Nanoparticles probe biosystems

by Paula Gould

Advances in nanofabrication techniques are opening up a wide array of highly sophisticated biomedical applications for smaller and smarter magnetic particles. Areas under investigation include targeted drug delivery, ultra-sensitive disease detection, gene therapy, high throughput genetic screening, biochemical sensing, and rapid toxicity cleansing. Each of these disparate applications hinges on the apparently benign relationship between magnetic fields and biological systems; field strengths required to manipulate nanoparticles have no deleterious impact on biological tissue and the biotic environment does not shield efforts to detect internal magnetism. This makes magnetic nanoparticles highly attractive as *in vivo* probes or *in vitro* tools to extract information on biochemical systems.

Magnetic particles with micron or submicron dimensions have long attracted researchers' curiosity. Early applications focused on tiny iron oxide particles, which were found to have zero remanence; applying a magnetic field induces a small dipole moment in the ferrite particles, but this moment disappears once the field is removed. This observation led to the use of iron oxide particulate suspensions (ferrofluids) in mechanical applications, for example, as machine clutches.

Over the past decade, a number of biomedical applications have begun to emerge for magnetic micro- and nanoparticles of differing sizes, shapes, and compositions[1]. Many applications still rely on the use of iron oxide particles (usually Fe_2O_3 or Fe_3O_4), like the original ferrofluids. These particles are available with diameters ranging from ~300 nm to less than 10 nm. They exhibit superparamagnetic behavior, magnetizing strongly under an applied field, but retaining no permanent magnetism once the field is removed.

This on/off magnetic switching behavior is a particular advantage in magnetic separation, one of the simplest applications. Magnetic separation is now well established as a viable alternative to centrifugal separation of complex chemical or biological solutions. Iron oxide particles are first encased in a biocompatible coating to form tiny beads. The beads are then 'functionalized', that is, their surfaces are treated with a biological or chemical agent known to bind to a specific target. On placing the beads in solution, any target cells or molecules will latch onto the functionalized surfaces.

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A permanent magnet placed at the side of the solution beaker induces a magnetic moment in each of the freely floating beads and sets up a field gradient across the solution. The now-magnetized beads will move along the field lines and clump together by the magnet, separating their bound targets from the bulk solution.

Iron oxide nanoparticles can also be used to enhance the signal from magnetic resonance imaging (MRI) examinations. MRI scans yield high-quality anatomical images for clinical diagnosis, pre-surgical assessment, and therapy monitoring. The technique works by applying a series of short-lived radio frequency (RF) pulses to a body placed within a constant magnetic field. Measured changes in the magnetization of hydrogen protons in water molecules are used to derive a picture of anatomical structures. So-called 'contrast agents', which alter the behavior of nearby molecules, can be administered to sharpen image detail. Suspensions of coated magnetite particles are now available commercially for this very purpose. The agents tend to be classed as superparamagnetic iron oxides (SPIO) if individual particles are larger than 50 nm or ultrasmall superparamagnetic iron oxides (USPIO) for particles smaller than 50 nm.

An alternative class of magnetic nanoparticle is being produced from pure transition metals, such as Fe, Ni, and Co, which exhibits ferromagnetic behavior. Unlike the SPIO and USPIO particles, these pure metal particles retain their magnetization once an external magnetic field is withdrawn, causing particulate clustering. Ferromagnetic nanoparticles also tend to have a larger magnetic moment than their superparamagnetic counterparts. Ultrasmall Fe particles are, thus, likely to produce a better signal in magnetic sensors or respond more readily to an applied field gradient than iron oxide particles of the same size.

But ferromagnetic nanoparticles are not without their disadvantages. Most crucially, they can be extremely tricky to make. Nanoscale Fe particles are so unstable that they will spontaneously burst into flames when exposed to air, says Charles J. O'Connor, distinguished professor of chemistry and director of the Advanced Materials Research Institute at the University of New Orleans. Co particles are less reactive, but an oxide coating will still form if exposed to air. The magnetized particles' inherent clumping or coercivity can also present problems during manufacture.

