

A Trojan Horse in the Wound Dressing

Operation successful – patient dead. In German hospitals alone, 30,000 patients die every year from antibiotic-resistant infections that attack injuries and wounds or develop on implants. Researchers working with **Renate Förch** at the **Max Planck Institute for Polymer Research** in Mainz aim to outwit these bacteria with the help of specially coated dressings.

TEXT **CHRISTIAN MEIER**

The idea comes directly from medical practice – or more precisely, from the South West UK Children’s Burn Centre in Bristol, where pediatrician Amber Young treats hundreds of children with burn and scald injuries every year. And every year, the number of children seeking treatment increases. The cause of their injuries is often, for example, a boiling-hot cup of tea or coffee that the children tried to retrieve from an unreachable kitchen counter.

Time and again, the parents end up returning to the hospital with the children after they have been discharged because the young patients have developed a high temperature. This may be a harmless immune reaction, but it can also be a sign of a bacterial infection

that may prove fatal if the dressing is not removed and further treatment administered. The doctors can only guess, and face a difficult decision based on this: change the dressing or not? Changing the dressing means torture for the young patients – something the doctor would really rather not have to inflict.

INNOVATIVE DRESSINGS AS AN INFECTION INDICATOR

Amber Young wondered whether it might not be possible for researchers to develop a dressing that would reliably indicate whether an infection had set in. Or, even better, a dressing that would treat the infection before it can become established and force the child to return to the hospital.

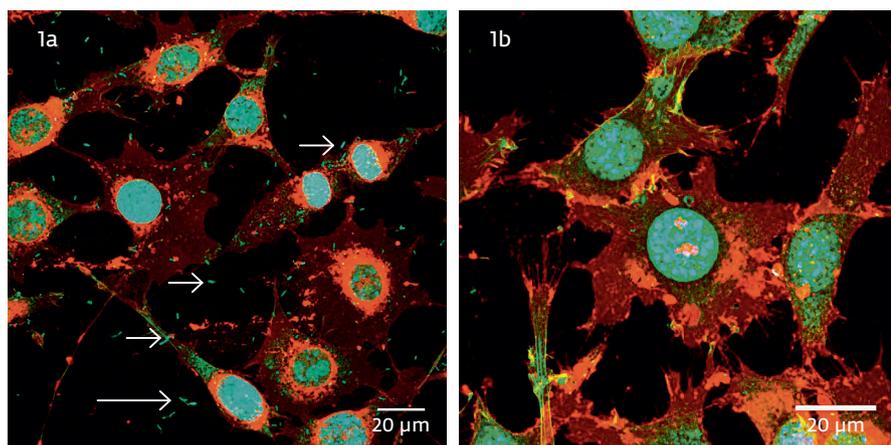
The pediatrician’s request found its way to the chemists working on the *Embek1* and *BacterioSafe* EU research projects, which are coordinated by Renate Förch from the Max Planck Institute for Polymer Research in Mainz. The scientist’s previous work involved the application of printable coatings to plastic cups and bags and technical textiles. Plastics also play an important role in her new research field: “Plastic bags and wound dressings are often made of the same material,” says Förch. Although they look and feel different, they both consist of such materials as polypropylene, polyester and nylon.

As part of the *BacterioSafe* project, the researchers are developing quasi-intelligent dressings and bandages that can indicate that a wound is infected



Dressing without bacterial protection: Burns are treated using Smith & Nephew's Biobrane dressing. The researchers from the *BacterioSafe* project are developing a dressing that prevents, or at least indicates, bacterial infections.

