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A versatile plasmonic thermogel for disinfection of antimicrobial resistant bacteria

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ABSTRACT

The increasing occurrence of antimicrobial resistance among bacteria is a global problem that requires the development of alternative techniques to eradicate these superbugs. Herein, we used a combination of thermosensitive biocompatible polymer and gold nanorods to specifically deliver, preserve and confine heat to the area of interest. Our data demonstrates that this technique can be used to kill both Gram positive and Gram negative antimicrobial resistant bacteria in vitro. Our approach significantly reduces the antimicrobial resistant bacteria load in experimentally infected wounds by 98% without harming the surrounding tissues. More importantly, this polymer-nanocomposite can be prepared easily and applied to the wounds, can generate heat using a hand-held laser device, is safe for the operator, and does not have any adverse effects on the wound tissue and healing process.

1. Introduction

Antimicrobial resistance (AMR) is a global problem that represents a major threat to human and animal health. Multidrug resistant superbugs are often hospital or community acquired sources of infection of the lungs, urinary tract, gastrointestinal tract, skin and soft tissue. It is estimated that AMR causes 2 million illnesses and 23 thousand deaths annually in the United States [1]. The clinical management of skin and soft tissue infections alone represents an increasing economic burden, estimated to surpass $4.8 billion/year in health care costs in the United States [2]. Complications associated with these infections are common, occurring in up to 25% of cases, and include bacteremia, endocarditis, and sepsis [3]. Taken together, there is an urgent need to develop effective alternative antimicrobial therapies for AMR infections.

At the correct temperature, heat can be used to destroy bacteria through disruption of the cell membrane, fatty acid melting, and protein denaturation [4]. However, one challenge in using heat to treat wounds is to locally and selectively concentrate and deliver the heat to the infected area without damaging the surrounding tissue. Inorganic nanoparticles (NP) offer fine control and homogenous distribution of heat over conventional heating probes, such as ultrasound, microwaves, and radiofrequency [5]. There are several types of heat-producing NPs including gold nanomaterials, magnetic NPs, carbon-based nanostructures, and porphysomes [6–9]. Typically, these particles are excited by light or magnetic fields and the resulting energy is converted into heat through atomic scale electronic and orientation transitions [10]. When NPs

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are excited by light, the process is known as photothermal therapy (PTT). The predominant applications of PTT have been: 1) localized ablation of diseased tissues; 2) as diagnostic probes; and 3) to manipulate and control the release of drugs [6,11,12]. Previous studies have demonstrated that PTT can be used to selectively kill AMR bacteria in vitro [13–16]. However, to apply this technique to the treatment of infected wounds in-vivo, an appropriate NP dispersion medium is required. Since the NP dispersion medium used to perform PTT experiments is water, a major limitation is evaporation, resulting in poor heat delivery and preservation.

In this study, we devised a PTT technique to specifically deliver, preserve, and confine heat to a defined area. We used a combination of a thermosensitive biocompatible polymer, n-vinyl-polycaprolactam (PVCL), as a dispersion medium, and gold nanorods (NRs) excited by a low intensity laser. PVCL is a biocompatible polymer that exhibits phase transition from sol to gel upon heating. Once in gel form this polymer can confine and preserve heat generated from NPs dispersed within it to a defined area of interest. NRs were selected because of ease of synthesis and functionality, as they possess high absorption cross-section and are able to absorb light at between 700 and 1000 nm (a region of the electromagnetic spectrum with low biological scattering and absorption). We hypothesized that this PTT method could have potent bactericidal effect when applied to experimentally infected wounds created using a standard biopsy punch in rats.

2. Materials and methods

2.1. Gold nanorods synthesis, functionalization and characterization

Gold nanorods were synthesized using a seed-mediated method described by Nikoobakht and El-Sayed and Gou and Murphy [17,18] with modifications. Briefly, the seed solution was prepared by adding 1.2 ml of 0.01 M sodium borohydride (Sigma-Aldrich, St. Louis, MO, USA) to 20 ml scintillation vial that contains 500 μL of 0.01 M gold chloride (Sigma-Aldrich, St. Louis, MO, USA) and 19.5 ml 0.1 M cetyl trimethylamonnium bromide (CTAB) (Sigma-Aldrich, St. Louis, MO, USA) under vigorous stirring. In a clean bottle, 49.5 ml of 0.01 M gold chloride was added to 950 ml of 0.1 M CTAB. To that, 5 ml of 0.01 M $AgNO_3$ (Sigma-Aldrich, St. Louis, MO, USA) and 7 ml of 0.1 M ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) were added respectively under stirring. Finally, 20 ml of the above prepared seed solution was added and the entire solution was left overnight with constant stirring. The obtained gold nanorod solution was then purified by centrifugation twice at 17000 g for 20 min and re-dispersed in deionized water to remove excess CTAB. The CTAB-NRs were further characterized using UV–Vis spectrophotometer (Shimadzu, UV-1601PC) and zeta potential. For surface functionalization, 5 ml of the above concentrated CTAB-NRs solution was added to clean bottle containing a solution of m-PEG-SH (MW: 5000 Daltons, Laysan Bio) so that the final concentration of PEG was 0.5 mg/ml. The mixture was incubated at room temperature for 4 h. PEG coated NRs (PEG-NRs) were further purified twice by centrifugation at 17000 g for 20 min to remove excess PEG and CTAB. PEG-NRs were further characterized using UV–Vis spectrophotometer, zeta potential (Nano-Zs) and Transmission Electron microscopy (TEM) (Hitachi 7000).