O'Connor is trying to optimize the fabrication of nanoscale ferromagnetic particles, experimenting with

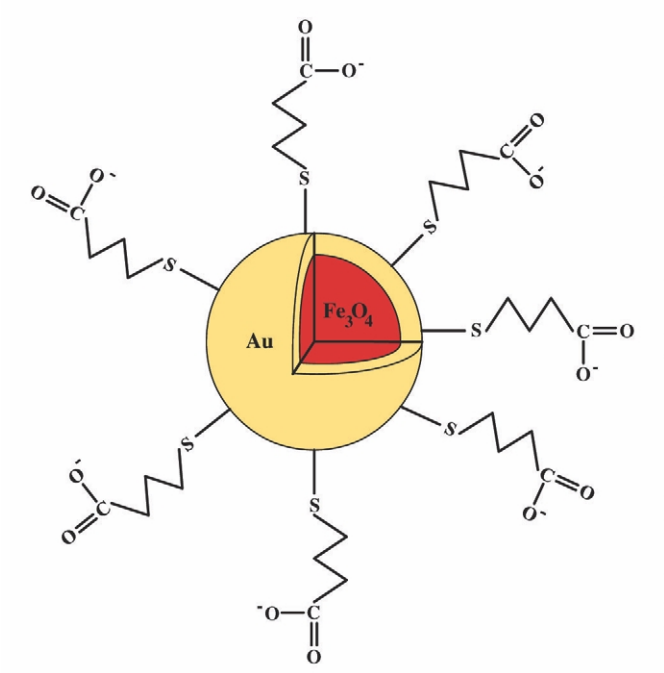


Fig. 1 Au coated ferrite nanoparticles can be attached to functional groups through Au-S bonds. (Credit: Charles O'Connor.)

particle synthesis in aqueous and nonaqueous solutions. The particles are grown within microdroplets and then coated with a thin Au layer to prevent any reaction with the external environment. The Au also provides a good surface for subsequent functionalization with chemical or biological agents (Fig. 1). "For example, if we want to attach an organic molecule to the particle, we can take a thiol or S group and use a covalent bond with the Au to attach it," he explains.

The Au coating is not sufficiently thick to keep the particles from aggregating, though. Ionic capping ligands, which bind to the particles' surface, must also be added during nanoparticle synthesis. The ligands' electrostatic charge causes the particles to repel, countering the magnetic attraction pulling them together.

While the majority of magnetic nanoparticles produced at present for biomedical applications are bead-like or spherical, some research groups are fabricating nanoscale wires. Daniel H. Reich, professor in the department of physics and astronomy at The Johns Hopkins University, is part of a team developing ferromagnetic nanowires for use in biomedical research or therapy. The group's fabrication procedure enables them to make wires with diameters of 20-400 nm. Wires can range in length from 100 nm up to several tens of microns. The wires' high aspect ratio gives them a greater dipole moment than spherical particles of the same volume,

which means they can be manipulated at lower field strengths.

Material composition can also be varied along the wire's length[2], allowing a single class of magnetic nanowire to have multifunctionality, says Reich. "You can make a wire where part of it is Ni and part Au, or even a multilayered structure. That allows you to tune the magnetic properties," he says. "And by taking advantage of differences in surface chemistry, say between the Au and Ni segments, you can bind different molecules to different parts of the wire, and arrange to have the wire functionalized to carry out different tasks."

Direct detection

The size, shape, and composition of magnetic nanoparticles being trialed as biochemical probes depends on their intended application, as well as the practicality of fabrication. But balancing the advantages and disadvantages offered by superparamagnetic versus ferromagnetic particles, or homogeneous beads versus multilayered wires, is not always clear-cut. Consequently, many researchers are experimenting with differing particulate compositions, which may later be patented, to find the optimum probe for their particular application.

"Our goal with cancer... is to detect individual cancer cells instead of waiting for tumors to become large." (Gang Bao, Georgia Tech)

Gang Bao, professor and principle investigator at Georgia Institute of Technology's Laboratory of Molecular Bioengineering, is assessing a range of options in his quest for smart imaging contrast agents. As part of this research, Bao is trialing bead-like 5-10 nm USPIO particles that are sufficiently small to reach intracellular markers of disease or viral infection. The iron oxide probes are coated and then functionalized, for example, with antibodies, oligonucleotides, or peptide ligands. Once administered to the body, by injection into the bloodstream for example, the nanoparticles should recognize the target molecular markers present inside cells, says Bao. Any USPIO particulate clustering as a result of target recognition should then induce a specific signal in MRI (Fig. 2).

