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Safety reflector technology from footwear getting new life in detecting bioterror threats

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Tiny versions of the reflectors on sneakers and bicycle fenders that help ensure the safety of runners and bikers at night are moving toward another role in detecting bioterrorism threats and diagnosing everyday infectious diseases, scientists said today.

Their report on progress in using these innovative "retroreflectors"—the same technology that increases the night-time visibility of traffic signs—was among almost 12,000 on the agenda of the 245th National Meeting & Exposition of the American Chemical Society.

"Our goal is the development of an ultrasensitive, all-in-one device that can quickly tell first-responders exactly which disease-causing microbe has been used in a bioterrorism attack," said Richard Willson, Ph.D., who leads the research. "In the most likely kind of attack, large numbers of people would start getting sick with symptoms that could be from multiple infectious agents. But which one? The availability of an instrument capable of detecting several agents simultaneously would greatly enhance our response to a possible bioterror attack or the emergence of a disease not often seen here."

Willson's team is developing another version of the technology intended for use in doctors' offices and clinics for rapid, on-site diagnosis of common infectious diseases before patients leave. Eliminating the need to wait for test results from an outside laboratory could allow patients to get the right treatment sooner and recover sooner, Willson noted.

One of those tests focuses on detecting norovirus, the dreaded "cruise ship virus," or "winter vomiting virus," which strikes more than 20 million people annually in the United States alone. Norovirus was in the headlines last December when it struck 220 people on the Queen Mary II.

Balakrishnan Raja, the member of Willson's team at the University of Houston (UH) who presented the report, pointed out that retroreflectors may be the most visually detectable devices ever made by humanity. They work on the project with colleagues at UH, the University of Texas Medical Branch in Galveston and the Sandia National Laboratories branch in Livermore, Calif. The devices reflect light directly back to its source in a way that produces extreme brightness. One version of retroreflection effect occurs when someone shines a flashlight in a mirror. The reflection is so bright that looking at it hurts.

Although most people have never heard the term "retroreflector," these devices are not new, Raja pointed out. The Apollo 11 astronauts, for instance, left a laser-ranging retroreflector on the moon during the first lunar landing mission in 1969. Scientists still use the device to study the moon's orbit. And they are ubiquitous fixtures in road signs, traffic lane markers and elsewhere in everyday life.

Willson's collaborator Paul Ruchhoeft of UH has developed a way of making retroreflectors so small that more than 200 would fit inside the period at the end of this sentence. The retroreflectors then become part of a lab-on-a-chip, or a microfluidic device, with minute channels for processing "microliter"-scale amounts of blood or other fluids. A microliter is one-millionth of a liter (a liter is about one quart). A drop of water contains about 50 microliters.

When a sample of fluid that doesn't contain disease-causing viruses or bacteria flows through those channels to the retroreflectors, they shine brightly. A sample containing bacteria, however, makes portions of the reflectors go dark, signaling a positive test result. Raja explained that the change from bright to dark is one of several advantages of the retroreflector technology, compared to existing ways of detecting disease-causing microbes. It can be detected with simple optical devices, rather than expensive, complex optics. The retroreflector technology also avoids the need to specially prepare samples for analysis and is faster.

"Right now, we have seven channels in our device," Raja said. "So we can test for seven different infections at once, but we could make more channels. That's one of our long-term goals—to multiplex the device and detect many pathogens at once."

They have demonstrated clinically useful sensitivity on samples containing *Rickettsia conorii*, a bioterrorism threat that causes Mediterranean spotted fever, and others are on the agenda. A new version of the technology involves retroreflector cubes that can be suspended in samples of fluid. Willson's team initially will use it on norovirus with the goal of developing a device that can raise a red flag on norovirus viral contamination and prevent the disease's wildfire-like spread.

More information: Abstract

Ultrasensitive and rapid pathogen detection generally relies on nucleic acid extraction followed by amplification, or labeling with dyes, enzymes or fluors, which require elaborate instrumentation. This work introduces embedded, microfabricated linear retroreflectors as bio-sensing surfaces, using micron-sized magnetic particles as light-blocking labels in a highly sensitive diagnostic immunoassay. Retroreflectors return light directly to its source and are easily detectable using inexpensive optics. The pathogen is immunocaptured by a sensing surface following immunomagnetic separation and concentration from a

complex sample. An automated difference imaging algorithm that detects single 3.0 μm magnetic particles without optical calibration is used to quantify the number of labels bound to each from each 1 sq. mm. array of retroreflectors. An assay for the detection of Rickettsia conorii is implemented in a microfluidic format with fluidic force discrimination to enhance reproducibility and specificity, with a current limit of detection of less than 4000 bacteria per mL.

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