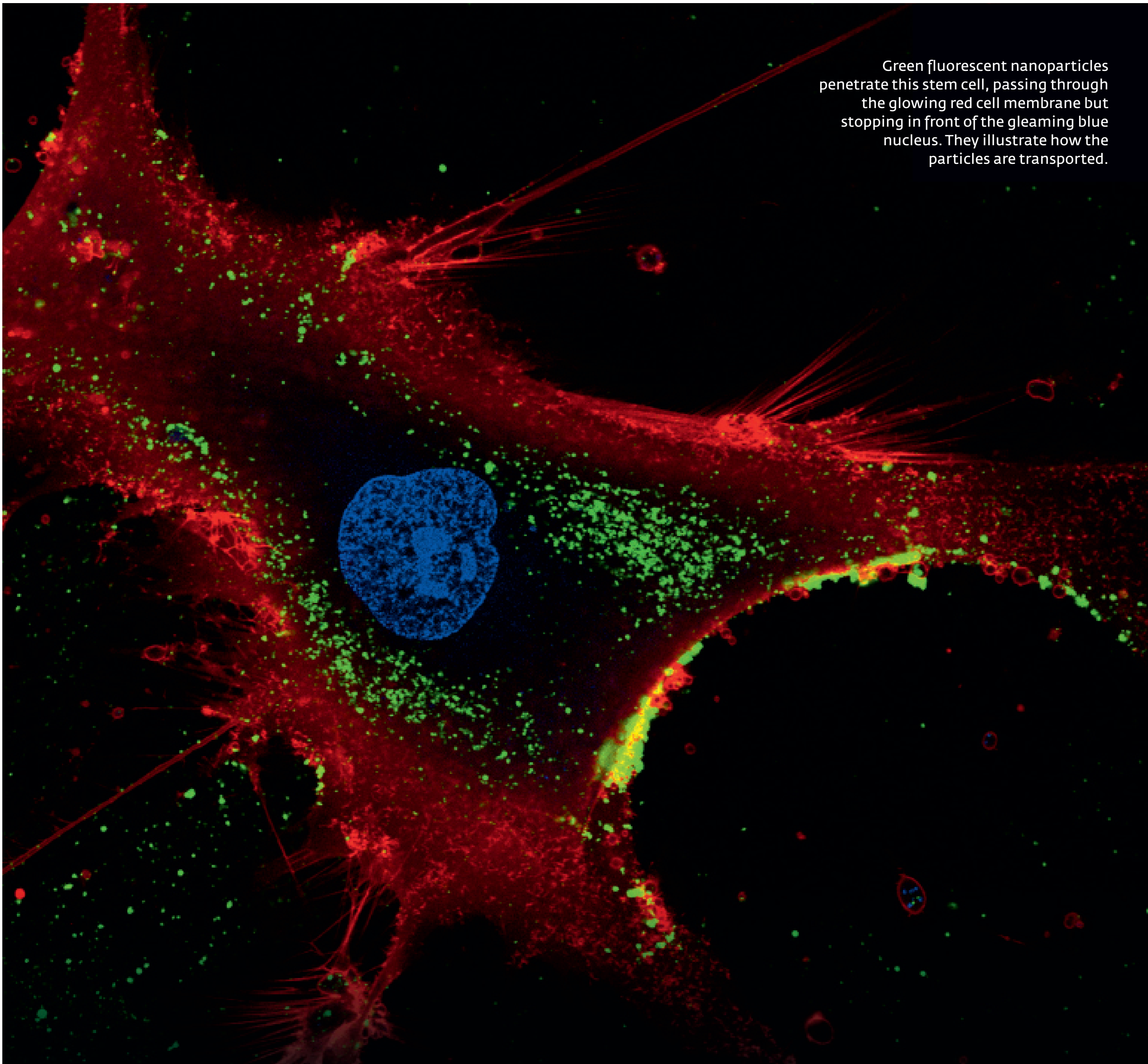


Green fluorescent nanoparticles penetrate this stem cell, passing through the glowing red cell membrane but stopping in front of the gleaming blue nucleus. They illustrate how the particles are transported.