The technique could prove especially useful for detecting extremely early signs of disease. "Our goal with cancer, for instance, is to detect a cluster, let's say 10-100 tumor cells," says Bao. "That would be a very early-stage malignancy. We want to detect individual cancer cells instead of waiting for the tumors to become large. Typically, a 10 mm tumor will have at least one hundred million cancer cells."

Targeted detection could also play an important role in AIDS research, says O'Connor. Some drug cocktails used to treat AIDS patients effectively reduce levels of HIV infection to undetectable concentrations. But if patients stop taking the drugs, the virus simply returns in strength, leading to full-blown AIDS again. Au-coated ferromagnetic nanoparticle probes, tagged with an HIV antibody via an Au-S covalent bond, could track down those viral particles left untouched by conventional AIDS drugs, he says. This could be the first

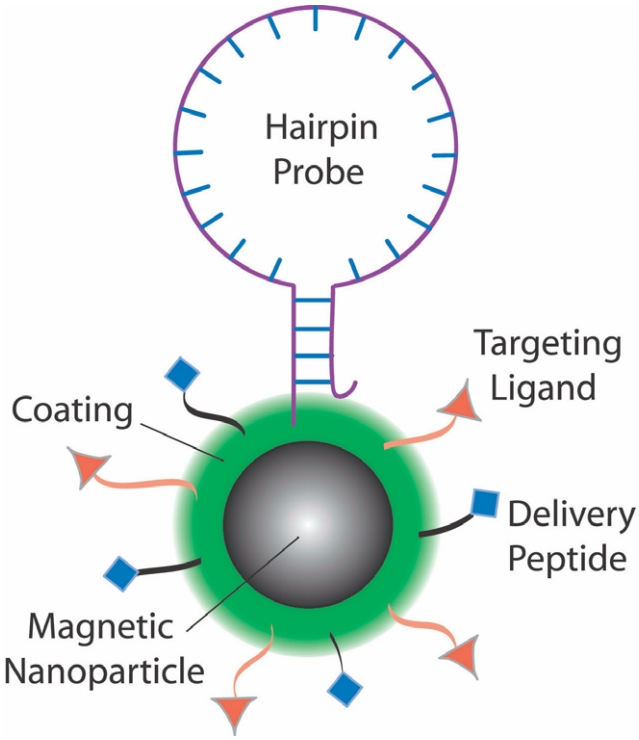


Fig. 2 Illustration of a multifunctional magnetic nanoparticle probe for deep tissue molecular MRI. The magnetic nanoparticle (5-10 nm in diameter) has an oligonucleotide hairpin probe on its surface. Cell-penetrating peptides (for deep tissue delivery) and ligands (for targeting specific cell types) are also conjugated on the nanoparticle surface. Binding of two or more nanoparticle probes on an mRNA target inside a cell should generate a measurable change in the MRI signal. Cells expressing specific molecular markers of disease, infection, or injury are detected in this way. (Credit: Gang Bao.)

step toward future targeted therapy regimes, which could eliminate the body of its infection altogether.

Supersensitive, targeted detection has a long way to go before reaching clinical trials, let alone daily practice. Lab bench tests demonstrate the ability of magnetic nanoparticles to cluster onto disease target markers in solution and tissue samples[3]. Experimental studies must now confirm that a marker-specific signal from just a small number of diseased cells is sufficiently strong to be detected reliably by MRI. Researchers must also ensure the magnetic probes reach their intended target and guarantee that the signal contrast is really a result of particulate clustering induced by probe-target binding, not because of overly large, unattached particles or nonspecific interactions.

Despite the many challenges ahead, Bao remains confident that magnetic nanoparticles offer the best option for sensitive, deep tissue molecular imaging using MRI. Fluorescent and luminescent markers can visualize gene expression in cells with far higher spatial resolution than MRI. But tissue absorption of light precludes the use of optical probes in most clinical deep tissue imaging applications, where target sites could be a few centimeters beneath the skin. "The optical-based probes are very effective in cellular studies, but they cannot be used for deep tissue imaging because, of course, the tissue typically absorbs light, so we cannot get much signal," says Bao.

