Nanoparticles – invisible threat?

TEXT: Rainer Klose and Selina Chistell / PICTURES: Empa, iStockphoto, agefotostock

The production of tiny particles and their industrial use is deemed a key technology of the 21st century. Nanoparticles revolutionize many applications – from industrial products such as sunscreens or waterproofing agents for wood all the way to therapeutics. However, their manufacture, usage and disposal can also harbor risks for humans and the environment.

In order to pinpoint and minimize these hazards, but also to seize the opportunities the technology has to offer, the Swiss National Science Foundation (SNSF) launched the National Research Program "Opportunities and Risks of Nanomaterials" (NRP 64) in 2010. The five-year interdisciplinary program is due to finish at the end of 2015. Empa has played an instrumental role in NRP 64 and is in charge of 5 of the 23 projects.

Before the results are published next spring, we'd like to seize this opportunity to take stock. In the following pages, we present the five Empa projects and some of the results achieved so far.

Final destination: sediments

Nanoparticles are already found in numerous consumer products such as cosmetics and textiles, and enter the wastewater when we wash and shower. From there, they gradually spread in nature. What impact do they have on various ecosystems? Where do the particles accumulate? Empa researcher Bernd Nowack and his team set about studying material cycles in Australia.

Tracking nanoparticles in the environment is no mean feat: Currently, there aren't any methods to determine trace concentrations of nanoparticles in environmental samples, so researchers have to trace material flows and perform computer calculations. One interesting model region is South Australia. Firstly, the region is highly developed; secondly, it recycles urban wastewater and uses sewage from municipal treatment plants to fertilize fields. As it rarely rains there, barely any of the sewage is washed into the rivers. Hence, a kind of "closed loop" for nanoparticles exists.

In a study published in the journal Environmental Science: Nano, a team headed by Bernd Nowack calculated the annual mass flow for four different nanoparticles on fields and in sediments from various bodies of water. The model calculation revealed that 54 tons of nano-titanium dioxide, 10 tons of zinc oxide, 2.1 tons of carbon nanotubes, 180 kilograms of nano-silver and 120 kilograms of fullerenes – so called buckyballs – are processed in South Australia each year and eventually end up on the market as components in other products.

The fate of the particles varies greatly: fullerenes and carbon nanotubes are predominantly used for synthetic composites. These particles remain embedded in the synthetic parts and wind up with – or rather in them – on a garbage dump. Nano-zinc oxide, which is used in cosmetics, for instance, is chemically converted into other zinc compounds in sewage treatment plants. As a result, the nano-effect is lost and it can no longer be distinguished from "normal" zinc. It's a similar story with nano-silver, which is turned into silver sulfide – a black metal salt that is not readily soluble and is also formed from "normal" silver.

Nano-titanium dioxide – a popular component in sunscreens – on the other hand likes to go walkabout. Titanium dioxide itself is a non-toxic, white substance used in its conventional form in white wall paint and toothpaste. Titanium dioxide nanoparticles are extremely stable. According to the model study, almost three tons of South Australia's annual consumption (around 5.5 percent) ends up in the ocean. The rest is sprayed on the region's fields in the form of sewage or compost. The model calculation reveals that the concentration in certain soils rose from 9.5 micrograms per kilogram of soil to 450 micrograms per kilogram in just seven years – that's over 40 times more. Whether this permanent "disposal" of nanoparticles in the soil affects health or the environment, however, remains to be seen.

According to the researchers, not only will it in future be necessary to calculate the nanoparticles' path in dry regions like South Australia, but also the transportation of the particles in rivers and marine sediments. This is the only way to assess exactly where and in what amounts these materials eventually accumulate. //

Every sunbathing session spreads nanoparticles in the environment. The long-lived particles, which contain titanium dioxide, eventually end up on agricultural land and in sediments in the sea.

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Gone with the wind

Waterproofing agents that contain copper salts protect wood against rot and wood-destroying fungi. They have been used all over the world for more than a century. Compounds with copper nanoparticles have been on the market in the USA since 2006 and thousands of tons of these sprays are used every year. But what happens when this timber is recycled or does eventually rot?

Children's playgrounds, walkways, fences, poles and wooden pylons along the roadside all need to be protected against wood-destroying fungi. There is only one substance that combats soft rotting fungi from the soil: copper. Back in the early days, toxic solutions such as copper vitriol, chromated copper arsenate and copper chrome boron were used. Meanwhile, wood is waterproofed with copper carbonate or copper citrate (the copper salt of citric acid), which inhibits the metabolism of fungi but is harmless for mammals. Various wood preservatives containing copper carbonate particles that measure between one nanometer and 25 micrometers have been on the market in the USA since 2006. According to the manufacturers, the particles soak deeper into the wood during waterproofing than conventional liquid copper salt solutions, which is why the protection they offer lasts longer. The manufacturers would now like to expand their business on the European market, where refractory types of wood, such as Norway spruce (Picea abies) or Swiss pine (Abies alba), are primarily processed.

