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Short communication

Bacterial biofilm elimination using gold nanorod localised surface plasmon resonance generated heat

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ABSTRACT

Antimicrobial resistance is an increasing global health concern and the world is facing a major challenge to develop novel ways of replacing antibiotics. Gold nanorods exhibit localised surface plasmon resonance upon optical irradiation. During relaxation, absorbed energy is dissipated as heat, which has been utilized to kill bacteria. In this study, 10 × 45 nm gold nanorods were attached to glass surfaces using silanisation. Then biofilms were cultured on the surfaces and studied using microscopy. On average, 71% of the early biofilm bacteria were eliminated after 5 min of near infrared radiation (LED emission peak at 850 nm) of the gold nanorod coated surfaces, showing the potential of this novel antibiofilm technique. Most notably, the best individual result showed 97% biofilm elimination. This study demonstrates that nanoplasmonic generated heat offers a novel way of eliminating bacterial biofilms. In future applications, this method may be used to eliminate bacterial contamination during implant surgery.

1. Introduction

Once discovered, antibiotics revolutionised treatment of bacterial infections and were outstanding in reducing mortality and morbidity. However, there is an emerging antibiotic resistance in society today [1] and the need of finding alternative ways of fighting bacteria is pressing. One alternative that is currently explored is the use of various nanoparticles to fight bacterial infections. This may be achieved in combination with biological, chemical or physical agents such as localised surface plasmon resonance.

The biological activity of nanoparticles used for antibiofilm strategies depends on factors such as chemistry, size, charge and hydrophobicity. Nanoparticles can act as carriers of drugs, concentrate drugs on their surface or specifically attack biological targets [2]. Silver and gold nanoparticles are the most investigated nanoparticles used for antimicrobial activities. For example, gold nanoparticles can be modified with antibiotics [3–5], or be surface modified to increase bacterial elimination. By varying the surface charge and hydrophobicity of gold nanoparticles, bacterial elimination has been obtained while still retaining eukaryotic cell membrane integrity [6,7].

The use of gold nanoparticles for hyperthermic destruction is explored for their potential in hyperthermal cancer therapy [8–10], but so far it is largely unexplored in destruction of bacteria [11]. In 2006 Zharov et al. were the first to test hyperthermic destruction of bacteria using gold nanoparticles [12]. They selectively targeted *Staphylococcus aureus* by mixing planktonic bacteria with gold nanorods onto which antibodies had been immobilized, and then irradiated the solution (532 nm, 0.1–5 J/cm^2) to eliminate bacteria. A few additional studies on the effect of eradicating bacteria using heat generated from gold nanoparticle localised surface plasmon resonance, some in combination with other chemicals, have been performed [13–17]. These studies show promising antibacterial effects even on multiresistant bacteria. However, in these studies planktonic, free-swimming, bacteria were investigated. Implant-associated infections suffer from bacterial biofilm growth, *i.e.* bacteria attached to a surface and encapsulated in slime consisting of extracellular polymers. Few hyperthermic biofilm elimination studies have been performed. In one study, bacterial colonies on semisolid agar plates were irradiated by 808 nm wavelength for 5 min resulting in 60% colony elimination [18] and in a study using antibody-coated gold nanocrosses incubated within a preformed biofilm prior to 808 nm laser irradiation for 5 min, complete biofilm destruction was induced [19]. In the present study gold nanorods were pre-attached to the surface onto which biofilms were cultured.

When gold nanoparticles are irradiated, the photons can be either absorbed, reflected or scattered in relative amounts depending on size, shape and composition of the nanoparticles. Photothermal applications rely on absorption of visible light/infrared radiation and subsequent plasmon formation. When decaying, the plasmon energy is transferred to electrons of the conduction band, creating high-energetic

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or hot electrons. The hot electrons will equilibrate with the lattice and transfer their energy to the surroundings, in a picosecond time frame. This heat may be used to eliminate bacteria.

Biofilm bacteria are inherently resistant to antimicrobials and they can be up to 1000 times more susceptible to antibiotics compared to their corresponding planktonic counterparts [20]. This susceptibility may be due to factors such as restricted penetration of drug through the biofilm, antimicrobial destroying enzymes, efflux pumps, slow growth rate and presence of persister cells. Thus, infections caused by biofilms are difficult to treat, and they often involve surgical implant removal or debridement, where necrotic tissue and foreign material is removed.

This study is showing bacterial biofilm elimination utilising heat generated from localised surface plasmon resonance. Biofilms were grown onto gold nanorod covered glass surfaces and exposed to near IR irradiation. The bacterial biofilms were analysed using fluorescent optical microscopy for detection of viable and dead bacteria and scanning electron microscopy (SEM) to assess bacterial morphology.

Future applications may include minimization of antibiotic usage in treatment of implant-associated infections, see Fig. 1. Unlike systemic antibiotic treatment, which may struggle with difficulties to achieve high local antibiotic concentration in the biofilm, a gold nanorod covered implant will strike at the biofilm foundation upon irradiation.