Targeting treatment

Functionalized magnetic nanoparticles are being considered as smart treatment agents, as well as diagnostic tools[4]. The idea here is to bind therapeutic drug molecules onto the surface of a nanoparticle, then use a magnetic field gradient to draw tagged particles towards the intended treatment site. Present-day drug treatment mechanisms rely on a certain proportion of an injected or orally administered pharmaceutical reaching its intended target. But a significant number of drug molecules never make it. Use of magnetic 'tag and drag' could improve treatment efficacy, while simultaneously reducing administered doses.

Targeted drug delivery regimes offer particular advantages for cancer patients. Chemotherapy has notoriously unpleasant side effects because the highly toxic agents used to kill cancerous tumors also damage healthy cells. Attaching chemotherapy agents to magnetic nanoparticles and then focusing a magnetic field on the target tumor should, quite literally, pull the drug towards malignant cells, explains O'Connor. "If you can get all the chemotherapy aggregated around the tumor, and then hit it with an RF pulse, you can release the drug, so the tumor would get a high concentration of the drug and the rest of the body would get a relatively low concentration. That's really what you want to have happen," he says.

Magnetic tag and drag could improve drug treatment efficacy and simultaneously reduce administered doses.

O'Connor's group is developing Au-coated Fe, Ni, and Co ferromagnetic particles, with diameters of 50-100 nm, for potential drug delivery applications. The larger the magnetic particle, the stronger the force it can exert against blood flow when delivering its pharmaceutical tag. But particles must be sufficiently small to rule out any risk of clogging small capillaries, which could be just a few microns wide. Magnetic nanoparticles designed for drug delivery must also be completely biocompatible. Iron oxide particles are known to be nontoxic, and are eventually broken down to form blood hemoglobin. Au-coated ferromagnetic particles are a slightly trickier issue. The small amounts of Au would likely pass through the body eventually, and any Fe would also be metabolized, O'Connor says. But Co, which is less reactive and hence easier to fabricate, is toxic, making it unsuitable for *in vivo* applications.

The same concept could be used in gene therapy, according to Christian Plank, who is leading research into nonviral gene delivery at the Institute of Experimental Oncology, Technical University of Munich. Plank and his team have been assessing the viability of iron oxide nanoparticles as a vehicle for delivering nucleic acid into cells (transfection)[5]. SPIO particles coated with polyelectrolytes are mixed with so-called gene vectors, such as DNA or recombinant viruses, in a salt-containing solution. The gene vectors, which also carry an electrostatic charge, collect around the magnetic particles owing to salt-induced colloid aggregation, explains Plank. These tagged particles can then be directed along a magnetic field gradient to transfect