The problem: there are also wood-destroying fungi that are copper-resistant. These fungi from the pore sponge family (such as the Antrodia, Postia and Serpula species) store the copper in their cells and shut it away, which begs a question: can the fungi also store copper nanoparticles, multiply and eventually scatter the nanoparticles in the environment in their spores? After all, humans breathe in between 20,000 and 30,000 fungal spores every single day. Empa researcher Chiara Civardi set about investigating this hypothesis with support from Peter Wick, a specialist in nanoparticles, and Francis Schwarze, an expert in wood fungi.

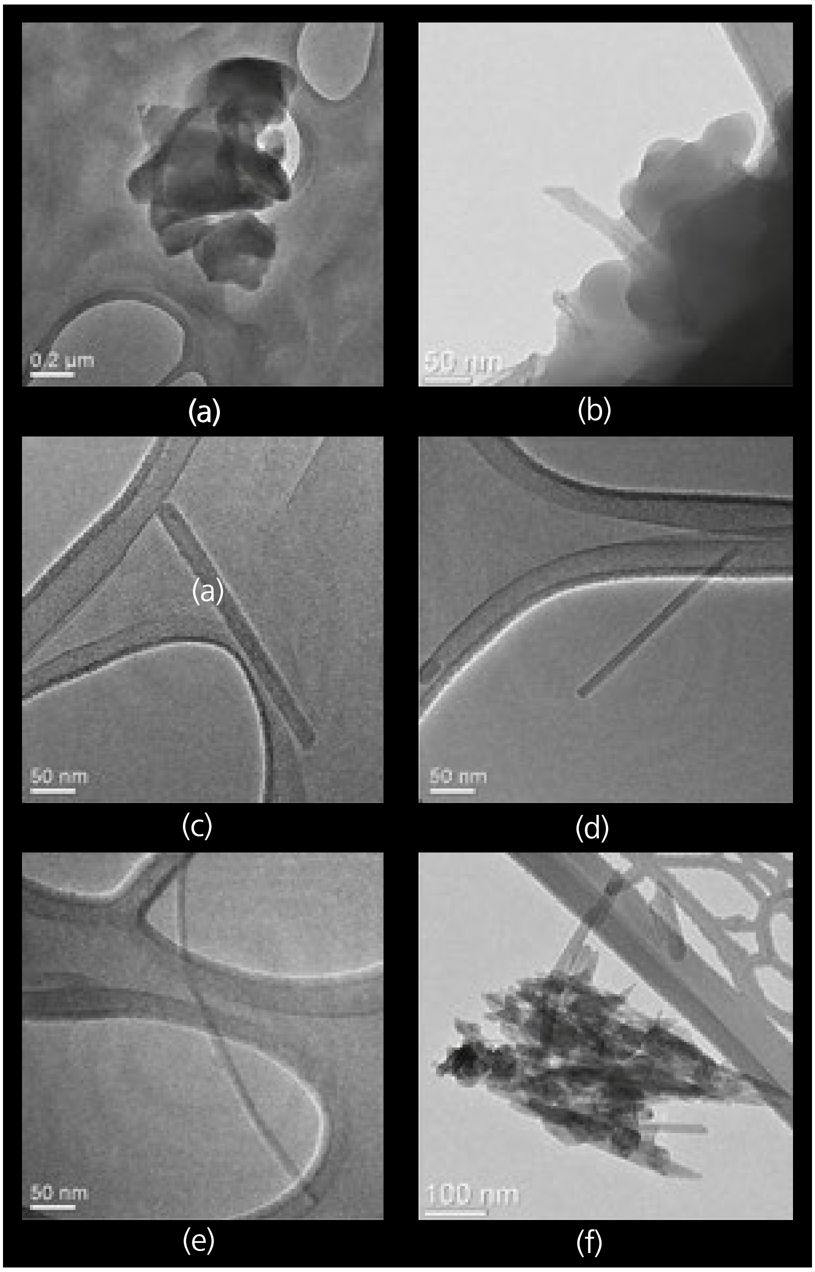
Their first move was to treat spruce and fir wood with the novel waterproofing agents and incubate it with fungi. The researchers then examined questions such as: does the fungus absorb the smallest particles, which are one nanometer in size? Or only the larger microparticles? Do the particles actually penetrate the wood more effectively and, if so, does this increase the wood's durability? The team's next move was to follow the "fate" of the copper particles absorbed by the fungus. How quickly do the particles dissolve in the fungal cells? Do they get into the spores? Do they leave the waterproofed wood and enter the air, soil or even the food chain? //

Wooden telephone poles are still a common sight, especially in the US. As they are meant to last for decades, they are waterproofed with copper nanoparticles, which are supposed to keep rot fungi from the soil at bay. But where do these nanoparticles end up when the wood does eventually rot?