- 1 | Comparison of bacterial growth: (a) Both human cells and bacteria (arrow) grow on a biocompatible coating. If the surface releases zinc, it prevents bacterial growth but allows for the growth of healthy cells (b).
- 2 | Renate Förch and Martin Heller coat titanium, which is used in implants, with an antibacterial covering in the glass plasma reactor. The plasma contains the components of polymers, which react to form the polymer coating on the metal.



by releasing dyes. What's more, they can kill bacteria through the targeted release of antibacterial substances. Renate Förch firmly believes that this can be achieved with the help of nanotechnology. Therefore, although the *BacterioSafe* researchers are pursuing Amber Young's objective – the pediatrician is also a member of the consortium – the problem that the group would also like to contribute to resolving goes much deeper: the fact that the traditional weapons against bacterial infection, antibiotics, are becoming less and less effective.

NEW WEAPONS FOR FIGHTING RESISTANT PATHOGENS

The hospital-acquired infections *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which infest healing injuries and clinical wounds and cause infections, are a particular cause for concern. Almost one-third of bacterial strains are resistant to antibiotics. The German Society for Hospital Hygiene (DGKH) estimates that, in Germany alone, such

resistance costs the lives of 30,000 patients annually. The pathogens are also introduced to patients through implants. For example, some patients with artificial hip joints have to endure several operations due to bacterial infections at the site of the implant.

"We need new weapons against resistant pathogens," summarizes Förch. "If possible, weapons that kill infections at their onset." What the researcher has in mind are materials or implants with coatings that release antibacterial substances in the early stages of an infection.

The substance favored by the Mainz researchers is zinc. As part of the *Embek1* project, the team developed a polymer coating that releases the heavy metal. "Heavy metals are a potent weapon against bacteria," says Renate Förch. Silver and zinc kill bacteria and are thus good candidates for spearheads in the war against hospital pathogens. Zinc is particularly suitable, as the heavy metal has a concentration range in which it is toxic to bacteria but not to humans – even after prolonged application. Zinc

also has a clear price advantage over silver. However, it remains to be clarified whether the long-term use of zinc eventually leads to resistance.

A NANO-LAYER STIMULATES CELL GROWTH

The Mainz-based scientists have already produced the first coatings, for example for titanium plates used by physicians to treat bone fractures. The scientists have even succeeded in coating the screws with which the plates are attached to the bone with a nanometer-thin polymer layer that has zinc nanoparticles embedded in it. The method used to apply this coating is called the plasma process. "It can be used to coat any three-dimensional structure," explains Förch.

Doctoral student Martin Heller demonstrates how the plasma process works. He turns a valve that connects a pear-shaped glass flask with a much larger glass tube around which a cable is wound in a regular spiral. The latter contains a small titanium screw that is to be coated. "Molecules known as monomers are now streaming out of the small flask into the bigger one," says Martin Heller. He then turns a regulator. "And now I'm setting it at 100 watts."

Alternating current now flows through the spiral, such that an alternating electric field activates the molecules inside the larger flask. The field removes electrons from the molecules, leading to a mixture of charged particles and electrons: a plasma. "The ions are highly reactive due to the loss of electrons," says Heller. The alternating field also activates the surface of the screw, so that this, too, becomes reactive. As a result, molecules bond to the surface and react further with mole-



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» An implant that damages human cells would be useless.

cules from the gas phase, gradually forming a thin surface coating. The monomers consist of a complex compound in which organic molecules surround a zinc ion. The alternating field dissociates the molecules into different reactive components. The organic components bond to form a network of molecular chains, like in a polymer; in this case, however, the chains are of different lengths and form an irregular network of polymer-like molecules called a plasma polymer. The zinc is embedded in its meshes in the form of nanoparticles.

“We also opted for the plasma process because it has already been in use in industry for decades, for example for the modification of polymer webs,” says Renate Förch. “From the outset, we placed particular emphasis on the practical applicability of our developments.” The researchers in Mainz have also proven that the coatings actually

have an antimicrobial effect. They applied a zinc-containing coating in a thickness of 500 nanometers (millionths of a millimeter) to cell culture dishes and seeded the hospital pathogen *Staphylococcus aureus* on them. The bacteria were all killed. In a control experiment, the microorganisms thrived on a control dish with no zinc coating.

