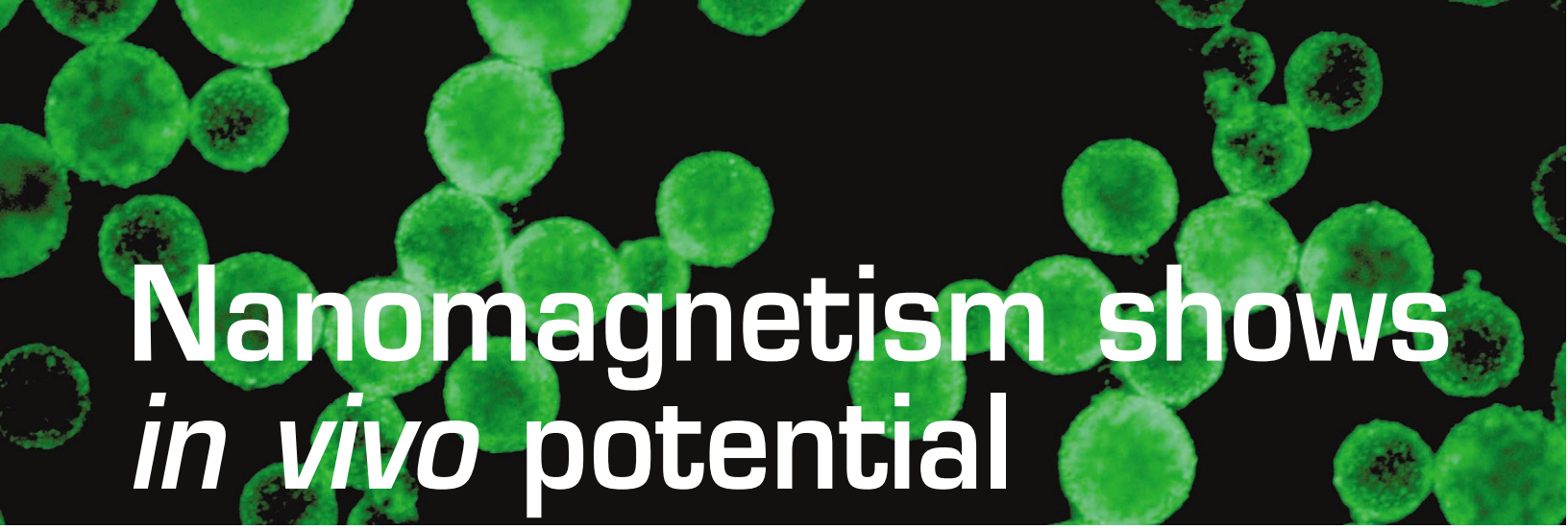

Nanomagnetism shows *in vivo* potential

The *in vivo* use of magnetic nanoparticles is attracting considerable interest as a means of delivering personalized medicine. Biocompatible nanoparticles that can be drawn toward a magnet are being investigated as site-specific drug delivery agents. Transfection of cells with nanosized particles observable by magnetic resonance imaging (MRI) offers a way to monitor experimental cell therapies. However, one size does not necessarily fit all. Realizing the clinical potential of these novel nanocarriers means finding the correct magnetic nanoparticle for each particular job.

Paula Gould

To date, most interest in the clinical use of magnetic nanoparticles has focused on iron oxide. This is because of the chemical stability, biological compatibility, and relative ease of manufacture of magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) nanoparticles.

Mixtures of Fe_3O_4 and γ-Fe_2O_3 can be synthesized in a single step by alkaline co-precipitation of Fe^{2+} and Fe^{3+} salts. Synthesis is generally performed in an aqueous solution of an appropriate macromolecule. The macromolecule limits the growth of the magnetic core, while also forming a coating that helps control particle dispersion and aggregation. *In vivo* tests have shown that the iron oxide component of such mixtures will gradually be recycled naturally. The human body contains around 3-4 g Fe, for example, in the proteins ferritin, hemosiderin, transferritin, and hemoglobin. As the magnetic nanoparticles start to break down, any soluble Fe becomes part of this normal Fe pool, which is then regulated by the body[1]. Given that a clinical dose would likely include just a few milligrams of Fe per kilogram body weight, the prospect of Fe overload is highly unlikely.