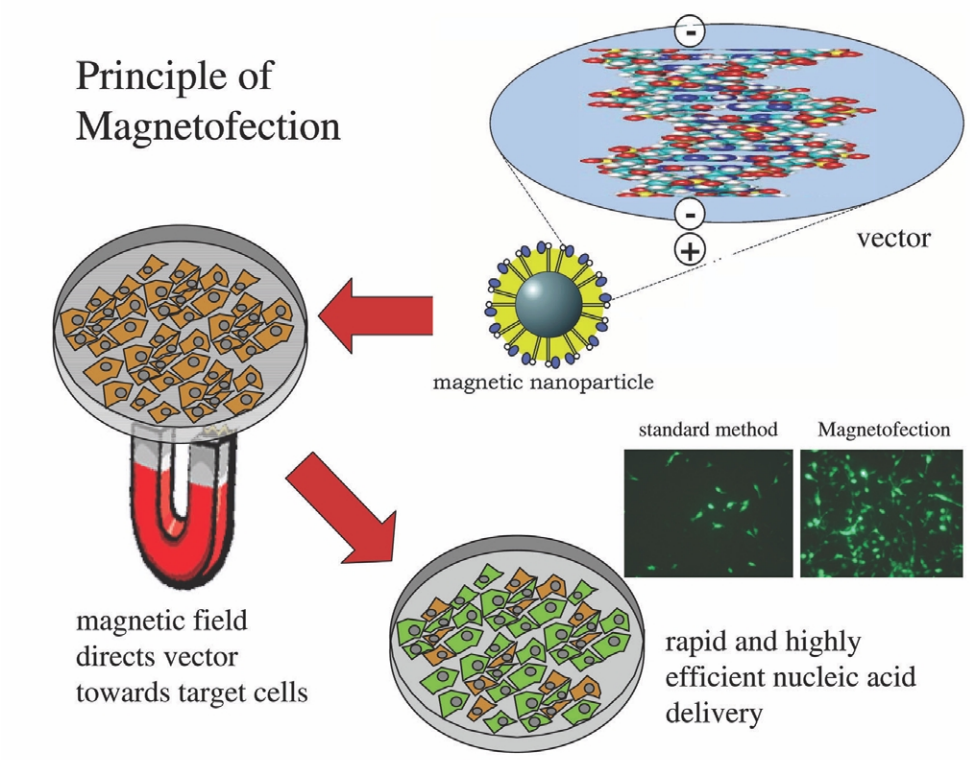


Fig. 3 Proposed mechanism for 'magnetofection'. (Credit: Christian Plank.)

appropriate cells (Fig. 3). The researchers have shown that their 'magnetofection' method works for *in vivo* applications. But substantial improvements are required to direct the magnetic gene vectors to specific target sites in the human body without invasive surgery.

Ex vivo applications could come sooner, though. Plank's group has already demonstrated that cells incubated with vector-tagged SPIO particles under the influence of a magnetic field can be transfected efficiently within a few minutes. "To my knowledge, there is no other transfection method that is as simple in practice," says Plank. "The method can be implemented in robotic systems and will, therefore, be a useful tool in high throughput approaches in functional genomics, which involve the transfer of nucleic acids into cells."

Remote control

Donald E. Ingber, professor of pathology at Harvard Medical School and Children's Hospital Boston, agrees that magnetic nanoparticles can play an important role in *ex vivo* biomolecular work[6]. He describes how coated magnetic beads, often microparticles rather than true nanoparticles, can reveal the effect of physical stresses on cells. "Biologists are interested in how mechanical forces affect cells because these forces play a central role in the control of cell form and function, as well as in many disease processes," he says.

Biological materials can be subject to either pulling (tensional) or twisting (torsional) forces, explains Ingber. The former relies on the well-established process of magnetic separation, where functionalized particles drag their target across a magnetic field gradient. "The advantage over other techniques, for example, optical tweezers or atomic force microscopy, is that higher stresses can be applied with twisting forces, and whole populations of cells can be stressed at once, rather than single cells," he notes.

Investigations into torsional force involve coated ferromagnetic beads. The beads are first magnetized with a strong, short-lived magnetic pulse, then subjected to a weaker but sustained magnetic field in a perpendicular direction. The beads try to realign along the second field direction, exerting a twisting force on bound receptor molecules on the surfaces of associated cells. Measuring the extent of the rotation yields information about the stiffness and viscoelasticity of the surrounding cells.

Researchers in Ingber's Harvard laboratory have refined these procedures to probe the effects of the mechanical force within individual cells. The goal is to assess how external forces are transferred across specific cell receptors and how this translates into changes in intracellular biochemistry and gene expression. They coat commercially-available magnetic beads with adhesive proteins and antibodies, which bind to cell surface receptors known as 'integrins', explains Ingber. Results indicate that these transmembrane receptors facilitate the mechanochemical transduction process. "We have obtained stress-dependent changes in biochemical signal transduction, structural rearrangements inside the cell, and alterations in the expression of specific genes in these studies," he says.

A number of other groups are using magnetic nanoparticles to design smart sensors. Paulo Freitas, professor of physics at the Instituto Superior Técnico in Lisbon, has been testing different superparamagnetic iron oxide particles as markers for a table-top genetic screening device[7]. The particles are first functionalized, for instance, with streptavidin, to enable binding of targets containing biotinyl groups. Meanwhile, a surface is prepared with a coating of biomolecular probes that will also bind with complementary target species, creating a 'gene chip'. The intended end result is similar to a sandwich: the target hybridizes with the fixed molecular probe and links to the functionalized magnetic label. Its capture is recorded by magnetoresistive sensors beneath the gene chip (Fig. 4).

Sensor development is still at a very early stage and many challenges remain. Researchers have yet to reach a consensus on the optimal particle for magnetic labels. The tendency of Fe or Co particles to aggregate would prevent a sensor from working effectively. Polymer-coated iron oxide particles have the advantage of moving freely, but they have a far smaller magnetic moment for their size. Micron-sized particles may impede biomolecular recognition and interaction, hampering the sensor's efficacy, but particles smaller than 100 nm may prove difficult to detect individually.