HOLLOW SPHERES RELEASE ACTIVE SUBSTANCES

The researchers also tested the effect on human cells. An implant or dressing that could kill harmful bacteria but that would simultaneously damage the patient’s cells would be useless. Therefore, Renate Förch’s team tested whether the antibacterial coating inhibits the growth of the endothelial cells, which play a crucial role in wound healing. Initially, this proved to be the case – a setback.

However, the researchers managed to find a solution to this problem. They added another layer, this time just 15 nanometers thick. It consisted of plasma-polymerized allylamine (ppAA), which has been shown to be biocompatible. The endothelial cells grew even better on this coating than on a base consisting of unmodified titanium.

The scientists solved two problems with this additional coating. Without a coating, the zinc reservoir emptied itself within a period of 24 hours. This period is often too short for practical applications: “Surgeons tell us that the reservoir should last two weeks,” says Förch. The wound needs this long to heal following implantation, and the increased risk of infection also lasts this long. The biocompatible top layer delayed the release of the zinc. “By modifying the thickness of the coating, we are able to control the release period to one, two or three weeks dur-

ing which the zinc is to be released,” says Renate Förch.

In any case, the chemists are pursuing the idea of producing multifunctional coatings by layering several coatings. The ideal coating should not only release zinc over a specified period, but also act as a sensor that detects the presence of harmful bacteria and releases the

active substance only if they are present. At the same time, it must promote the growth of the body’s tissue, and thus wound healing, as well. Moreover, the coating of implants should be such that it either enables or disables biointegration – depending on whether the implant is intended to remain in the body or to be removed at a later stage.

Renate Förch explains why the latter option is sometimes desirable: “In the case of a wrist fracture, a titanium plate must be implanted, but only for a few weeks.” The doctor then has to stand in the operating room for hours, cutting out the implant that has by then been integrated into the tissue, and creating a new wound in the pro-

»» The ideal coating should act like a sensor.



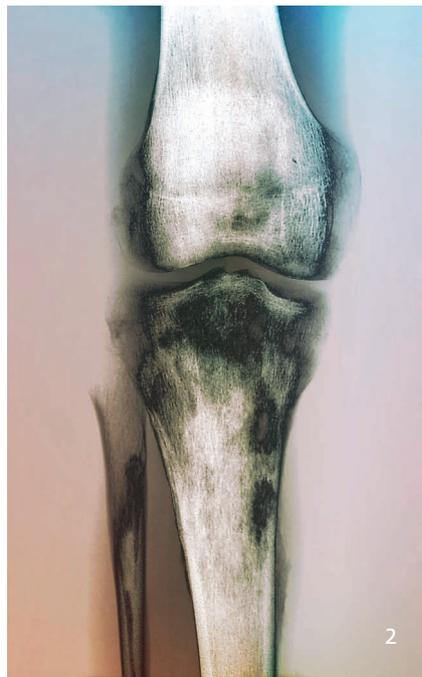
- 1 | Doctoral student Martin Heller turns a coated screw in a pig bone. Afterwards, he will examine under the electron microscope whether the coating on the screw has been damaged.
- 2 | Hospital bugs infected the knee of a 39-year-old patient following orthopedic surgery. The dark areas on the colored X-ray indicate the infected areas.
- 3 | The catalyst for the research project: Young Isambard's burn wound developed a bacterial infection under the dressing. The case involving the young patient at the South West UK Children's Burn Centre triggered the development of an antibacterial dressing.

cess. Förch's team is currently working on a coating that will prevent the integration of titanium implants into body tissue. "This coating will contain a chemical compound containing silicon and oxygen."