Magic Spheres from Oil and Water

As vehicles for drugs, in dyes, or for the manufacture of data storage devices – the nanospheres and nanocapsules that **Katharina Landfester** and her fellow scientists at the **Max Planck Institute for Polymer Research** produce promise a variety of applications. This was made possible only by fundamental insights into the way they are made.

TEXT **PETER HERGERSBERG**

When Katharina Landfester held a small glass of the milky fluid in her hand for the first time, she had no idea what was in it. A mixture as unremarkable as its name and sharing more than just its appearance with milk sloshed around in the vessel. It was a miniemulsion, of which milk is a very good example: tiny droplets of fat are dispersed in a large amount of water and kept afloat by proteins and fats, among other things.

But a miniemulsion from Katharina Landfester's laboratory does more than milk can. The chemist's team produces ingenious nanospheres and nanocapsules from their droplets, constructing vehicles for many things: the particles could transport drugs specifically to tumors, or mark diseased tissue in order to make it visible in computer tomography. They could also help pack data onto memory chips at higher density.

The history of the multifunctional particles began in 1997. Katharina Landfester was then a junior researcher working with colloids: nanoscale or microscale particles or droplets that float in a different medium. "At that time, I wanted to study abroad to gather ideas for my research," says Katharina Landfester, now Director at the Max Planck Institute for Polymer Research in Mainz: "But there are not many places in the world for colloid chemists to go." So she went to work with Mohammed El-Aasser at Lehigh University in Bethlehem, USA.

In the 1970s, the Egyptian chemical process engineer was the first to produce miniemulsions in order to open up a simple route to obtaining fine dispersions: mixtures that contain very small particles in a liquid medium, just like wall paint or printer ink. El-Aasser mixed the starting materials for polymers into the oily phase and allowed them to react in the miniemulsions to form polymers, which immediately assumed the form of nanospheres. But he had actually wanted to produce just simple spheres, and spheres that consisted of a single type of polymer.

In conventional emulsions, in which the chemical industry has already been producing little polymer spheres for decades, the possibilities are also limited. Only simple particles, barely smaller than one thousandth of a millimeter, are created in those emulsions, and their size also frequently varies greatly. It is not possible to encapsulate any kind of freight in such oil-water mixtures because the starting materials of the polymers quickly escape from the droplets, which serve as chemical reactors.

NANOPARTICLES WITH OPENERS, ANCHORS AND STEALTH EFFECT

In the miniemulsions mixed by Mohammed El-Aasser's group, however, the droplet-shaped nanoreactors stayed tightly sealed. Katharina Landfester thus sensed quite early on that it also had to be possible for them to create more complex chemical constructions. But she never dreamed back then that they would be able to make many of nanotechnology's promises a reality. That is exactly what is happening today.

The particles that Katharina Landfester and her fellow scientists build come quite close to a vision that, in the 1990s, was associated with the world of tiny things. At that time, many researchers redesignated their experiments on the nanoscale to an independent research field they called nanotechnology. This quickly created the idea that, in the not-too-distant future, robots smaller than a thousandth of a millimeter could carry out precision medical work within our body: delivering medicines, cleaning blood vessels, destroying proliferating tissue. This idea will probably remain a vision, because nanorobots for these tasks will not be available for a long time yet. Still, Katharina Landfester and her team teach their nanoparticles to do some of these jobs one step at a time – even if they are less spectacular in appearance than nano-science fiction envisaged for the tiny machines.

It's the milk that does it: Katharina Landfester discovered what keeps miniemulsions stable, and has been producing versatile nanoparticles in these emulsions ever since.

The researchers headed by Katharina Landfester equip the particles with an opening mechanism, provide them with an anchor for certain cells, or a stealth layer, so that the particles can move though the body unhindered. The particles owe these special accessories to a variety of chemical tricks that the polymer researchers use. But this was made possible only by the fundamental insights Katharina Landfester gained as head of a research group on miniemulsions under Markus Antonietti, Director at the Max Planck Institute of Colloids and Interfaces. >

Photos: MPI for Polymer Research (left), Bernd Schuller

Anna Musyanovych pipettes the essential ingredients for the nanospheres and nanocapsules into the glass beaker before homogenizing the emulsion with an ultrasonication tip – and chemistry does the rest.

