Nanoparticles offer hope for TB detection

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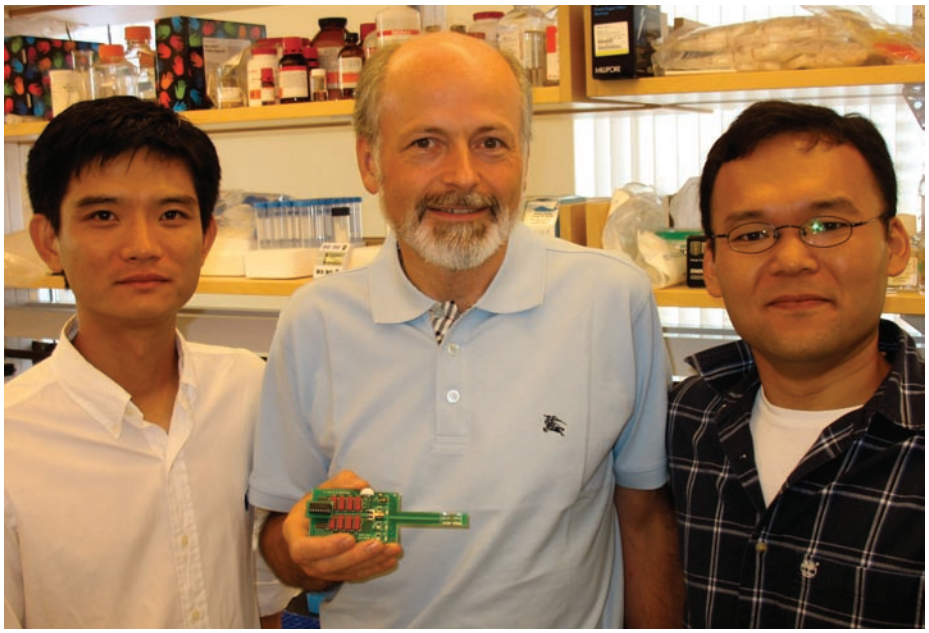
Combining magnetic nanoparticles, microfluidics and nuclear magnetic resonance could transform the way tuberculosis and other diseases are diagnosed.

Tuberculosis (TB) is an infectious disease that kills millions of people each year. The disease is caused by the bacteria *Mycobacterium tuberculosis*, which mainly attacks the lungs, and is spread when an infected person coughs, sneezes or spits. Symptoms of the disease include chronic coughs with blood in the sputum (phlegm), fever, night sweats and weight loss.

A number of methods are available for detecting TB (ref. 1) but they all suffer from drawbacks. In smear microscopy — a technique developed in the 1870s — sputum samples are smeared on a microscope slide and stained with a dye that binds to the bacteria that cause the disease. A lab technician then counts the stained bacteria and uses a grading system to estimate how serious the illness is. Although smear microscopy is rapid and relatively inexpensive, it suffers from low sensitivity, and the sensitivity is decreased further in cases with HIV co-infection, which is a major disadvantage because TB is the leading killer for people infected with HIV (ref. 2).

A better way to detect TB is to culture the sample in the lab to see if the relevant bacterial colonies are formed; this method can also assess the response of the mycobacteria to antibiotics that are used to treat TB. Although this approach is more sensitive than smear microscopy, it can take between one and three weeks. Moreover, facilities for culturing bacteria are not readily available at the point-of-care level in most developing countries. There is, therefore, a need for new diagnostic tests that are sensitive, fast, inexpensive and capable of working with unprocessed biological samples (such as blood, sputum or urine).

With this aim in mind, Ralph Weissleder, Hakho Lee and Tae-Jong Yoon[3] of Harvard Medical School and Massachusetts General Hospital (MGH), both in Boston, have developed an easy-to-use and portable chip-based diagnostic system that can detect as few as 20 bacteria per millilitre of unprocessed sputum sample in 30 minutes. Conventional culture-based tests, by comparison, can detect about 10–100 bacteria per millilitre depending on the specimen-processing method and culture medium used, and this takes more than two weeks (ref. 1).



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Helping to fight TB — Tae-Jong Yoon (left), Ralph Weissleder (centre) and Hakho Lee (right) with their new diagnostic device.

Building on previous work by Weissleder, Lee and others[4], the prototype has three key components: iron-based magnetic nanoparticles tagged with antibodies that will bind to the target bacteria (tuberculosis in this case); a microfluidic system to deliver the bacteria and buffer solutions; and a nuclear magnetic resonance (NMR) unit containing a microcoil and a membrane filter to capture, concentrate and detect the bacteria. The new features in the latest prototype are the magnetic nanoparticles and the membrane filter system.

The specificity of the system depends on the antibodies, and the Harvard/MGH team achieved a signal-to-noise ratio of approximately 200:1. The high detection sensitivity is possible because the magnetic nanoparticles have very high magnetic moments and high values of transverse relaxivity. "Initially, we had a problem with the iron nanoparticles oxidizing very quickly, which considerably reduced their magnetic moments," says Weissleder. "This problem was solved by putting a ferrite shell over the iron core."

Many pathogens are infectious at very low counts (~1–2 bacteria per millilitre or lower in blood) but concentrating the sample with the membrane filter can markedly improve the detection sensitivity. "The idea of adding the membrane filter came while preparing TB samples for electron microscopy, where

samples are loaded in a syringe and pushed through a membrane filter to capture the bacteria," says Weissleder. By applying the same concept to NMR detection, the team was able to concentrate and detect a small number of bacteria from a large sample volume and also wash the bacterial samples to remove excess nanoparticles directly on the chip.

As well as sputum samples the device can also measure blood and tissue samples, and because the readout is an electronic NMR signal, the assay is less prone to human errors and can be performed with minimal training. The NMR component can be reused and costs less than $200, and the microfluidic chip, which is disposable, costs less than $1. The Harvard/MGH team, in collaboration with the Harvard Public School of Health, is now evaluating various ligands that are specific for TB, in place of antibodies, and is also working on improving the sensitivity to the single bacterium level.

Other types of nanosensors exist for TB detection but most require extensive sample purification. "Nanowire-based sensors are susceptible to stray electric charges from background objects, and cantilever-based sensors use high-purity samples to minimize false positives from, for example, cellular debris," says Weissleder. Because biological samples show virtually no magnetic background, it is possible to make highly sensitive magnetic measurements even in turbid and unprocessed samples.

"At the [sensitivity] levels demonstrated, I am convinced that this technology has excellent opportunities," says David Gorenstein of the University of Texas Medical Branch.

"This technology is significant and smart and therefore extremely welcome, given that current diagnosis is reliant on old technology," says Mario Raviglione, director of Stop TB at the World Health Organization, "but we still have to wait and see if this new device could be used at the point-of-care level. If it could be developed into something simple, cost effective and easy to use, this tool could be a genuine breakthrough in global TB control."

"The big hurdle is in the use of antibodies, which are expensive," says Mauro Ferrari of the University of Texas Health Science Center. "Problems with antibody purification can also often lead to unsatisfactory cross-reactivity and, moreover, antibodies are simply not available for many, many molecules of interest." However, Ferrari believes that using different recognition moieties, such as thioaptamers or peptides, instead of antibodies could overcome some of these problems, and might also allow the Harvard/MGH approach to be used for the early detection of cancer.

Indeed, Weissleder and co-workers are already moving in this direction and have started to explore the potential of their approach for detecting and profiling cancer cells drawn from tumours[5].

Ai Lin Chun is an Associate Editor for Nature Nanotechnology.

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