Rapid elimination of *Staphylococcus aureus* achieved by sonodynamic Au@Cu_2O hybrid nano-cubes

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INTRODUCTION: Although efforts have been devoted to develop new antibacterial agents and techniques, the challenge of bacterial infection remains unresolved and is even increasing. In recent years, the exogenous antibacterial approach has become a promising antibiotic-free antibacterial strategy with high maneuverability and an ability to rapidly eliminate bacteria. Sonodynamic therapy (SDT) driven by ultrasound (US) has demonstrated effectiveness in terms of penetration and it can help to clinically address the problem of deep tissue bacterial infection. In recent years, a variety of sonosensitizers, which were originally designed for photodynamic therapy, have been adopted for SDT. Yet, their unstable chemical stability and ineffective electron–hole separation are not favorable for clinical applications. To improve the low electron migration rate and SDT activity, we propose to couple an enhancer with Cu_2O nano-cubes, namely nano-gold particles (Au) that can rapidly produce reactive oxygen species to eliminate the bacteria, while stimulating by US (**Figure 1(a)**).

METHODS: A hydrothermal method was employed to prepare Cu_2O. Briefly, 500 mL of $CuCl_2$ solution (0.01 M) was placed in a water bath at 55 °C, and then 50 mL of NaOH solution (2 M) was added dropwise to the $CuCl_2$ solution under vigorous stirring. Afterwards, 50 mL of ascorbic acid (0.6 M) was added to the mixed solution under continuous stirring for 5 h. The Cu_2O nano-cubes were collected via centrifugation (7000 rpm). The Au@Cu2O hybrid nano-cubes were prepared via the photo-reduction of $HAuCl_4$ on Cu_2O. The red blood cell membranes were then coated onto Au@Cu_2O. The surface morphologies of different samples were examined using a field emission scanning electron microscope (FE-SEM) and transmission electron microscope (TEM). The phase structure and chemical composition of different samples were characterized using X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). The reactive oxygen species (ROS) generation, antibacterial capability and biocompatibility of RBC coated Au@Cu_2O nano-cubes were also characterized by ROS assay kit, *in vitro* bacterial culture with *Staphylococcus aureus* (*S. aureus*) and MTT assay, respectively.

RESULTS SECTION: When US stimulation was applied, the electrons from Cu_2O could be excited at the junction and transferred to Au. Since the formed Schottky barrier could block the backflow of US- excited electrons, a prolonged electron–hole separation can be successfully established. Additionally, because of the boosted sonocatalytic activity, the Au@Cu_2O hybrid nano-cubes could produce a large amount of ROS, which are subject to US stimulation. Furthermore, we found that the sonocatalytic activity of the Au@Cu_2O hybrid nano-cubes could be reinforced by increasing the amount of Au, enabling 99.67% of *S. aureus* to be killed by US stimulation for 15 minutes (**Figure 1(b-d)**). The cytocompatibility of Au@Cu_2O hybrid nano-cubes was improved by a RBC coating over the surface, and the membrane did not sacrifice its superior antibacterial properties.

DISCUSSION: In this study, a series of effective sonosensitizers for rapid bacterial elimination that rely on the construction of a Schottky junction between Au nanoparticles and Cu2O nano-cubes have been proposed. The Au@Cu2O hybrid nano-cubes with higher loading amounts of Au exhibited better sonocatalytic properties. A Schottky contact between Au and Cu2O facilitated superior electron–hole separation, which significantly improved ROS generation and antibacterial efficiency against *S. aureus* under US stimulation. With a coating of an RBC membrane, the cytotoxicity of Au@Cu2O hybrid nano-cubes was substantially reduced without compromising their original antibacterial properties. We believe that this concept (i.e., construction of a Schottky contact between the metal and semiconductor counterparts to eliminate bacteria) can be extended to other types of metals and semi- conductors. We hope our work paves a way for the development of better sonosensitizers for a variety of bacterial treatments.

SIGNIFICANCE/CLINICAL RELEVANCE: As compared with other antibiotic treatment, our RBC coated Au@Cu2O nano-cubes approach is relatively versatile to disintegrate the membrane structure and proteins of *S. aureus* effectively.

IMAGES AND TABLES:

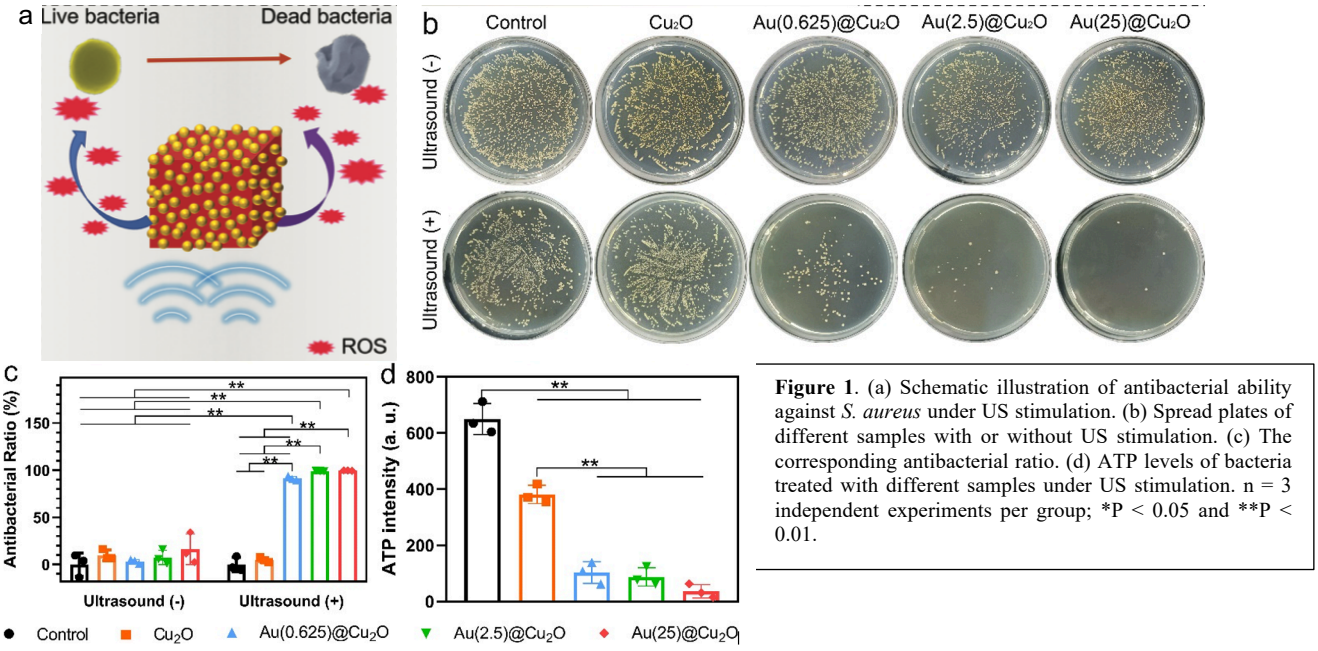

Figure 1. (a) Schematic illustration of antibacterial ability against *S. aureus* under US stimulation. (b) Spread plates of different samples with or without US stimulation. (c) The corresponding antibacterial ratio. (d) ATP levels of bacteria treated with different samples under US stimulation. n = 3 independent experiments per group; *P < 0.05 and **P < 0.01.