"The experiments you see at the moment are done with iron oxide microparticles, and ferromagnetic particles with diameters down to 130-250 nm. That's our limit at the moment," says Freitas. "I expect within the next year you'll see data coming out with labels around 100 nm and below. We need to see if the signals from these particles and the signal-to-noise ratio are good enough to give biologists and biochemists the information they require."

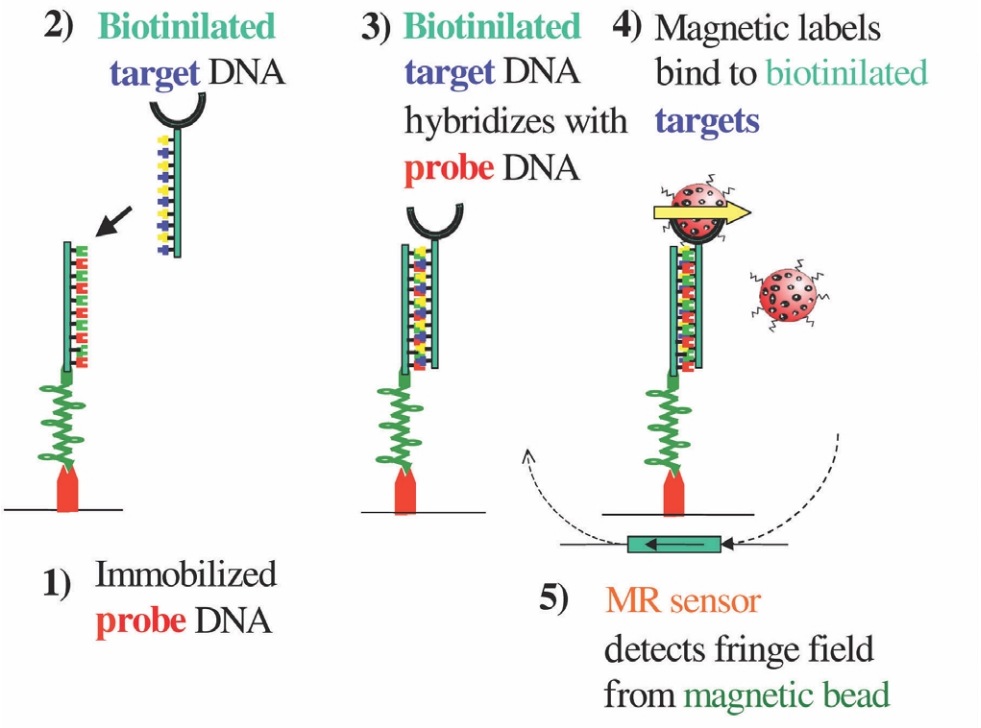


Fig. 4 Magnetoresistive (MR) sensors rely on linkage between immobilized biomolecular probes and magnetically-labeled targets. (Credit: Paulo Freitas.)