2. Experimental

2.1. Gold nanorod surface assembly

Gold nanorods were immobilized onto cover glass surfaces (10 × 10 mm, no. 1) using the following procedure; glass microscopy slides were washed in basic piranha, H_2O, NH_4OH, H_2O_2 (30:9:9), rinsed in water and methanol, and dried in nitrogen gas. Then 200 μl (3-mercaptopropyl)-trimethoxysilane was mixed in 1 ml methanol and added in a beaker next to the glass surfaces within the enclosed volume of a desiccator for steam silanisation. Silanisation was allowed for 24 h and then the glass slides were immersed in a gold nanorod solution (10 × 45 nm, citrate capped, surface plasmon resonance peak 850 nm, Nanopartz) containing 1.5 M NaCl. Citrate stabilized gold nanorods were chosen instead of cetyltrimethylammonium bromide (CTAB)-stabilized rods, due to the lower cytotoxic potential of the former [21]

Gold nanorod-coated surfaces were visualized using SEM (Leo Ultra 55 FEG SEM operated at 4 kV, magnification 50,000–100,000×) after being gold sputtered.

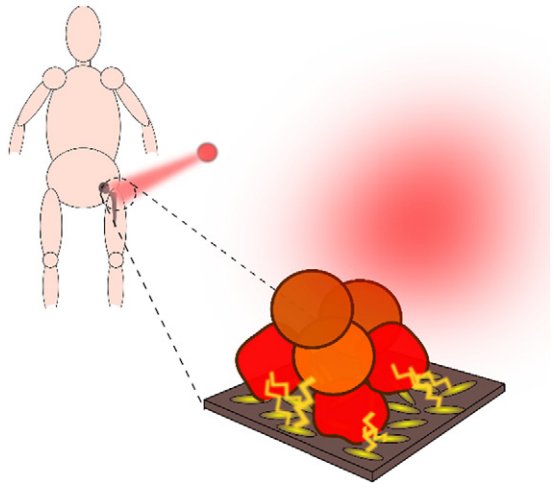

Fig. 1. Plasmon generated heat bacterial elimination. During implant surgery, heat generated from localised surface plasmon resonance in gold nanorods may be utilized for elimination of bacterial contamination.

2.2. Biofilm formation and plasmon generated heat elimination

To create biofilms, *Staphylococcus epidermidis* ATCC 35984 was cultured on brain heart infusion agar in 37 °C overnight after which one colony was cultured in tryptic soy broth until mid-logarithmic phase (OD 0.7). To minimize protein adhesion, bacteria were harvested (2500 rpm, 10 min) and suspended in physiological saline (0.85%) supplemented with 0.5 g/l glucose to a concentration of $2.1 \cdot 10^8$ CFU/ml.

The freshly prepared bacteria were cultured on the gold nanorod-coated glass surfaces for 3 h, 37 °C, and then rinsed twice in 1 ml saline (0.85%). This procedure created an early stage biofilm, essentially covering the surface by a monolayer with some thicker areas. Bare glass surfaces and non-irradiated gold coated surfaces were used as control. Biofilms were then exposed to overhead NIR irradiation (LED ML850L3, 900 mW, ~800–870 nm, $\lambda_{max} = 850$ nm, Thorlabs, see Fig. S1) for 2, 5, 10 or 20 min, with the LED output area covering that of the glass samples, 1 × 1 cm. At 850 nm a power of at least 200 mW was measured. Samples were then stained with *Bac*Light LIVE/DEAD (Thermo Fisher Scientific) according to the manufacturer and studied in fluorescence microscopy, 100×. Area covered by dead (red) or live (green) bacteria were evaluated using ImageJ [22].

2.3. SEM morphological studies

To investigate the heat killing effect of the bacteria in more detail SEM was performed on the NIR-irradiated biofilms. For the SEM sample preparation, biofilms were fixed in 4% paraformaldehyde in room temperature overnight, then washed three times in phosphate buffer saline and dried in an ethanol gradient (50, 60, 70, 80, 90 and 100% ethanol) for 10 min per step. A final drying was performed in 50:50 hexamethylsilazane (HMDS)/ethanol for 20 min and 100% HDMS until air dried. Samples were gold sputtered for 2 min and studied in an Ultra 55 FEG SEM (Leo) operated at 1–2 kV.

3. Results and discussion

The SEM micrographs of the gold nanorod covered surfaces revealed the nanorods to be evenly spread and totally covering the glass surface, as seen in Fig. 2. There is relatively low NIR absorption in tissue chromophores such as melanin, hemoglobin (decreasing absorption with increasing NIR range) and water (increasing absorption with increasing NIR range). At 850 nm, the radiation may penetrate up to 10 mm through tissue, depending on the tissue optical properties [23]. Due to this therapeutic window of relatively large penetration depth NIR absorbers are often used in biomedical applications. Since rod-shaped nanoparticles exhibit excellent NIR absorption cross section [24] and an efficient NIR photothermal heat conversion [25] rods were chosen for this study rather than gold nanospheres. Furthermore, as seen in Fig. 2, only few and rather small aggregates were observed. This is of importance since the presence of aggregated particles resulted in a red shift of the plasmon oscillation frequency.

A majority of orthopaedic implant-related infections are caused by the skin bacteria *S. epidermidis* and *S. aureus* [26]. The risk of orthopaedic device-related infections is 1–2%, and as the population is aging and is in need of more implants, the absolute numbers are increasing [27]. *S. epidermidis* was used in this study due to its abundance in implant related infections.

Biofilms irradiated with 850 nm for 2–20 min died (red colour), without a dose-response relationship (Fig. 3). This result indicates that the bacterial heat elimination was rapid. The overall temperature change during NIR irradiation was 2–3 °C in solution (data not shown), which is a moderate increase, although the surface temperature at the gold nanorods is considerably higher. For an estimate of heat added to each bacterium, see scheme S1. Earlier studies on bacterial suspensions with gold rods have reached temperatures of 48–100 °C