The miniemulsions of the first generation were not suitable for producing a large number of different polymer particles. When the chemists changed their composition, the oil droplets on the water merged to form a layer of oil before the polymer had formed. And when they did stay stable, this was just a matter of chance. At least that is how it appeared. "This made it clear that we had to take a close look at the physical-chemical processes in the miniemulsions," says Landfester. If these were understood, she thought, it might be possible to specifically select the composition of the miniemulsions so that multifunctional nanoparticles could be produced.

When she explains these connections today, she starts with the factors that keep milk homogeneous: it begins with the proteins that enclose the fat droplets. They act as a surfactant, just like a detergent, which prevents the droplets from merging. Nevertheless, the cream quickly separates out in milk that comes fresh from the cow. Some of its fat droplets are large, and due to their low density, they rise and form a layer of cream. The milk is therefore homogenized: it is sprayed onto a metal plate so that the fat droplets split to form smaller spheres. These are so small that their buoyancy is no longer sufficient to move them to the surface.

The chemists from Mainz homogenize their miniemulsions with an ultrasonication tip. This is routine work for Anna Musyanovych. She heads the group working on chemistry in miniemulsions. The lab specifically equipped for this purpose houses several metal cabinets, each one about the size of a wall cupboard in a kitchen. Anna Musyanovych fixes little glass vessels containing a mixture of oil, water and a surfactant under the ultrasonication tip in such a way that it hangs just above the bottom of the vessels. With a hissing sound and vibrations that are not very strong but very fast, the ultrasonication tip breaks up the oil droplets into nanodroplets.

STABILITY FOR TINY DROPLETS

But surfactant and droplet size are not enough to keep an emulsion stable over several hours, or long enough to produce polymers in them. The miniemulsion becomes stable only with the addition of a co-stabilizer: a substance that dissolves almost exclusively in the oil droplets. "I had already assumed that this reagent would have some kind of effect," says Katharina Landfester.

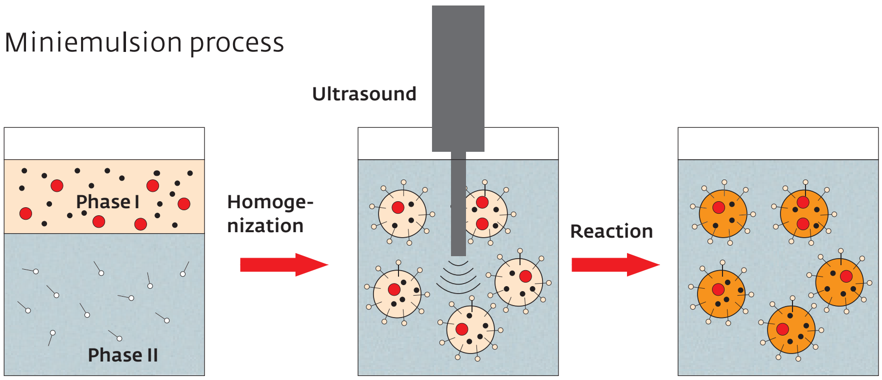
And indeed she discovered that these ominous substances acted as osmotic reagents – if they were chosen correctly. In milk, certain fats also act like osmotic reagents. The ones that do this are insoluble in water. Landfester discovered this after studying the processes that slowly separate oil and water in an emulsion. In addition to the contacts between the droplets that the surfactant prevents, Ostwald ripening also contributes to this: larger oil droplets grow at the expense of smaller ones. "In small droplets, there is a higher internal pressure," explains Katharina Landfester: "We see a similar thing when blowing up a balloon: it's more difficult to inflate while it's still small." The oil escapes the pressure in the smaller droplets by diffusing into the larger droplets through the water.

The diffusion of the oil molecules can be prevented with a counter-pressure – and this is precisely what the osmotic reagent that is dissolved in the oil droplets builds up. Since nature always tries to achieve equilibrium, the reagent feels best when it is present in all droplets at the same concentration. Otherwise, osmosis occurs.

