP from reacting or oxidizing while in use and the silica helps to prevent the GNP from losing its shape while it is heated. ***Escherichia coli*** is a gram-negative bacteria making it relatively resistant to antibioticsdue to its nature of their cell wall. For instance, the bacteria can adjust its porins opening so that antibiotics and other molecule unable to penetrate. Also, it is suitable for experimental purpose, because it is one of the best-defined groups of bacteria. Moreover, the bacteria have demonstrated ability to survive adequately in the environment and has a history of safe commercial application. ***E. coli*** is easy to grow, because it can grow with pensury amount nutrient and multiples at an exponential rates. Because of its wide use as a model organism in research in microbial genetics, physiology, and in industrial applications, ***E. coli*** K-12 is one of the most extensively studied microorganisms. ***Escherichia coli*** causes life-threatening bloodstream infections, urinary tract infections, and other common infections. Antibiotic resistance rates in ***E. coli*** are rapidly rising, especially with regard to fluoroquinol ones and third- and fourth-generation cephalosporins. Astoundingly, most of these multidrug-resistant strains are acquired in the community rather than in hospital setting. Drug-resistant ***E. coli*** are readily acquired in our food and water supply. When people eat sterile food, there is a rapid and substantial fall in the numbers of drug-resistant ***E. coli*** these people carry. (gram-negative) ***Staphylococcus epidermidis*** is one of the crucial reason and cause of clinical infections. Because of potential ability in biofilm formation and colonization in different surfaces, also using of medical implant devices in immunocompromised and hospitalized patients the related infections have been increased. In recent decades, the clinical importance and the emergence of methicillin-resistant ***Staphylococcus epidermidis*** strains have created many challenges in the treatment process. (Daniel, 2) Because ***Staphylococcus epidermidis*** is seen as an opportunistic pathogen, it is the frequent cause of nosocomial infections. (gram-positive)

Hypothesis/Goal

If the disk that was dipped in the 20 nm silica-coated Au NanoSphere zipped by the infrared laser with 808 nm with 500 mW was placed in the Staphylococcus e. and E. coli strain k12, then the bacteria will be eradicated by the heat produced by the resonating gold through the photo-physical processes when it comes in contact with the 808 nm and 520nm wavelengths.

Independent Variable/Dependent Variable/ Constants

- Independent Variable: species of the bacteria, wavelength of laser
- Dependent Variable: the zone of inhibition
- Constants: petri dishes, size of PBS gold nanoparticles, light, heat, microscope, nutrient, amount of dosage

MATERIAL AND METHODS

100mW 520nm Green laser, 500mW 808nm red laser, 20 ml of 20 nm silica-surface gold nanoparticles (GNP), Tube of E. coli strain k12, Tube of ***Staphylococcus epidermidis,*** petri dishes, microscope, lab coat, gloves, goggles, mask

Sources of Material Supply

http://nanohybrids.net/products/silica-coated-gold-nanospheres?utm_medium=cpcandutm_source=googleplaandgclid=CJWmhOL1z8gCFQiraQodVggHgw (Gold Nanoparticles)
http://www.carolina.com/bacteriophage-hosts/escherichia-coli-k12-living-bacteriophage-host/124500.pr?question=e+coli (***E. coil strain k12***)
http://www.carolina.com/bacteria/staphylococcus-epidermidis-microkwik-culture-vial/155556A.pr?question=Staphylococcus+epidermidis (***Staphylococcus Epidermidis***)
http://www.atcc.org/products/all/PCS-999-002.aspx (***Penicillin)***http://www.highlasers.com/500mw-808nm-infrared-laser-portable/ (Infrared Red Laser)

Procedure

1. Sterilize the working areas and all the equipments using clorox wipes and alcohol solution, wear gloves and laser safety goggles at all time
2. The E groups contain the bacteria ***Escherichia coli k strain 12*** and the S groups contain the bacteria ***Staphylococcus epidermidis***
3. All the bacteria will be stored in an incubator at 37 Celsius and the bacteria will be plated to get the maximum cover of the petri dish surface
4. The Gold Nanoparticles (GNP) will be stored in a closed box in a refrigerator at 3 degrees Centigrade
5. The Control Groups are E1 and S1 will contain the agar and the bacteria and the experimental

groups are all the other groups (E2-E7, S2-S7)