When produced in nanoparticulate form, both Fe_3O_4 and γ-Fe_2O_3 exhibit superparamagnetic behavior at room temperature. In other words, they magnetize strongly under an applied magnetic field but retain no permanent magnetism once the field is removed[2]. This magnetic behavior has raised hopes that iron oxide nanoparticles could improve the accuracy of drug delivery by literally dragging attached therapeutic agents to specific areas in the body under the influence of an applied magnetic field. The on/off switching means that particles are unlikely to clump together during manufacture, or once an applied magnetic field is removed, leading to easy dispersal.

Magnetic-nanoparticle-aided drug delivery is still very much a work-in-progress. However, Fe_3O_4/γ-Fe_2O_3 combinations have already been approved for clinical use as MRI contrast agents. MRI agents work by altering the relaxation rates of water protons that are trying to realign with a static magnetic field following the application of radiofrequency (RF) pulses. Iron oxide-based contrast agents affect transverse relaxation times, or what is known as T2 decay. This leads to 'negative contrast', or dark spots, on T2-weighted MR images. They have little impact on longitudinal relaxation, or T1 decay. The agents tend to be termed superparamagnetic iron oxides (SPIO) if individual particles are

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larger than 50 nm, or ultrasmall superparamagnetic iron oxides (USPIO) if the particles are less than 50 nm in diameter.

SPIO contrast agents are of particular use for imaging organs associated with the reticuloendothelial system (e.g. liver, spleen), which is where they tend to amass shortly after intravenous administration. The smaller USPIO agents are proving of interest for MR-based lymphography, owing to their tendency to accumulate in the lymph nodes. However, the true strength of iron oxide-based MR contrast may come with the development of cell tracking. This emerging *in vivo* application is expanding the scope of MRI as a tool for monitoring novel cell-based treatments.

For example, researchers at the Johns Hopkins University School of Medicine, Baltimore, are investigating the role of SPIO-based contrast in monitoring the fate of dendritic cells *in vivo*. Mature dendritic cells can generate an immune response in lymph nodes if 'primed' with an appropriate tumor antigen. This has raised hopes that they could be used as a possible 'cancer vaccine'. Trials of such vaccines have to date proved disappointing, though. An investigation in collaboration with researchers at the University of Nijmegen, the Netherlands, has now shown that the cells themselves may not necessarily be to blame. MRI of eight melanoma patients following administration of SPIO-labeled dendritic cells revealed problems with the initial injection technique, which had been performed under ultrasound guidance[3].

The Johns Hopkins team plan to repeat the investigation, but starting with MR-guided injection of the SPIO-labeled cells. They are also using SPIO-labeling to track bone marrow stem cells administered via intramyocardial or systemic injection in dogs. This kind of stem cell therapy is believed to show promise for treating patients with acutely impaired cardiac function. Another promising area for SPIO-based contrast is the *in vivo* monitoring of transplanted pancreatic islet cells. This kind of islet therapy could release patients with type I diabetes from their dependence on insulin, but without increasing the incidence of hypoglycemic events[4].

"The success of all these therapies essentially comes down to ensuring correct cell delivery," says Jeff Bulte, professor of radiology at Johns Hopkins. "This means real-time monitoring of targeted injection with MRI."

Maximizing magnetism

But are iron oxide nanoparticles the best material for MR-guided cell tracking? Not necessarily, says Taeghwan Hyeon, director of the National Creative Research Initiative Center for Oxide Nanocrystalline Materials at Seoul National University in Korea. Negative contrast from the iron oxide particles sometimes extends way beyond their immediate surroundings. This can lead to distortions in the background image, or large 'blooming artifacts' that obscure adjacent anatomy. "This could be a significant drawback to the utilization of SPIO-based contrast for tracking stem cells or transplanted cells, where the exact location and extent of the cells are important parameters," Hyeon says.

Doubts are also being aired about the suitability of Fe_3O_4 and γ-Fe_2O_3 in magnetically targeted drug delivery. The behavior of iron oxide nanoparticles within an external magnetic field may be sufficient for imaging purposes, but could they really be moved around the human body by magnetic force? Probably not, says says Jian-Ping Wang, associate professor at the Center for Micromagnetics and Information Technologies, University of Minnesota, Minneapolis. "The saturation magnetization, and hence the magnetic moment, per unit volume of SPIO nanoparticles is too low," he says.