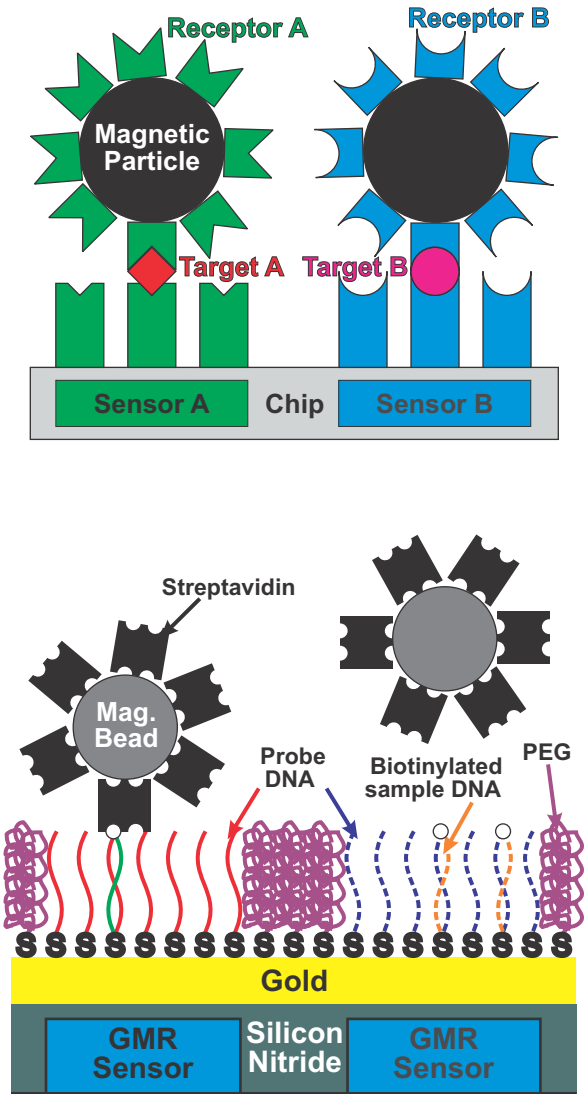


Fig. 5 Magnetoresistive sensor chips coated with zoned arrays of single-stranded DNA capture probes may be used to detect toxic biochemical agents. A biocompatible polymer film (polyethylene glycol or PEG) coating outside DNA probe spots prevents rogue signals from nonspecific binding. (Credit: Lloyd Whitman, Naval Research Laboratory.)

Researchers must also move from proof-of-concept studies to quantitative experiments, and generate reproducible results for comparison with existing biosensor technology. The planned magnetoresistive biochips offer many theoretical advantages, according to Freitas. These include the detection of low concentrations of genetic mutations or even single target markers. They could provide an easy-to-use, portable alternative to conventional screening technology. "The goal is to produce smaller kits, perhaps linked to PCs or laptops, so health centers can screen newborns, for example, for cystic fibrosis," he says.

Portability is one of the chief benefits that magnetic biosensors can offer, says Valerie M. Browning, who manages DARPA's (Defense Advanced Research Projects Agency) Biomagnetics Interfacing Concepts program for the US Department of Defense. "If we can begin to think about and develop magnetic tools that will allow us to manipulate, control, and detect individual cells and biomolecules with great precision, then we have this great supporting magnetoelectronic infrastructure. In terms of taking biotechnology out of the laboratory, I think magnetism holds great promise," she says.

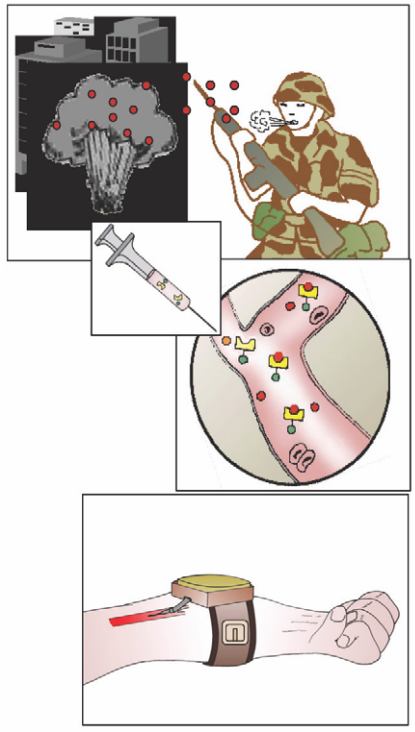
DARPA is currently supporting research into the use of magnetic biosensors to detect chemical and/or germ warfare agents. The proposed sensors would use DNA microarrays to capture specific toxic agents, such as anthrax. Multisensing chips capable of detecting a range of different agents are also being designed (Fig. 5). Rather than creating the magnetic labels themselves, some of the investigators are working with bacteria that produce magnetic nanoparticles naturally, says Browning. If the bacteria can be genetically programmed to produce the particles when placed in certain chemical environments – and at no other times – they could be used to alert military officials to the presence of toxins, or quickly confirm release of certain agents.

Detox regime

Magnetic nanoparticles could also help with treatment after a poison gas attack. DARPA-sponsored researchers at Argonne National Laboratory and the University of Chicago are also assessing whether the magnetic 'tag and drag' mechanism suggested for smart drug delivery could be used to detoxify contaminated military personnel or civilians. The proposed process would involve injection of magnetic nanoparticles that had been functionalized to bind with the foreign toxin. An external magnetic field would then be used to draw the toxin-tagged particles out of the patient's body (Fig. 6). "The goal would be to completely filter somebody who had been exposed to some sort of toxin within 10-15 minutes," says Browning.