Katharina Landfester again uses an example from everyday life to explain osmosis: "As long as lettuce is in water, it stays crisp. It collapses in the vinaigrette because the ion concentration in the vinegar is higher than in the lettuce leaf." In order to achieve equilibrium of the concentration, water seeps out of the lettuce. Something

Photos: Bernd Schuller (2)

Miniemulsion process



A formula for filled polymer particles: The oily phase I contains the starting materials for the polymer and the substances to be encapsulated. Ultrasonication transforms them into nanodroplets, which float in the aqueous phase II surrounded by the surfactant. The polymer is then formed in a chemical reaction.

similar would happen in a miniemulsion if the osmotic reagent were to concentrate in a shrinking droplet while diluting in a growing one. The Ostwald ripening is thus finished before it has really begun.

"When this became clear, we were able to produce miniemulsions using the starting materials for many different reactions," says Landfester. The possibilities for chemical nanotinkering now knew almost no bounds because the miniemulsions provide almost everything the plastics industry has to offer: polyacrylate, used in acrylic glass, for example, polyurethane, used as foams for insulating houses, polyester, nylon and biodegradable polymers such as starch, and even semiconducting polymers.

If the researchers create nanoparticles out of two polymer materials that do not mix, even so-called Janus particles are formed, which have two polymer faces. "Nanoparticles like these can result in coatings that possess the properties of both materials," explains Anna Musyanovych. Or they combine two semi-conducting polymers for a solar cell.

Katharina Landfester's team of chemists started to use the great variety of polymers that could now be produced in the miniemulsions to encapsulate dye pigments, such as carbon black particles. "We mix them into the oily liquid with the ingredients of the polymer material," explains Anna Musyanovych. The researchers then trigger the formation of the polymer, which encapsulates the pigments. Some manufacturers of printer ink are already using this method to prevent the pigments from agglomerating. Their inks then produce images that have a better resolution, and that do not smudge.

"So we thought: if it works with pigments, it should also work with magnetite," says Landfester. A patient must be injected with a contrast agent in the form of nanoparticles containing fer-

POLYMER PARTICLES IN THE ELECTRON BEAM

Electron microscopes, which have a long history of success primarily with metal, ceramic and biological structures, can be used to determine the shape and size of polymeric nanoparticles and nanocapsules. They can also image chains of molecules, for example, protruding from the surfaces of the particles. The polymer scientists in Mainz use three transmission electron microscopes (TEM), which illuminate the sample with an electron beam and thus also provide information about the internal structure of the nanoparticles, for example about the thickness of capsule walls. They also use two scanning electron microscopes (SEM) in which the electron beam scans the surface of the sample and produces a topographical image.

Integrated X-ray fluorescence and electron energy loss spectrometers also provide insight into the distribution of elements in the nanoparticles.

It is difficult to study polymer spheres in the electron beam, however, because it destroys the polymers more or less quickly. Ingo Lieberwirth's microscopy team is one of the few in the world that specializes in the microscopy of soft matter. This requires throttling the beam of electrons down to an extremely low intensity. They also fix the polymers so that they can withstand the electron beam for a longer time.

However, two disadvantages remain: Electron microscopes provide images of only a few particles. Moreover, the particles must be dried or frozen in their dispersion. In order to observe the particles in their natural medium, the researchers in Mainz must use complementary methods (see box on p. 64). The microscopy group is thus working on using high-resolution laser scanning microscopy (STED) for polymers.

The smaller the object, the bigger the microscope: Ingo Lieberwirth investigates nanoparticles with a two-meter-tall transmission electron microscope, which illuminates a sample with an electron beam.

rous, and thus magnetic, magnetite before a doctor can examine his liver by magnetic resonance imaging, for example. Pure magnetite particles need a protective capsule because they are not stable in water, and thus not in blood, either, and they can also dissolve.

Packaging them in a miniemulsion makes it possible to send the particles into the body as magnetic probes for different types of tissues and tumors. To this end, the chemists in Mainz must equip the nanoparticles with antibodies that act simultaneously as address labels, anchors and door openers for specific body cells. They therefore encapsulate the contrast agent in a polymer capsule with chemical hooks protruding from its surface. The antibodies can then adhere to these.