Increasing the particles' size would undoubtedly aid attraction to an external magnet. Investigators are wary of upping the size of their SPIO particles too much, though, for fear of raising the likelihood of blood vessel blockage. Larger particles are also likely to be cleared from the body more quickly. Smaller particles offer a proportionally larger surface area for absorption, reducing the amount of magnetic carrier required to deliver a fixed drug dose. And the smaller the magnetic carrier, the higher the efficiency of cell uptake is likely to be.

So what other materials might do the job better? One option would be to use transition metal nanoparticles, such as pure Fe and Co, or metallic alloys or compounds, such as FeCo. These metallic nanoparticles tend to have a larger magnetic moment than their iron oxide counterparts. The saturization magnetization of FeCo is particularly high. Using the same mass of magnetic carrier would then produce a far greater driving force, improving the efficacy of drug delivery. Alternatively, smaller concentrations of magnetic material, or smaller particles, could be used to produce the same magnetic effect. "This will allow us to use ultrasmall nanoparticles, perhaps less than 5 nm or 10 nm, which are critical for the delivery of small molecules and pieces of DNA," says Wang.

However, this class of nanomaterials carries its own set of disadvantages. Synthesis of stable, monodisperse transition metal nanoparticles that are suitable for use in aqueous environments is not necessarily that easy given the elements' reactivity. These pure metal nanoparticles are also ferromagnetic at room temperature, rather than superparamagnetic. This means that once magnetized, they will remain that way regardless of whether an external magnetic field is withdrawn, making the particles more likely to clump together.

A number of investigators are consequently seeking suitable coatings that will prevent particulate aggregation and ensure chemical stability. Options under consideration include inert metals, such as Au and Ag, peptide capping ligands, and silica[5,6] (Fig. 1).

Researchers from the Aragón Institute of Nanoscience (INA), University of Zaragoza, and the Aragón Institute of Materials Science (ICMA), Zaragoza, Spain are also experimenting with carbon as a possible coating for transition metal nanoparticles. They are producing Fe@C nanoparticles using arc discharge methods similar to those used to produce fullerenes and carbon nanotubes. Simultaneous evaporation of Fe and graphite in argon plasma has resulted in a mixture of carbon-coated Fe and iron oxide nanoparticles with an average size of 200 nm