Efficient detoxification will require particles with a reasonably high magnetic moment that can be pulled – together with any attached toxin molecules – across a magnetic field gradient. Browning consequently favors micron-sized ferromagnetic particles for this role. However, she has some doubts as to whether Au is the optimal coating.

(a)



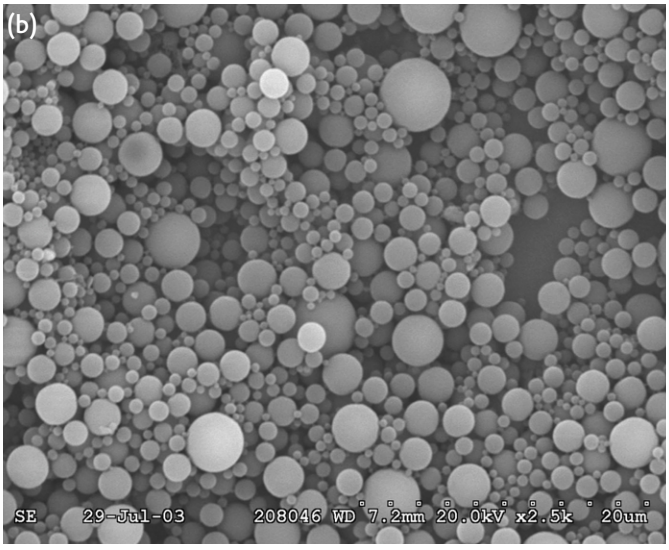


Fig. 6 (a) Proposed toxic screening mechanism using magnetic nanoparticles. (b) Electron micrograph shows a collection of biodegradable microspheres, roughly the size of red blood cells. Smaller versions of these would be injected into the blood stream to selectively remove blood-borne toxins. (Credit: Argonne National Laboratory and the University of Chicago.)

"A big part of this program is precision surface chemistry," she says. "Thiol chemistries have been developed for attaching DNA and antibodies, but Au doesn't wet very well to the nanoparticle surface, so it doesn't form a good, stable coating. Researchers are now looking at other materials to form that barrier layer."

O'Connor shares the view that Au coatings may eventually be superseded with something more user-friendly. The inert metal is not only expensive, but even a thin film layer adds considerable weight to an Fe nanoparticle. "If you want to attach a molecule to your particle, and it's going to be moved around, you're dragging all of this dead weight and so the magnetic force needed to move your particle is going to have to be larger," he says. An organic coating, on the other hand, would be cheaper and lighter. Researchers just need to find a suitable candidate that will coat the ferromagnetic nanoparticles effectively and also bind with functionalizing agents.

Many challenges clearly remain before magnetic nanoparticles achieve their full potential in the biomedical arena. Rapid realization of this novel technology in robust, reliable systems seems unlikely. But adoption of an interdisciplinary team approach may speed things up, says Catherine Berry, a researcher in the Centre for Cell Engineering, University of Glasgow.

"The search for new synthesis routes, or improving on previous ones to produce reliable particles with the correct characteristics, is vital. But it is equally important to try and understand and, therefore, have an element of control over the biological reactions that occur," she says. "I work with other cell biologists, molecular biologists, chemists, physicists, electronics engineers, and materials scientists, and I find that this interaction is invaluable. With collaboration of mixed expertise and good communication, we can move research along at a more rapid rate." MT

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REFERENCES

1. Berry, C. C., and Curtis, A. S. G., *J. Phys. D: Appl. Phys.* (2003) **36** (13), R198
2. Chen, M., *et al.*, *Appl. Phys. Lett.* (2003) **82** (19), 3310
3. Perez, J. M., *et al.*, *J. Am. Chem. Soc.* (2003) **125** (34), 10192
4. Alexiou, C., *et al.*, *J. Drug Target.* (2003) **11** (3), 139
5. Plank C., *et al.*, *Biol. Chem.* (2003) **384** (5), 737
6. Ingber, D. E., *Ann. Med.* (2003) **35** (8), 564
7. Ferreira, H. A., *et al.*, *J. Appl. Phys.* (2003) **93** (10), 7281