A LAYER THAT MAKES THE PARTICLES HYDROPHOBIC

The packaged particles of magnetite also need a stealth layer so that the immune system guards do not fish the foreign particles out of the blood vessels on the way to their target cells. The cornucopia of polymers also provides the researchers with a suitable material: polyethylene glycol, PEG for short, which does not set alarm bells ringing in the immune defense system. The researchers then also stir constituents with PEG appendages into the magnetite packaging mix. The finished particles now not only carry hooks for the biochemical address labels, but also camouflaging PEG chains.

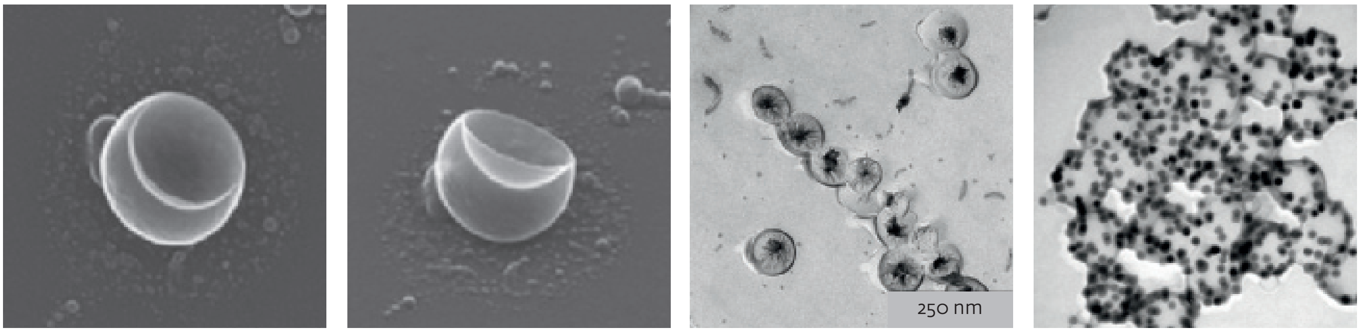
There is only one problem with the idea of ingenious multifunctional packaging for the magnetite particles: the particles initially oppose any type of polymeric capsules. If Anna Mus-

VIEW OF NANOPARTICLES IN THE WATER BATH

Wall paint, printer ink, drugs or contrast media – in practice, nanoparticles are usually used in dispersion form. Their size and shape in the liquid medium can be determined with different methods of light scattering, for example. The scientists in Anja Kroeger-Brinkmann's analytical group are specialists in this field. The methods are based on illuminating a dispersion of the nanoparticles with a laser beam. The nanoparticles then scatter the light. The intensity and direction of the scattered light depends on the shape and size of the particles.

Dynamic light scattering is one method that allows reliable statements to be made about this. The researchers analyze the fluctuations of the scattered light, which resemble a TV picture with poor reception. This is a function of the Brownian motion of the particles, which in turn is a function of the size. When the researchers analyze the scattered light deflected toward large scattering angles, this technique can also tell them something about the inner structure of the nanoparticles. To this end, they are now working on developing the method further. If a dispersion contains mixtures with different particle sizes, the particles must first be separated or sorted before reliable statements can be made about their size distribution.

One of the methods the researchers use is flow-field-flow fractionation: they pump a dispersion of the particle mixture through a flow channel. A second current, the cross-flow, meets the channel current at a perpendicular angle. This current forces the larger particles more strongly toward the channel wall than it does the smaller ones. Under the influence of this force field and the counteracting diffusion, the different sizes of particles separate. The particles sorted in this way can then be analyzed with light scattering methods.



left | Capsules with openers: The researchers in Mainz integrated chemical ingredients into the polymer capsules of these nanocontainers, and the ingredients escape in the form of gaseous nitrogen when the temperature rises. The capsule becomes perforated.

right | The solid inorganic particles can be packaged individually or collectively, depending on whether they will be used as dyes, coatings or medical contrast media. But sometimes the particles have to be smuggled in with chemical tricks.

yanovych were to mix them with oil, water and the other constituents, they would then float only in the aqueous part and not in the oil droplets: magnetite clearly prefers to spend its time in water. "We must therefore hydrophobize the magnetite particles," says Anna Musyanovych. The chemist reverses the preference for an aqueous environment into a preference for an oily environment by first surrounding the magnetite particles with a film of oleic acid before packing them into the multifunctional capsule.