When polymers bare their teeth

Embedded in synthetic polymers, carbon nanotubes convey new properties to the composite material: the part becomes more break-proof and conducts heat and electricity a lot better. But what happens if the part is sawed, sanded or drilled? Are carbon nanotubes released in the process? And if so, is this harmful to our health?

Many synthetic materials are reinforced with carbon nanotubes. If the surface is sanded or holes are drilled in the material, parts of the nanotubes are exposed. They are clearly visible under the electron microscope.



There is a wealth of toxicity studies on free carbon nanotubes (CNTs) and many more on dust formation when CNT-reinforced synthetic polymers are sanded. A number of research teams analyzed this dust in cell and animal experiments, and failed to detect any additional health hazard compared to normal synthetic dust. However, nobody counted exactly how many CNTs are actually released during the sanding and sawing process.

An Empa team headed by Jing Wang and Lukas Schlagenhauf has now succeeded in doing so for the first time. For their tests, the researchers added a certain quantity of lead ions to industrially manufactured CNTs and created a CNT-reinforced synthetic polymer. The test block was then rubbed down using a technique that Schlagenhauf already developed three years ago: he combines a standard scientific sanding device with a special suction system to capture the released dust particles.

Finally, the dust is treated with acid, which releases all the lead ions from the exposed CNTs as only free CNTs come into contact with the acid. Nanotubes that are still completely enclosed by the synthetic material mixture, on the other hand, do not give off any lead ions. Consequently, it is possible to accurately quantify the CNT dust for the first time; the amount of lead ions in solution is proportional to the number of exposed CNTs.

The researchers then used an electron microscope to verify the dust particles and document the free or partially exposed CNTs. Finally, they tested the dust on various cell cultures. The result: CNT dust is not acutely cytotoxic, which the researchers attribute to the fact that only very few free CNTs were detected in the dust particles. The connection between toxicity and the dust's surface properties was, therefore, established for the first time. Possible long-term health effects, however, are yet to be studied and cannot be ruled out.

In the next step, the researchers would like to investigate the mechanisms that release the nanotubes. They want to compare different material mixtures and analyze the dust at higher temperatures. //

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Shuttle service through the placenta

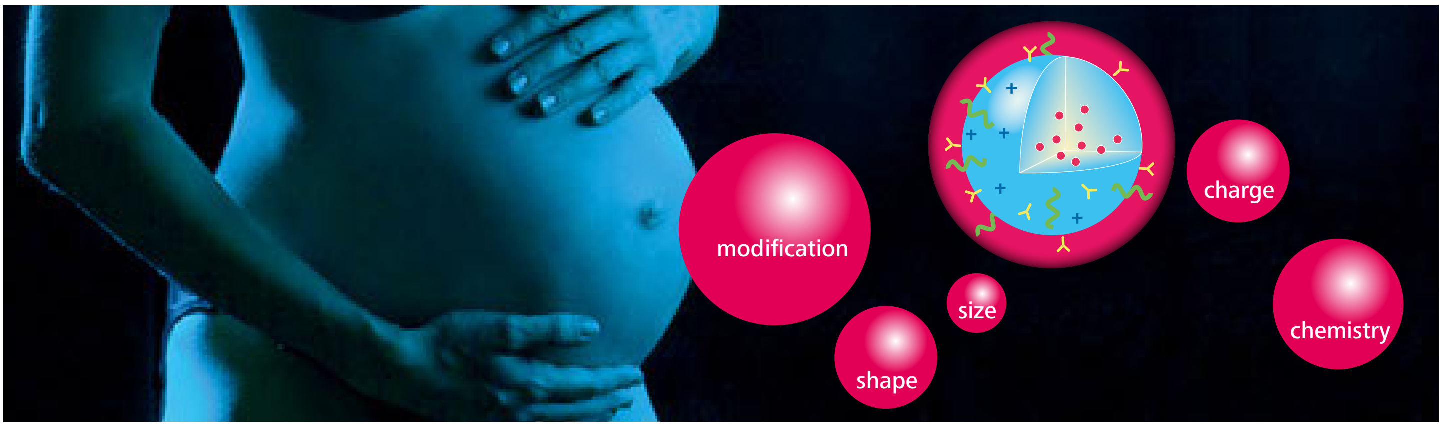
Barely a few decades ago, the placenta was regarded as an impermeable barrier between mother and child. Ever since the sleeping pill Contergan caused deformities, however, we know better. Nicotine, heroin and various environmental toxins also get through to the fetus. Does the same hold true for nanoparticles?