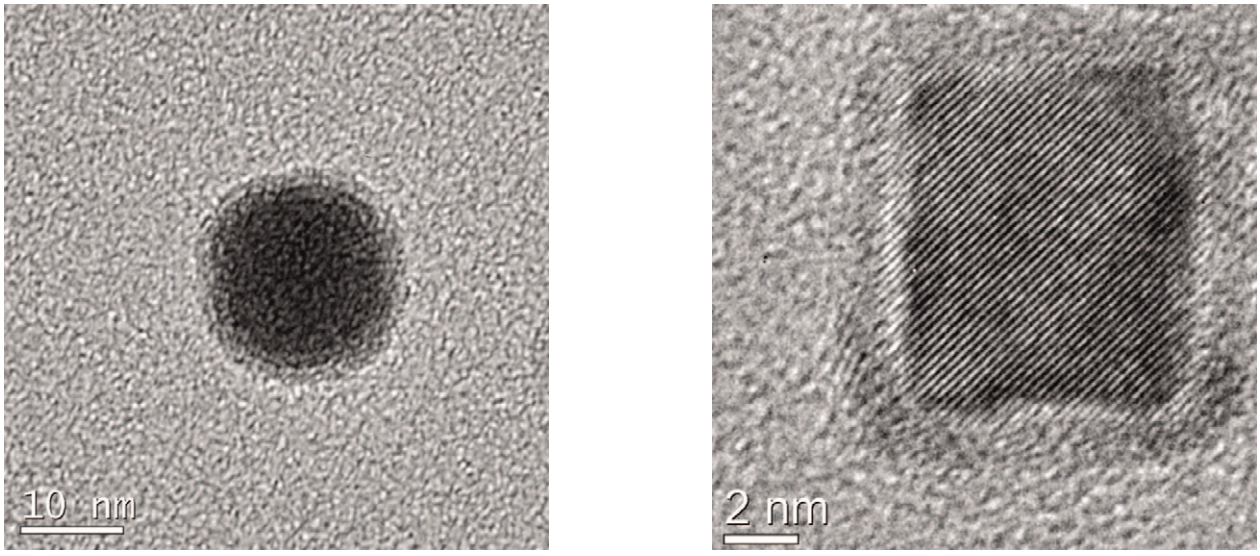

Fig. 1 University of Minnesota researchers are synthesizing FeCo nanoparticles of different shapes and sizes with a view to tuning their properties to different applications. These particles have a far higher magnetic susceptibility than SPIO. (Courtesy of Jian-Ping Wang, University of Minnesota.)

(Fig. 2). Preliminary hematological *in vitro* tests on New Zealand rabbit and human blood samples have indicated good biocompatibility.

The carbon-coated particles are currently being investigated in preclinical trials as possible vehicles for magnetically targeted chemotherapy delivery. The porosity and large specific area of the inorganic shell permits rapid adsorption of therapeutic agents, says Ricardo Ibarra Garcia, director of the INA. The drug molecules are then desorbed from the nanoparticles very slowly. So while the delivery agents can be loaded with their therapeutic armory quickly, early release of the payload into the bloodstream is largely avoided.

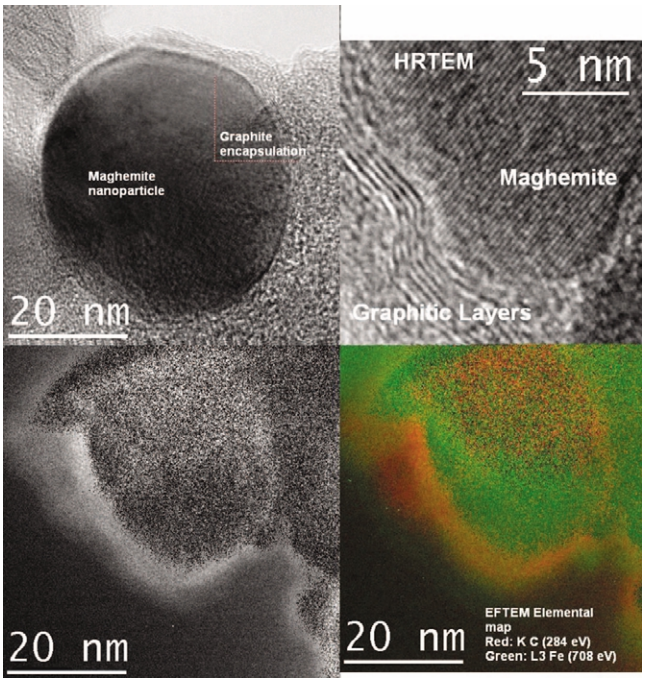

Fig. 2 High-resolution and energy-filtering transmission electron microscopy images of carbon-coated Fe and iron oxide nanoparticles. (Courtesy of Ricardo Ibarra Garcia, Aragón Institute of Nanoscience, University of Zaragoza, Spain.)

Carbon could, in theory, be used to coat Co nanoparticles too. However, many clinical researchers are wary of trialing these elements for *in vivo* applications, since unlike Fe, they are not already present in the body in significant quantities. "The toxicity of elements such as Co is an open question. Scientists have different opinions about this, but to date, there have been no detailed investigations or scientific proof either way," says Nina Matoussevitch, who is working on synthesizing biocompatible Co, Fe, and FeCo nanoparticles at the Institute for Technical Chemistry, Karlsruhe Research Center, Germany.

Nguyen T. K. Thanh, Royal Society university research fellow and lecturer at the Centre for Nanoscale Sciences, University of Liverpool, UK is more confident about clinical prospects for coated transition metal nanoparticles. "Low levels of Co are beneficial to human health. For instance, it is essential for vitamin B12 formation, and Co compounds are used in the treatment of anemia. In the long term, Co compounds are excreted and do not accumulate in the body," she says. "However, there is no data on the toxicity of Co in the form of nanoparticles, and further research is necessary."