If the nanoparticles allow themselves to be specifically sent to particular cells, it stands to reason that they could also be used as drug carriers. But active substances that are encapsulated in solid polymeric spheres are of no help here. They really need to arrive at the place they are to do their work in liquid form. And there is the additional point that some contrast agents for magnetic resonance imagers are available only in liquid form. "Our next thought was thus to encapsulate a liquid," says Katharina Landfester. And an aqueous one, if possible, because the capsule contents with a dissolved active agent should ultimately mix with the aqueous medium of the cell interior.

At that time, the researchers already had a solution ready by which to create polymers in droplets of water: the inverse miniemulsion. It consists primarily of oil containing floating droplets of water. The starting materials of the polymer that form in the droplets must now dissolve in water instead of oil. And there are plenty of these substances around, as well.

AN ORDERLY WAY TO BUILD DATA STORAGE SYSTEMS

When the chemists want to equip the water droplets with a solid capsule, they are also helped by the fact that many polymers precipitate out of the liquid as solid particles as soon as they are formed. This does not usually occur at the edge of the droplet, but as minute polymer grains in the interior of the liquid droplets. But the rich diversity of chemistry provides a helping hand here: some polymers are formed from one constituent that dissolves in the aqueous liquid, and another that prefers an oily environment. The two come together to form a polymer chain only at the boundary of the liquids – in other words, at the edge of the droplet, so the growing polymer forms a capsule all by itself.

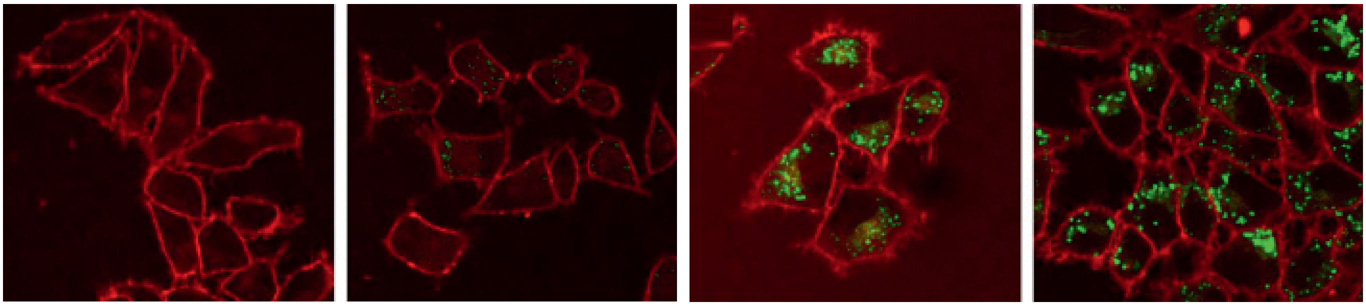
This capsule can thus be used to smuggle an active substance into, for example, a tumor cell – all that is missing is an opener to release the agent. But of course chemistry has a solution for this problem, too. In fact, it has several, depending on whether the capsule can be opened through an increased temperature, through a change in the acid-base properties of the surroundings, by enzymes or by UV light. The chemists working with Katharina Landfester provide the polymeric capsules with simple, predetermined chemical breaking points that release the contents when the appropriate mechanism perforates the capsule.

The development of the magic spheres has so far gone more or less according to plan. "One way or another, all the stages have usually worked just as we thought they would," says Katharina Landfester. There was only one point when it almost looked as if a project would fail. "We wanted to use nanoparticles for nanolithography," explains the researcher. The chemists tried to use nanocapsules to produce nanodots of a metal salt on a substrate, namely in a regular pattern that forms by itself. Such metal salts can be transformed into metal spots that could be used for data storage.

But the particles did not initially display any noticeable sense of order: "We first formed nanocapsules with a metal salt solution," explains Landfester. The capsules formed a regular pattern on a substrate, but as soon as the researchers dried them out, the salt did not deposit in an orderly pattern.