ENCAPSULATED ACTIVE SUBSTANCES FROM DEGRADABLE BIOPOLYMERS

There are also examples in which the integration of implants is desirable: Eye implants for the treatment of glaucoma consist of polymer materials and are required not to interact with the tissue, despite the fact that they are intended to remain there permanently. The organism treats them as foreign objects and encapsulates them by surrounding them with a layer of connective tissue. When this happens, the implant, whose purpose is to regulate the internal eye pressure, no longer functions. The Mainz-based researchers aim to change this by applying a polymer coating to the implants, the chemical structure of which resembles natural biological material.

In the meantime, Martin Heller is testing the practical suitability of the zinc coatings on the implant screws. "The layers must not increase the diameter of the screws perceptibly," explains the biologist. He has procured the thigh bone of a pig, because pig bones are very similar to human bones in terms of their solidity. Using a surgical screw driver, he screws an almost one-centimeter long screw into the bone and back out again. He then examines it under the electron microscope for scratches and abrasions, and tests whether the coating was damaged during the process.

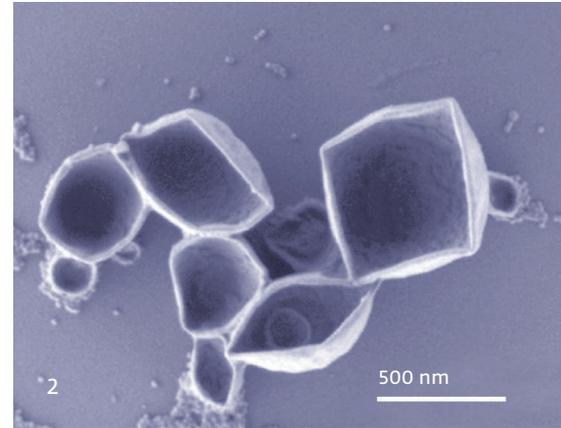


Renate Förch hopes that the first products based on the new technology will be available in five to ten years. The need for them already exists, as evidenced by an e-mail she recently received in which a woman enquires about the findings of the *Embek1* study. Her husband, who has an artificial hip joint, has undergone three hip operations and has suffered from repeated infections. "We also often receive inquiries from industry," says Förch. Although the *Embek1* project has been completed, the team continues to carry out research on the coatings for implants and dressings.

They are also working on the *BacterioSafe* project. "We are re-inventing the Trojan horse at the nano-level,"

says Renate Förch. The Trojans to be outwitted in this case are the hospital-acquired infections. The Trojan horse consists of nanocapsules, hollow spheres with a diameter of around 150 to 400 nanometers, which Katharina Landfester, Director at the Max Planck Institute in Mainz, is developing with her team. The researchers attach the spheres to a polypropylene non-woven, which acts as the basis for a dressing. The role of the soldiers hidden inside the horse is assumed by antimicrobial substances or antiseptics, which are locked inside the nanocapsules.

Although the hospital bugs don't perceive the nanocapsules as a gift, they are outwitted by them. Because the capsules have a shell that consists



of naturally occurring polymers, such as polylactic acid, to the harmful bacteria, they look like natural components of the body. Due to this mistaken identity, the hospital infections channel their aggression against the capsules.

The bacteria secrete toxins and enzymes that would normally attack the cells of the infected tissue. Typical examples are lipases, which readily digest the lipid membrane of healthy cells. This is usually observed in the form of damaged tissue around the site of infection. The substances released by the bacteria also digest the shells of the nanocapsules. The damaged containers then release their contents into the environment, and the antimicrobial substances are liberated.

It is intended to use this effect for different functions in innovative dressings coated with nanocapsules. “First, a dressing equipped with nanocapsules could indicate an infection through the release of a dye by the capsule,” says Renate Förch. Doctors like Amber Young could then be certain that a patient actually has a bacterial infection before subjecting him or her to the torture of changing the dressing. Second, an antimicrobial or another active substance that kills bacteria could flow out of the capsule and attack the pathogens directly. And third, an active substance could also be released that supports the regrowth of healthy tissue.