6. E2 and S2 will be treated with penicillin using the kirby-bauer disk diffusion method with three equidistant points
7. E3 and S3 will be treated with the infrared laser (808nm with 500 mW)
8. E4 and S4 will be treated with the green laser (520nm with 100 mW)
9. E5 and S5 will be treated with GNP using the kirby-bauer disk diffusion method with three equidistant points
10. E6 and S6 will be treated with GNP treated with green Laser (520nm with 100 mW) using the kirby-bauer disk diffusion method with three equidistant points
11. E7 and S7 will be treated with GNP treated with infrared laser (808nm with 500 mW) using the kirby-bauer disk diffusion method with three equidistant points
12. The infrared laser and green laser will be set up using a ring stand and a clamp to hold the laser
13. Set the distance from the bacteria on top of the agar to the laser initial output is 12 cm +/-. 05cm and the 6 minutes of exposure to the laser
14. Turn off the lights in the lab before taking out the GNP that was stored in refrigerator and leave the GNP in a room for 30 mins prior to usage so that it will be at room temperature (be sure it does not come into contact with any light) and right before experimentation shake well for 30 seconds
15. Use the micropipette to take out 2 ml of GNP and drop 1 ml of GNP on a sterile petri dishes, and then drop the other 1 ml back into tube
16. Use the tweezers to take the disks and dip them in GNP and dry it off for 30 seconds while holding it with tweezers, then place them three of them in each of the groups E5, E6, E7, S5, S6, and S7
17. Use a different pair of tweezers to take the penicillin discs gently and place 3 in E2 and S2 each
18. All the bacteria will be given its assigned treatment after plating and prior to incubation.
19. After plating and treatment record any qualitative observation and image of the bacteria in the petri dishes
20. After 24 hours and 48 hours exposure record image and qualitative data
21. Cleaning Method: Sterilize the working area and equipment, wash hands with soap, dispose the petri dishes with bacterial agents using autoclaved at 120 Celsius for 2 hours and then bleach it, then once the petri dishes are sanitized throw them out in the hazardous bin for safety.

Table 1: Zone of Inhibition (+/-0.5mm)

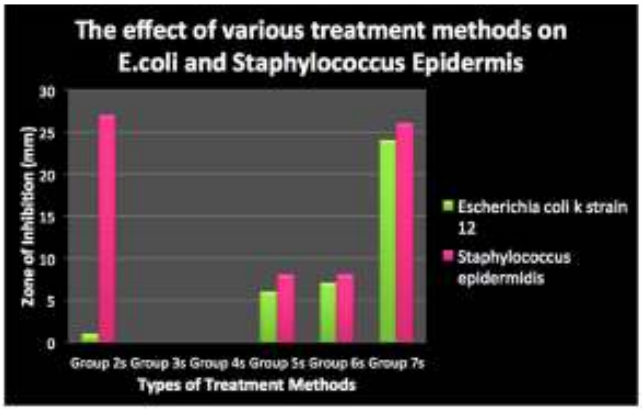
Species of Bacteria	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
E.coli Strain k12	NONE	0.0	0.0	0.0	0.7	0.7	2.3
Staphylococcu s Epidermidis	NONE	2.6	0.0	0.0	0.9	0.7	2.7

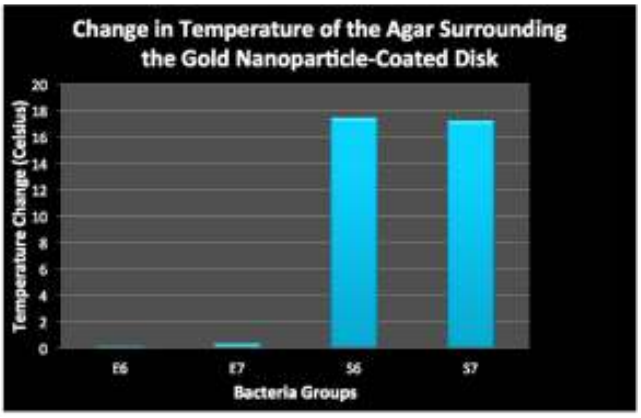
Species of Bacteria	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
E.coli Strain k12	NONE	0.1	0.0	0.0	0.6	0.8	2.2
Staphylococc Us Epidermidis	NONE	2.7	0.0	0.0	0.8	0.9	2.6

Species of Bacteria	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
E.coli Strain k12	NONE	0.2	0.0	0.0	0.6	0.8	2.5
Staphylococc Us Epidermidis	NONE	2.8	0.0	0.0	0.8	0.9	2.8

Temperature Changes

Group	E6	E7	S6	S7
Temperature Change (+/-0.1°C)	.1	.3	17.4	17.2





Equation

Heat Dissipation Efficiency Equation

$$B \equiv \sum \frac{hS}{m_w C_w}$$

Where, m is for mass of the water(w), C is the specific heat capacity of water(w), S is the cross-sectional area of the Au Nanospheres, h is the sum of heat dissipation at the beginning and end of the experiment.

Heat Dissipation Equation

$$Q = \sigma\left(T_{Final}^4 - T_{Initial}^4\right)A$$

Where, σ is the Stefan Boltzmann Constant, A is the surface area, ΔT is the change in temperature. This equation describes heat dissipation involving radiation.