The absolute quantity used is clearly important, notes Urs Hafeli, assistant professor in the Faculty of Pharmaceutical of Sciences, University of British Columbia, Canada. "As Paracelsus said back in the 16th century, it is the amount that makes the poison. While tens or hundreds of millions of magnetic nanoparticles might be administered

during targeted drug delivery, the actual weight will be small, most probably just tens of milligrams."

Effective delivery

Whatever the pros and cons of using nanoscale iron oxide for *in vivo* applications, (U)SPIOs remain the only magnetic nanoparticles that have been approved for clinical use. Investigators seeking to fast-track development of magnetic-guided therapy may consequently prefer to go for this tried-and-tested option.

And the drawbacks may not be entirely insurmountable. One solution to the nanoparticles' weak magnetic responsiveness is to maximize the magnetic field at the target site. Ibarra Garcia and colleagues would like to do this by implanting a Au-plated permanent magnet within the organ to be treated. This strategy, they hope, will enable nanoscale magnetic carriers to deliver chemotherapy agents to tumors deep within the body.

Preclinical studies are planned using the chemotherapy agent doxorubin tagged to 200 nm Fe@C particles (see above), and 80 nm to 2 μm Fe_3O_4/γ-Fe_2O_3 particles coated in silica. Early results from *in vivo* investigations with the carbon-coated nanoparticles in New Zealand rabbits appear promising. Histopathological analysis confirmed that the magnetic carriers could be drawn to a tumor in each animal's left kidney, close to an implanted magnet. Indeed, when the magnets were later extracted, they were found to be covered by the magnetic particles. No particles were observed in the animals' right kidneys (Fig. 3).

Yet the trial has thrown up some problems. "We usually find a concentration of nanoparticles in the liver and the Kupffer cells. We also find some in the spleen and the lung, though the maximum concentration is typically in the liver," Ibarra Garcia says. "If we are able to solve this problem, I think we are on the way to proposing an alterative method for delivering cancer therapy."

Another option is to optimize the shape and strength of externally placed magnets, as researchers at The University of Texas, MD Anderson Cancer Center, Houston, have discovered. Along with collaborators at the Edmond, Oklahoma-based NanoBioMagnetics, Inc. (NBMI), they are looking into using magnetically responsive nanoparticles to treat patients with advanced (stage III or IV) ovarian cancer whose malignancy has spread to the peritoneum. Intraperitoneal administration of taxane- and Pt-containing regimens has shown considerable benefits, but many patients dislike the temporary insertion of the catheter used for drug delivery. So the researchers instead plan to administer the chemotherapy agents via 20 nm silica-coated, magnetite-based nanoparticles under the direction of an external magnet. Additional anticipated benefits from this approach are the targeting of these drugs to the tumor or peri-tumoral environment, as well as reduced toxicity compared to the free drugs.

Initial trials in mice using a 22 mm, 5600 G cylindrical magnet confirmed that the particles could indeed be moved within the peritoneal cavity. Subsequent studies showed that the magnetic nanoparticles could also be directed toward a tumor in the peritoneal area. But some particles also clustered around the abdominal wall. This unwanted effect diminished when the cylindrical magnetic was switched with a pyramidal design, positioned with its 3 mm-wide point over the tumor site (Fig. 4).