The placenta is a complex organ that is responsible for the exchange of oxygen and carbon dioxide between the mother and child, as well as the transport of nutrients and metabolic products. But it also keeps the mother's bloodstream separate from her unborn child's. Anyone who wants to study how the human placenta works can use data from animal experiments only to a limited extent as the placenta functions very differently from one species of mammal to the next. One alternative is to conduct research on an *ex vivo* model, i.e. on placentas that mothers donate for research purposes after a C-section. Thanks to nutrient solutions, the organs can remain intact for several hours and document the transport of substances through the tissue. This ex vivo study method was first applied in the early 1970s and has been honed continually ever since.

Peter Wick and his team joined forces with MDs from the University Hospital Zurich and Kantonsspital St. Gall to investigate whether tiny polystyrene particles are able to pass through the placenta. The result: particles up to 80 nanometers in diameter passed through the barrier and would have reached the fetus. 500-nanometer particles, however, were stopped in their tracks. What's more, the team also discovered that the nanoparticle shuttling is not passive diffusion. In other words, the particles don't simply seep through the tissue, but are actively transported through the placenta via a mechanism that is yet to be elucidated. A considerable proportion of the particles accumulates in the so-called syncytium, the first cellular barrier layer.

Besides experiments using polystyrene particles, which remain chemically unchanged in the body, the researchers are now looking to study the transport of metal oxide particles or other chemically active substances. The goal is not merely to understand the exchange mechanism of the human placenta, but also to recognize rules with a view to using nanoparticles diagnostically or therapeutically in future. If the mother falls ill, for instance, the drugs could thus be prepared in such a way that the active substances are only administered to the pregnant woman and not to the unborn child. //

The placenta isn't a completely impenetrable wall. Alcohol and drugs can get past the barrier between the mother's and child's bloodstreams and damage the fetus. Nanoparticles of a certain size also pass through the placenta while others are stopped in their tracks. Might this effect be used to encapsulate medication so that only the mother actually receives the drug?



Bone replacement from nanofibers

Bone replacement implants need to be made of biodegradable materials to enable the body to incorporate them into its own bone tissue and eventually replace them during the healing process. Ideally, the replacement material should be as solid and resilient as real bones. Are nanofibers the answer? And how does the immune system react?

Can bones be fashioned from nanofibers? Pieces of bone can be lost through disease or accidents. These need to be replaced with an implant, which dissolves in the body. The problem: as yet, no such material exists.

If part of a bone is lost in an accident or due to illness, the missing piece can be replaced with artificial bone material. The bone substance is constantly built up and broken down again in the body, with the result that our entire skeleton is completely renewed within a few years. This is the only way the body can grow and adapt to physical strains as the bones don't become brittle as rapidly and bone fractures generally heal well.

A bone replacement material is supposed to "join in" this physiological construction and breakdown process and, in an ideal scenario, eventually dissolve and be replaced with the body's own bone. In the past, bone was removed from the patient's iliac crest and implanted for minor defects. Larger defects were frequently replaced with bone that had been removed from corpses, sterilized and used as an implant. Research is on the lookout for more ethically acceptable, synthetic materials made of ceramics. So far, however, both the strength and breakdown of the replacement material in the body has posed a problem in larger quantities.

Katharina Maniura's team examines the interfaces between synthetic materials and biological systems. Teaming up with the RMS Foundation and researchers from the University of Bern, the Empa team studies nanofibers made of biodegradable polymers. Such fibers could also give ceramic bone replacement cements the crucial break resistance that such a material needs.

In the first part of the project, thin fibers were produced in collaboration with Empa's textile experts. The fibers are only 200 nanometers in diameter and made of polylactide – a synthetic material that is broken down into harmless lactic acid in the body. The fibers are then mixed with ceramic nanoparticles and cut up in an ultrasonic bath to produce nanofibers that resemble short staples and hold the bone replacement material together more effectively.

The next step is to add these fibers to calcium phosphates and mix them into a cement. Calcium phosphates are also broken down by the body and converted into bone substance. Tests on cell cultures and animals are expected to reveal that these biodegradable composite materials are highly biocompatible. The aim of the research project is to produce molded bodies, supports, and even plates and screws from biodegradable yet solid material. Such an implant wouldn't need to be removed once fitted – which spares the patient a second operation. //