The result was the same for the attempt to encapsulate metal salt in polymeric material, distribute the filled grains over the surface and then etch away the polymer with a plasma beam. "In the beginning, nothing we tried

Photos: MPI for Polymer Research (4)



Many keys, easy access: The more functional groups the fluorescent nanoparticles carry, the easier it is for them to gain access to the cells. From left to right, their proportion in the shell increases from 0 to 10 percent.

worked out," recalls the chemist. But the researchers then dissolved the salt in the polymer material. Now, when they removed the polymer using the plasma beam, the spheres shriveled up like shrinking balloons until finally – precisely in their center – the spots of metal were left behind. The chemists now control their size via the amount of salt in the polymer, and the distance between them via the size of the polymer spheres.

They can now conjure up all sorts of capsules and particles for different purposes. Simple capsules could slowly release perfume in washing powder, or biominerals in toothpaste to regenerate damaged tooth enamel.

POISONOUS PARCELS FOR TUMOR CELLS

For doctors at the university hospital in Ulm, the chemists in Mainz have prepared more complex nanocapsules that could help heal damaged tissue. The nanocapsules contain substances that stimulate the stem cells to differentiate. The doctors smuggle stem cells and nanocapsules into the diseased tissue, where the nanovehicle's load stimulates the stem cells to form healthy heart tissue. As part of the same project, the Mainz-based chemists have also packed fluorescent substances and magnetic contrast media into nanoparticles that penetrate into the stem cells. The doctors in Ulm use suitable microscopes and MR imaging methods to enable the particles to show them the route the stem cells follow in the tissue.

Katharina Landfester's team has designed a container for active substances for biotechnologists at the University of Stuttgart. The container is designed like a hazelnut and could fight breast tumors. Its core is formed by a nanoparticle whose surface is coated with a strong toxin. The chemists encapsulate the core and address the toxic parcel to the cancer cells using appropriate antibodies so that it attacks only the tumor – but this it does with that much more vigor. Healthy cells are largely spared by the poison. The pharmaceutical industry would have to develop the active substance container further for it to become a marketable drug. "In my view, people here have become less willing in recent years to take risks involving approaches that are still at the basic research stage," says Katharina Landfester.

Maybe particles from several capsules will also be used in genetic engineering, or even in gene therapy. Such particles could serve as a vehicle for DNA or RNA. The outer shell could carry the door opener for the cell, while the inner shell could give the particles access to the nucleus. The colloid researchers are working with researchers from the University of Mainz to investigate the best way to smuggle genes into the genome. They are currently studying what form the surfaces of the particles must take in order to penetrate into the nucleus.

A similar problem confronts them in their attempts to smuggle nanoparticles through the blood-brain barrier. These physiological barriers protect the central nervous system from invaders and also prevent most drugs from gaining access. This is why many potential drugs for the treatment of nervous diseases are thwarted here. The polymer researchers in Mainz are now doing all the fine-tuning they can to provide their particles with the means to access the brain. Katharina Landfester suspects that, here too, the surfaces of the particles are key.

"I do think we should take another close look at the chemical details of the blood-brain barrier," she says. Medical researchers have concentrated too much on the system as a whole, she thinks. She therefore wants to approach this problem from the same point of view that once revealed the potential of the miniemulsions – namely the point of view of the colloid chemist. ◂

GLOSSARY

Miniemulsion
A mixture of oil and water in which droplets of one liquid float in the other in a fine dispersion. Unlike conventional emulsions, ultrasound tears the droplets apart and makes them a fairly uniform size on the nanoscale. Moreover, the miniemulsion is stabilized by the osmotic reagent: a substance that dissolves almost exclusively in the droplets and prevents larger droplets from forming at the expense of the smaller ones.

Polymers
Includes all synthetic materials. They consist of chain-like or reticular molecules comprised of building blocks of a monomer, or sometimes different monomers. They are distinguished by their starting materials and their chain type.

Surfactant
A substance whose molecules have a water-soluble and an oil-soluble end.

Photos: MPI for Polymer Research (4)