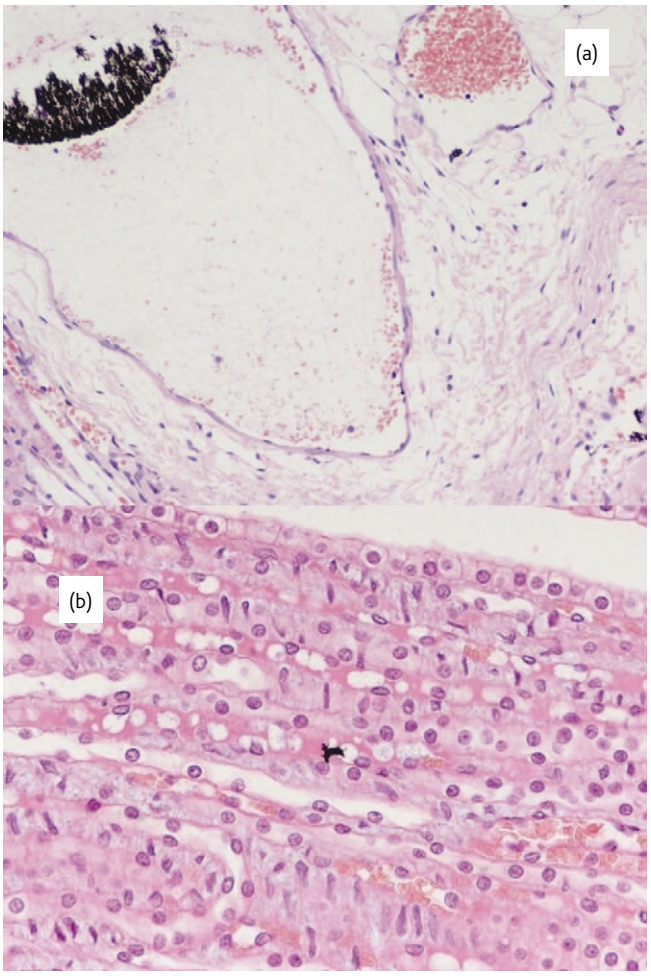

Fig. 3 (a) Histopathology analysis of the left kidney shows nanoparticles (stained with hematoxilin-eosin) aligned along the magnetic field lines of an implanted permanent magnet. (b) Practically no nanoparticles are observed in the right kidney, where no magnet was implanted. (Courtesy of Ricardo Ibarra Garcia, Aragón Institute of Nanoscience, University of Zaragoza, Spain.)

"It has become apparent to us that the design and selection of the vectoring device is a very important variable as well as the particle and coating chemistry," says Jim Klostergaard, professor of molecular and cellular oncology at MD Anderson, and leader of the study. "As history has shown, those who don't deal with both issues are not likely to be very successful in moving from the preclinical to the clinical scale."

Optimizing magnet design is not the only way to improve magnetic retention, according to Christian Plank from the Institute of Experimental Oncology, Technical University of Munich, Germany. Together with colleagues from the Ludwigs-Maximilians University,