2.2. Preparation and characterization of gold nanorods thermogel nanosol

21.5% w/v of Poly N-vinylcaprolactam (PVCL) (MW: ~176000 Daltons, PolySciTech Akina, Inc) solution prepared by dissolving equivalent amount of polymer in 1x phosphate buffered saline buffer (PBS) (2.7 mM potassium chloride, 173 mM sodium chloride and 1.76 mM potassium phosphate) at 4 °C overnight. This was followed by addition of the above prepared PEG-NRs to the polymer solution so that the final concentration of NRs and polymer was 5 nM and 20%, respectively. The thermogel nanosol solution was further characterized by UV–Vis spectrophotometric measurement and TEM.

2.3. Development of temperature profiles

The rise in temperature of 3 different solutions of the NRs was measured using an infrared (IR)-Camera (ICI 7320) for 20 min. First, 1.5 μL of 400 nM PEG-NRs were dispersed in 56.5 μL of 21.5% w/v PVCL, 1xPBS and 21.5% w/v PEG solutions respectively. 2 μL of 1xPBS was then added to all solutions so that the final volume was 60 μL. Then 50 μL of each solution was applied on the surface of 35 mm Petri dish followed by laser irradiation using 785 nm continuous wave diode laser as light source (0.65 W/cm^2) for 20 min. The rise in temperature (ΔT_f) was calculated as the difference between film temperature at time point t (Tf(t)) and initial film temperature (T_0 ~ 18.5 ± 1.5) on the surface. Blank solutions of PVCL, PBS and PEG were used as non-heating controls.

2.4. Heat dissipation and water evaporation measurements

1.5 μL of 400 nM PEG-NRs was dispersed in 56.5 μL of 21.5% w/v PVCL and 1xPBS. 2 μL of 1xPBS was then added to all solutions so that the final volume was 60 μL. Then 50 μL of each solution was applied on the surface of 35 mm Petri dish followed by laser irradiation at 0.65 W/cm^2 for NRs-PVCL and 1.5 W/cm^2 for NRs-PBS. After 10 min of exposure, the laser was turned off and the drop of temperature from the two solutions was measured as described above. For water evaporation measurement, 50 μL of each solution was weighed before and after laser exposure using analytical scale (METTLER TOLEDO, AL54, readability 0.0010), and the amount of evaporated water was calculated as:

$$\%\ of\ water\ evaporation = (W_b - W_a/W_b) \times 100$$

where W_b is the weight (g) of sample before laser exposure and W_a is the weight (g) of sample after laser exposure.

2.5. Antimicrobial effect of gold nanorods thermogel in vitro

Three antibiotic resistant bacteria have been used in this study. Ampicillin-resistant *Escherichia coli* NEB 10 beta was obtained from Dr. Aaron Wheeler's lab (Chemistry Department, University of Toronto, Canada). Vancomycin, ampicillin, and gentamicin-resistant *Acinetobacter baumannii* and vancomycin resistance *Enterococcus faecalis* (VRE) ATCC51299 were obtained from Dr. Justin Nodwell's lab (Biochemistry Department, University of Toronto, Canada). Overnight cultures of *E. coli*, *A. baumannii* and *E. faecalis* bacteria were prepared by streaking small amount of the frozen bacterial stock on Luria agar (LA) plates and incubated at 37 °C. Then a single colony of each bacteria was inoculated in Luria broth (LB) medium and incubated at 37 °C while shaken (200 rpm). Fresh cultures were prepared in LB medium by adding 30 μL of overnight culture to 2.97 ml of LB media and incubating for another 3–4 h at 37 °C while shaken or until the OD_{600} of the culture medium reached approximately 0.3–0.4 (logarithmic growth phase). The bacteria pellets were then collected by centrifugation at 3000 *g* for 10 min and then washed three times with sterile PBS. The pellets were then re-suspended in an appropriate amount of sterile PBS buffer for further use. To test the antimicrobial effect of NRs-PVCL, 2 μL of bacterial cultures was