Radius of the Laser at any Distance Equation

$$w(z) = w_0[1 + (z/z_0)^2]^{1/2}$$

Where, w (z) is the radius of the beam at a distance z away from the laser, w0 is the beam radius at the aperture and z_0 is the "Rayleigh range"

Angle of Divergence

$$M^2\lambda/(\pi w_0)$$

Where, λ is the wavelength (in the medium), w0 the beam radius at the beam aperture, and M is the beam quality. The infrared laser had a smaller angle of divergence due to its greater beam quality, and the green laser's angle of divergence was greater mostly due to the lesser beam quality. We factored this into our experiment by positioning the infrared laser 12 cm farther from the petri dish than the green laser to make the divergence of the beam negligible.

RESULT

The penicillin demonstrated the greatest effectiveness against the Staphylococcus Epidermis since the antibiotic is naturally effective against gram-positive bacteria due to penicillin ability to inhibit the production of peptidoglycan, which allows for the repairing of the cell wall and decreases the bacterium's ability to stave off the osmotic pressure of its environment, which is usually within a person. However, penicillin was not effective against the gram negative bacteria E. coli strain k12 because gram-negative bacteria have a lipopolysaccharide and protein coating that prevents the degeneration of the peptidoglycan layer that would usually be altered by the penicillin. The NIR laser and green laser demonstrated a negligible effect on the bacteria in groups E3, E4, S3, and S4. The gold dipped discs in groups E5 and S5 created a miniscule zone of inhibition of only averages of 6 mm in group E5 and 8 mm in group S5, which is much less than the zone of inhibition of the penicillin disks of 27mm in the group S2, but compared favorably to group E2, which saw no change due to the penicillin. The green laser groups (E6 and S6) shared similar results with groups E5 and S5 when measuring the zone of inhibition at widths of 7mm for E6 and 8mm for S6. The NIR laser groups (E7 and S7) demonstrated a greater effect on average on both groups with an zone of inhibition of 24mm on group E7 and 26mm on group S7. The NIR laser had a profound effect on the E. coli strain k12 group compared to the group E2 which the penicillin had no effect at all. However, it did not exceed the zone of inhibition created by the penicillin in groups S2 and demonstrated similar effects.

DISCUSSION

Penicillin was chosen as the antibiotic to treat the bacteria because it is the first antibiotic used to treat people. It has the limitation that it doesn't affect gram-negative bacteria. The gold nanoparticles are covered with silica and polyethylene-glycol (PEG) because those chemical compounds and elements have properties that allow the gold to remain in spherical shape after exposed to high level of temperature change and prevent any chemical reactions between the gold and their environment. Groups E5 and S5, with average zones of inhibition of 6mm and 8mm, demonstrated that the gold contains, to some extent, antibacterial properties. In addition, the group E6 and S6, containing the gold treated with green laser at 520 nm with 100 mW, results demonstrated that the green laser even though at the peak of the SPR wavelength for gold nanoparticles, which had average zone of inhibitions of 7mm and 8mm. Interestingly enough, groups E7 and S7 featured sizeable zones of inhibition of 24mm for E7 and 26mm for S7 compared to group S2, which had a zone of inhibition of 27mm. Furthermore, the penicillin had nearly no effect on the group E2 containing the penicillin. These results demonstrated that the NIR laser and gold nanoparticle treatment may have advantages to penicillin treatment as the NIR laser and gold nanoparticles treatment affects gram positive and gram-negative bacteria.

Also, using the peak SPR wavelength of any given gold nanoparticle is non-conducive to treatingbacterial infections.

APPLICATION

Cancer Therapy

The heat produced by the infrared laser and amplified by the gold nanoparticle will cause hyperthermia in cancer cells causing them to go through apoptosis. The radiation is less intense than the radiation used for radiation and is less toxic than the chemicals used in chemotherapy. Since gold nanoparticles can be injected into the bloodstream of

a patient to target specific areas of the body, this will allow the accurate targeting of tumors.

Treatment of bacterial infections

The treatment proved to be effective against necrotizing fasciitis (flesh-eating bacteria), as the bacteria will not be able to adapt to the harsh radiation due to the apoptosis the bacteria experience. The gold nanoparticles can be used as drug carriers or antibody carriers to accurately target the location of the foreign bacterial agent by covalently binding targeting agents and drugs to 5 nm gold nanoparticles.

Nanoparticle-assisted Selective Photothermolysis of Adipose Tissue Removal

The selective photo thermal heating of adipose tissue by polymer-coated gold Nanorods energized by an external near-infrared exposure at 800 nm is used to facilitate fat removal twice as fast as liposuction. The treatment can aid in the process of removing unwanted scars from liposuction and is safer for the patient.

Future Research

Further research will involve testing the photothermal therapy method on living organisms and eventually human trials. The test subjects, preferably mice, will be injected with superbugs that are resistant to most multiple antibiotics and then treated with PEG-coated silica gold nanoparticles energized by infrared laser. The sizes of the gold nanoparticles would be varied between trials to analyze their effects. The coatings of the gold nanoparticles will also be altered to test for varied results, such as swapping a PEG-coating for a surfactant stabilized model instead. The study doesn't have to be restricted to bacteria only, however, as tests will be performed to test the gold nanoparticles as a novel agent for eradicating viruses.

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