IMAGING

Gas vesicles enable ultrasound imaging

Acoustic reporter genes that produce gas vesicles offer a way to image and locate microorganisms in mammalian hosts.

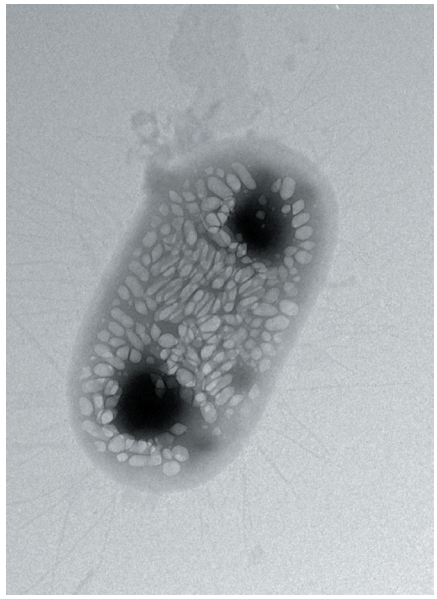
The location of microorganisms within a host organism is key to understanding many physiological and pathophysiological processes, but this has been difficult to image because of light scattering in deep tissue. Ever since Mikhail Shapiro from the California Institute of Technology first read about bacteria that produce gas-filled vesicles to regulate buoyancy, his group has been working on adapting these gas vesicles for noninvasive ultrasound imaging of so-far inaccessible tissues.

Gas vesicles consist of a shell of proteins that encapsulate a hollow interior filled with gas. In 2014, Shapiro's group published their discovery that purified gas vesicles scatter sound waves and thereby produce ultrasound contrast. Gas vesicles "are really beautiful and fascinating structures that nature evolved," says Shapiro. However, to transfer them into bacteria that are relevant not only for basic research but also for clinical studies proved to be even more challenging than anticipated. "Unlike fluorescent proteins, that are just encoded by single genes, gas vesicles are encoded by a cluster of genes that have to work together to form the structure," explains Shapiro. Simply transferring the genes from one bacterium to *Escherichia coli* did not lead to the expression of gas vesicles that could be detected by ultrasound. Rather, a hybrid-engineered gene cluster of gas vesicle genes from *Bacillus megaterium* and the cyanobacterium *Anabaena flos-aquae* was necessary to produce gas vesicles of sufficient size and quantity to be detectable by ultrasound imaging.

When gas vesicles are exposed to acoustic pulses with amplitudes above a critical pressure, they will collapse. "They essentially break irreversibly and the gas that was inside of them gets dissolved within milliseconds," says Shapiro. "And so their ultrasound signal disappears when this happens." His group took advantage of this property to increase the sensitivity of their approach. By first taking an image of the bacteria population with low ultrasound pressure and then subjecting the population to a higher sound pressure to collapse the gas vesicles, the researchers erased the ultrasound contrast. Subsequently, the high-pressure image was subtracted from the low-pressure image to obtain a background-free image that helps researchers detect the gas vesicles.

The researchers also used the collapsing pressure to distinguish between two populations of bacteria that express different types of gas vesicles, which collapse at different sound pressures. They first took an image of both populations and then applied an ultrasound pressure "that is above the critical collapse pressure of one of them but below the other," explains Shapiro. With this approach, they selectively eliminated the ultrasound signal from that particular population. After taking another image, they applied higher ultrasound pressure to also erase the signal from the second population. The researchers found that there was some overlap of the critical sound pressure between the gas vesicle populations, so they mathematically recovered the concentration of each of the bacteria types. "This is analogous to real optical spectral unmixing where you have two fluorophores that emit at overlapping spectra," explains Shapiro.

To illustrate the *in vivo* potential of this method, the researchers applied it to locate microorganisms in the gastrointestinal tract of mice. They transformed a probiotic *E. coli* Nissle strain to express gas vesicles. After introducing the bacteria into the colons of anaesthetized mice, the researchers used ultrasound imaging to see whether the bacteria were located in the center or the periphery of the colon lumen. This goes far beyond the resolution capabilities of other current methods such as bioluminescence imaging and illustrates the high resolution that is possible with ultrasound imaging of gas vesicles.



TEM images of whole *E. coli* cells expressing gas vesicles. Image adapted with permission from Bourdeau *et al.* (Springer Nature).

Although Shapiro notes that the technology is still at a very early stage, he hopes that it "will be used for *in vivo* cell imaging the way GFP is now used *in vitro*." Apart from the potential of gas vesicles in basic research, clinical diagnostics and therapeutic applications may also benefit from being able to locate microorganisms precisely within the body and employ them for efficient and targeted drug delivery.

Christian Schnell

RESEARCH PAPERS

Bourdeau, R.W. *et al.* Acoustic reporter genes for noninvasive imaging of microorganisms in mammalian hosts. *Nature* **553**, 86–90 (2018).