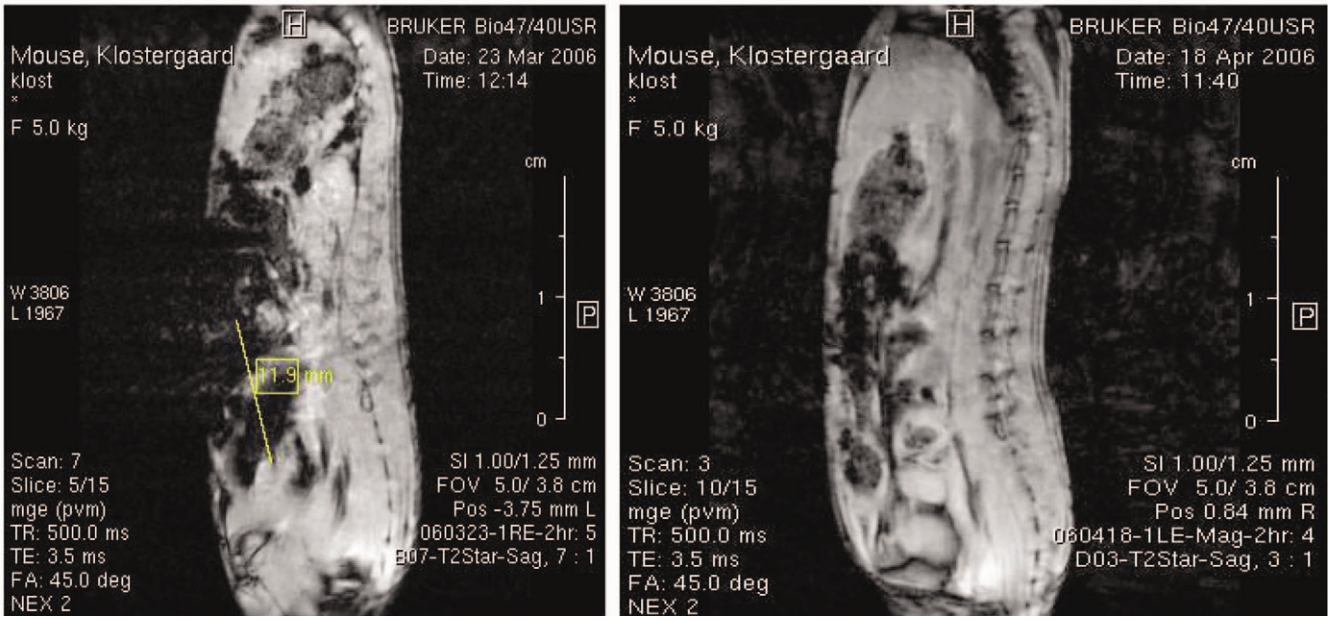

Fig. 4 T2-weighted MRI of nude mice previously injected intraperitoneally with HEY human ovarian adenocarcinoma cells. Once a tumor was established in the ventral abdominal wall, mice were injected intraperitoneally with magnetically responsive nanoparticles. An external magnet was placed next to the tumor for two hours prior to MRI. (left) A ~22 mm diameter cylindrical magnet was used where the cylinder axis was aligned with the center of the tumor. (right) The cylindrical magnet was superimposed with a pyramid magnet, with its ~ 3 mm peak positioned over the center of the tumor. This latter magnet assembly enabled much greater selectivity in the movement of the nanoparticles to the tumor/peri-tumoral environment, as opposed to the ventral abdominal wall. (Courtesy of Jim Klostergaard and James Banks at the MD Anderson Cancer Center and Charles Seeney and William Yuill at NBMI.)

Munich, he is investigating whether gas-filled microbubbles can help increase the magnetic responsiveness of SPIO-based drug delivery agents. The idea is to concentrate the particles together, but without causing clumping or blocking blood vessels. Flexible 2-5 μm diameter microbubbles offer a means of doing this, they believe.

Microbubbles are already used clinically to enhance ultrasound images. Their resonance under the influence of ultrasound improves visualization of areas where the bubbles are present. Trials are also underway at a number of sites to investigate the potential of microbubbles as drug delivery agents. However, demonstration of delivery using magnetically responsive microbubbles is entirely new, says Plank.

The German researchers are using 100-200 nm particles containing a high proportion of Fe_3O_4. These are incorporated into the lipid shell of C_3F_8-filled bubbles together with a therapeutic agent simply by vigorous shaking (Fig. 5). "You need to have tailor-made particles that are compatible with the other components of the bubble," Plank notes. "Some of the magnetic nanoparticles we are using are coated with a detergent, and these are incorporated into the lipid bubble shell very well."

Experiments have shown that the magnetic retention of bubbles is indeed far greater than retention of an equivalent dose of 'free' magnetic nanoparticles. *In vitro* tests have also confirmed that 1 MHz ultrasound pulses will burst the bubbles, releasing whatever drug molecule or genetic material is being carried. Further animal studies are now needed to confirm that the delivered therapeutic agents remain functional following their ultrasound-induced delivery. "100% retention at a target site will never be possible," says Plank. "Our goal is to have a carrier system that delivers the active agent in functional form only at the site where both a magnetic field and ultrasound are applied. For delivery of nucleic acids, this may be possible."

Investigators from the University of Chicago and Argonne National Laboratory, Illinois, are interested in the use of ultrasound to release a magnetically targeted medicinal payload too. Their approach differs from that adopted by Plank and colleagues in that the magnetic nanoparticles are coated with oleic acid, to promote hydrophobicity, and then embedded with a therapeutic agent in a polymer matrix. "We are now able to incorporate so much magnetite into the carrier that the magnetization value is much higher than any other reported carriers. This means that the carrier is much easier to direct and to hold in target positions against strong, arterial blood flow," says Axel Rosengart, assistant professor of neurology and surgery at the University of Chicago.

As before, application of ultrasound of an appropriate intensity causes the polymer beads to resonate and then break, releasing the therapeutic agent. But in this case, there is an additional benefit from using ultrasound. Rosengart and colleagues want to use the magnetic beads to deliver the 'clot-busting' thrombolysis agent rt-PA to stroke

