Silver Nanoparticle-Embedded Polymersome Nanocarriers for the Treatment of Antibiotic-Resistant Infections

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Statement of Purpose: The rapid rise and prevalence of bacterial genetic resistance to antibiotics is having a drastic impact on human health today. This study explored the development and optimization of a polymersome nanocarrier formed from a biodegradable diblock copolymer to overcome such antibiotic resistance. Here, the polymersomes (Ps) were designed with silver nanoparticles embedded in the particle's hydrophobic membrane bilayer, and an ampicillin solution encapsulated in the particle's aqueous core in order to provide a dual-mechanism, concentrated, and less cytotoxic localized treatment. These silver nanoparticle-embedded polymersomes (AgPs) were subsequently tested for bactericidal function against *Escherichia coli* transformed with a gene for ampicillin resistance (*bla*). Results showed for the first time that the AgPs killed the antibiotic-resistant bacteria, whereas free antibiotic, encapsulated antibiotic without the addition of the silver nanoparticles, and AgPs without the addition of ampicillin did not. In this manner, this study introduces a novel nanomaterial that can effectively treat problematic, antibiotic-resistant infections in an improved capacity which should be further examined for a wide range of medical applications.

Methods: A diblock copolymer of methoxypoly(ethelyne glycol)$_{5,000}$ and poly(*D*)-(*L*)-lactic acid$_{50,000}$ was utilized for the polymersome synthesis using a modified stirred-injection technique. Briefly, 1mL (0.25% w/v) of 5nm hydrophobically-functionalized silver nanoparticles were suspended in a solution of 10mg mPEG-PDLLA copolymer dissolved in THF. This was then quickly injected through a syringe atomizer into a stirring solution of 10mL ampicillin sodium salt in phosphate buffered saline (PBS). The resulting suspension was then allowed to dialyze against pure PBS to remove the organic solvent and any non-encapsulated drug. Formation of the AgPs was confirmed using TEM, and were further characterized for size distribution, zeta potential, and loading efficiency. After transformation with the *bla* gene, an *E. coli* solution was streaked for inoculation on tryptic soy agar + ampicillin (100µg/mL). 100µL of a 10^6 CFU/mL suspension of the bacteria was then combined with varying AgP treatment concentrations or controls in a 96 well plate, which was allowed to incubate at 37ºC inside a spectrophotometer. Growth measurements (OD_{600}) were taken every 2 minutes for 24 hours to establish the speed of proliferation and shape of the bacterial growth curve.

Results: Most importantly, results showed that the ampicillin-loaded AgPs displayed significant antibacterial action against the ampicillin-resistant *E. coli*. At low treatment concentrations, the addition of the AgPs resulted in a bacteriostatic effect, as seen from the delayed time taken for the bacteria to proliferate to exponential phase. This is extremely important, as it allows the body's immune system more time to fight the infection. Higher treatment concentrations resulted in a bactericidal effect, with the bacteria failing to proliferate within 48 hours (Figure 1A). The control treatments (free ampicillin, ampicillin loaded Ps, and AgPs without ampicillin) displayed no bacteriostatic or bactericidal effects even at the maximum concentrations tested (Figure 1B). This indicates restored efficacy of the antibiotic towards the resistant bacteria. Interaction between the *E. coli* and the AgPs was visually examined using TEM (Figure 1C). The "silver nanoparticle first" orientation of the polymersomes suggests the hydrophobic interaction between the nanoparticles and the bacterial outer lipopolysaccharide membrane helps the particles to penetrate.

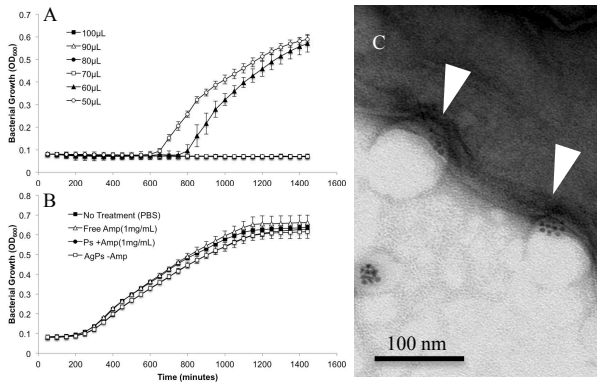

Figure 1. The proliferation of ampicillin-resistant *E. coli* was measured over 24 hours in the presence of differing concentrations of (A) AgPs loaded with 160μg/mL ampicillin and (B) control tests of AgPs synthesized from pure PBS, Ps synthesized from a 1mg/mL ampicillin solution, free ampicillin at a concentration of 1mg/mL and no treatment (pure PBS). (C) Micrographs reveal that the AgPs orient "silver nanoparticle first" in order to interact with the bacterial outer membrane (white arrows).

Conclusions: This work has many important implications. A dual-mechanism, concentrated treatment in one particle able to overcome antibiotic resistance would be extremely beneficial in the clinic. Importantly, this treatment does not rely on the discovery of a new pharmaceutical compound, which would be extremely expensive to commercialize. Instead, the efficacy of an existing antibiotic is restored by exploiting a synergistic combination with hydrophobic silver nanoparticles and nano-encapsulation. The absence of effect from the AgPs -Amp is a promising sign for limiting the toxicity from the silver nanoparticles themselves. Finally, the designed particle is also easily customizable, allowing for the potential of personalized infection treatments. The authors envision a scenario where a combination of different antibiotics and/or antimicrobial compounds could be loaded into the AgPs designed to optimally treat a patient's specific infection.

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