Bacteria eat nanoprobes for aggregation-enhanced imaging and therapy

Introduction

The gut microbiota plays a fundamental role in both health and disease, with dysbiosis of the microbiome being linked to conditions such as obesity, asthma, inflammation, multiple sclerosis, Parkinson's disease, and cancer. An extremely active area of research is to use engineered bacteria to diagnose or treat such illnesses. However, existing approaches for *in vivo* bacterial imaging use reporter genes, fluorescent dyes, or nanoprobes and the optical signals are often hard to visualize due to tissue attenuation. Improved methods to image bacterial therapies and diseases are therefore needed.

This study describes a new method for imaging bacteria which relies on laser-induced aggregation of a probe such that the signal strength builds intracellularly and improves both visualization and sensitivity. Notably, the LAMBDA™ UV/Vis spectrophotometer was used to monitor the absorption profile of the nanoprobes and the IVIS® Lumina III optical system was used to track probe biodistribution using *in vivo* fluorescence imaging.

Methods and results

First, the researchers synthesized nanoprobes composed of four modules: gold nanoparticles, glucose polymer, diazirine, and chlorin e6 (Ce6). Once developed, the nanoprobes were internalized into bacterial cells through the bacteria-specific ATP-binding cassette (ABC) transporter pathway.

The researchers report that upon 405-nm laser irradiation, the photoactive crosslinkers of diazirine facilitated the aggregation of internalized nanoprobes in the bacterial cells. Compared with non-aggregated counterparts, the aggregates displayed a ~15.2-fold enhancement in photoacoustic signal and a ~3.0-fold enhancement in antibacterial rate.

Publication authors

Yunmin Yang and Colleagues

Suzhou key laboratory of Nanotechnology and Biomedicine, Soochow University, Suzhou, China

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Publication highlights:

This study uses a new imaging technique whereby bacteria consume a gold nanoprobe which aggregates intracellularly upon irradiation. Aggregation enhances signal intensity enabling visualization of unprecedented low quantities of bacteria. Multimodal *in vivo* fluorescence was used to locate bacterial colonies.

To further explore the effectiveness of the strategy on *in vivo* imaging, the team used proof-of-concept models of bacteria in tumor xenografts and the gastrointestinal tract. For the tumor xenograft model, mice were injected in the right back region with 5 x 10^6 4T1 cancer cells. They were then injected with 50 µL of *S. aureus* (SA) or *P. aeruginosa* (PA) into just the left thigh region, or into both the left thigh and the right tumor regions. For the gastrointestinal tract model, agarose gel containing *E. coli* (EC) was injected into the gut lumen. For both models, 100 µL of nanoprobe was

injected intravenously and the probe's *in vivo* biodistribution was monitored using *in vivo* fluorescence imaging of label, Ce6, and Revvity's IVIS Lumina III instrument.

Results

The researchers report successful detection of diverse bacteria at cell concentrations of ~1.0x10^7 CFU residing in the tumors and gut. In the tumor model, the strategy enabled the discrimination of bacteria from tumor, and signals were simultaneously detected at the infected and tumor sites containing bacteria. Consistently, the detected signals from both the infected and tumor sites containing bacteria treated with 405-nm laser irradiation were much stronger than counterparts without 405-nm laser irradiation. In the gastrointestinal model, distinct signals were measured in the gut containing EC. Furthermore, the intensity from the gut containing bacteria treated with irradiation was ~5.5-fold higher than the counterparts without laser irradiation.

Conclusion

The described technique, which utilizes *in vivo* fluorescence imaging, enables the visualization of diverse microbial populations and holds promise for the study of the microbial ecosystem in tissues and the development of new diagnostic and therapeutic agents.