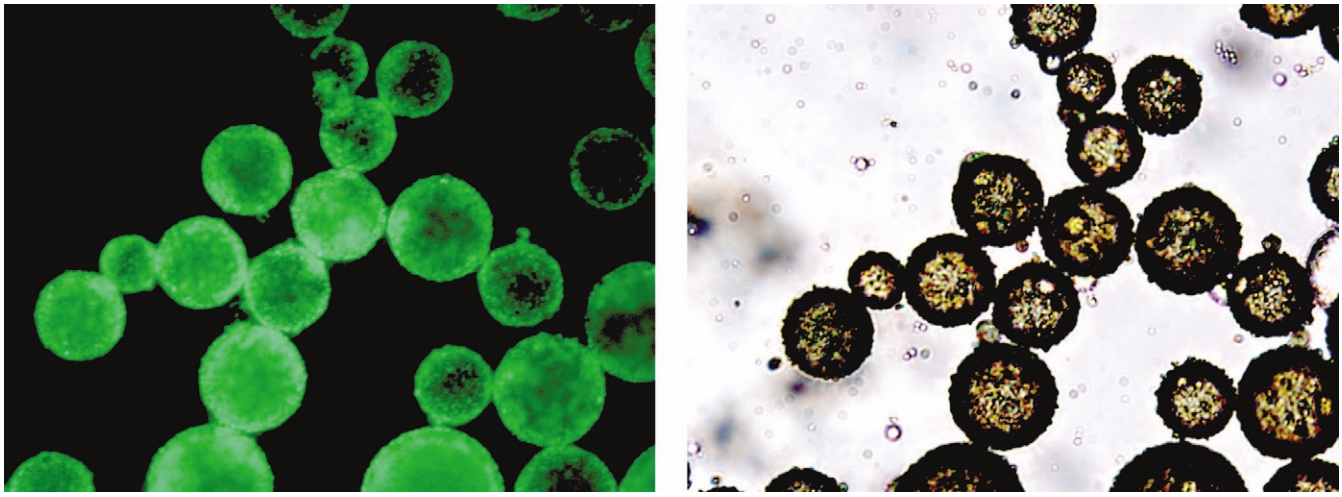

Fig. 5. Microbubbles (diameter ~10 μm) loaded with detergent-coated magnetic nanoparticles and fluorescently labeled plasmid DNA. (left) Fluorescence microscopy image. (right) Bright-field image. The brown color indicates the high load of magnetic nanoparticles. The bubbles also contain a lipid mixture and a cationic lipid transfection reagent. (Courtesy of Christian Plank, Technical University of Munich, Germany.)

and heart attack patients. The porosity of blood clots tends to increase when they are subjected to ultrasound, which in itself helps to speed up lysis, Rosengart explains. So using ultrasound-triggered delivery should increase the efficacy of targeted rt-PA delivery still further.

A six-month study in rat models is now planned to assess the *in vivo* feasibility of the scheme. "We have been focusing for the past three years on making the magnetic carrier, and I think we have succeeded now in developing a prototype that will run well *in vivo*." Rosengart says. Work will also continue on improving the stability of the rt-PA so its reactivity is not reduced by ultrasound heating effects.

Fit for purpose?

It is clearer than ever before that one size – and one composition – will not fit all when it comes to *in vivo* applications for magnetic nanoparticles. For instance, an agent best suited to hunting down widely spread metastatic cancer cells using MRI is not necessarily going to be the same agent selected to drag chemotherapy molecules toward a well-defined tumor site.

"The design of magnetic carriers requires a true multidisciplinary approach," says Etienne Duguet, professor at the Bordeaux Institute of Condensed Matter Chemistry, France. First there is the question of core composition. Is its magnetic behavior appropriate, and sufficient? Is it likely to be toxic in the administered dose? Then there is the coating. How will the coated particles interact with bodily fluids, biomolecules, and cells? Can drug molecules be attached and released where required?

Urs Hafeli suggests that designers work backwards from the application, rather than synthesizing a clever magnetic nanoparticle and then trying to find an *in vivo* use. "No part of magnetic drug delivery is more important than any other. We can't just combine the most magnetic particles with the best drug-release matrix and make perfectly monosized particles. Each drug and each application have physicochemical properties that require adaptations in areas that are not fully understood yet," he says. nt

Acknowledgments

This article draws on research presented at the 6th International Conference on the Scientific and Clinical Applications of Magnetic Carriers, 17-20 May 2006, Krems, Austria. Full proceedings of the meeting will be published in J. Magn. Magn. Mater. For information about forthcoming meetings in this area see www.magneticmicrosphere.com.

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