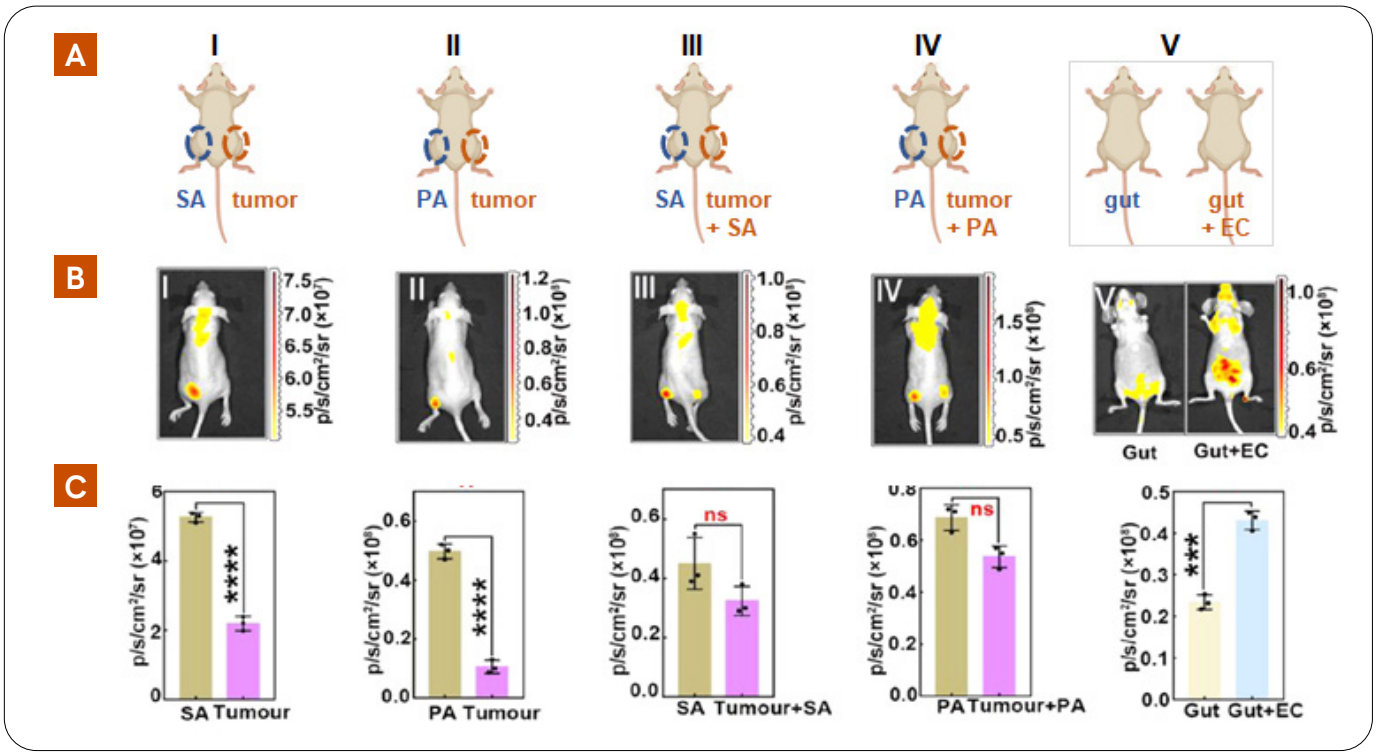

Fig. 1 *In vivo* imaging studies. A) Schematic illustrating different animal cohorts. Mice were either inoculated subcutaneously with 1 x 10^7 CFU S. aureus (SA; gram-positive bacteria), 1 x 10^7 CFU P. aeruginosa (PA; gram-negative bacteria), or 5 x 10^6 4T1 cancer cells. In a separate cohort, agarose gel containing E. coli (EC) was injected into the gut lumen. B) The probe's *in vivo* biodistribution was monitored using *in vivo* fluorescence imaging of label, Ce6, and Revvity's IVIS Lumina III instrument. C) Fluorescence quantification shows the radiant efficiency is significantly enhanced in both Gram-positive and Gram-negative bacterial colonies.

Revvity, Inc
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com