The *BacterioSafe* researchers have already demonstrated two of the functions using prototype dressings. For the first function, the capsules release a dye when their shells have been digested, as the researchers working with Toby A. Jenkins from the University of Bath demonstrated.

MATERIALS AND PROCESSES FOR INDUSTRIAL PRODUCTION

The British team is also part of the *BacterioSafe* consortium and also demonstrated the second function: In the experiment, the dangerous hospital bugs actually broke into the nanocapsules, but the comparatively harmless intestinal bacteria *Escherichia coli* were unable to do any damage to the capsules. That was good for them, as the capsules contained the antimicrobial substance sodium azide. The aggressive hospital pathogens, in contrast, did fall victim to this substance in the experiment. “The experiment showed that the active substances are released only when they are actually needed,” explains Jenkins. In his view, this could result in the more directed use of antimicrobials or antibiotics and thus reduce the risk of antibiotic-resistant pathogens.

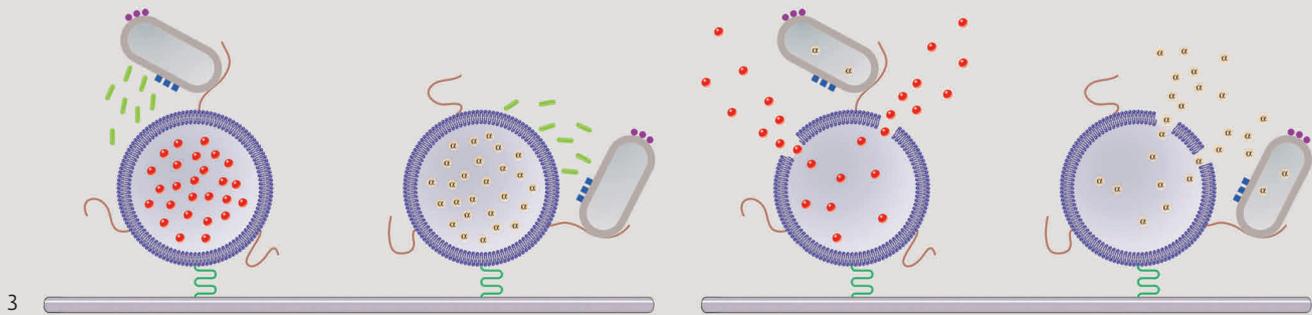
At the Max Planck Institute for Polymer Research, Katharina Landfester’s team is developing other variants of the nanocapsules. Their shells consist of different biological molecules, poly-

lactic acid or hyaluronic acid, which combine to form a polymer-like network. The shells thus consist of degradable bioplastic.

“Predetermined breaking points are broken down by the bacterial enzymes,” explains Landfester. Hyaluronic acid is such a predetermined breaking point: it is broken down by a particular enzyme called hyaluronidase. “The most important strains of the hospital bugs secrete this enzyme in high concentrations,” explains the Max Planck researcher. Therefore, the use of a hyaluronic acid shell is very promising.

Nonetheless, the scientists are testing capsules made of different materials and have a good reason for doing so. First, not every drug can be locked into every shell; for example, a hydrophilic drug needs a hydrophilic shell. Moreover, the researchers are generating a pool of knowledge about the production and properties of different kinds of nanocapsules and finding the answers to a series of questions: How stable are the nanocapsules? Can they be exposed to air or do they have to be stored in water? Can they survive in the conditions that prevail in hot countries?

In addition, different shells can be used for multifunctional dressing materials. If one capsule is placed inside another, like a Russian matryoshka doll, according to Landfester, it is possible



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- 1 | Doctoral students Kerstin Malzahn and Alexander Lotz test different surfaces for their antibacterial effect and influence on mammalian cells. The tests are first carried out in cell culture dishes.
- 2 | Trojan horses in the fight against bacteria: The scanning electron microscope image shows the nanocapsules, which are filled with antibacterial substances or dyes that indicate the development of an infection, and embedded in a dressing.
- 3 | Bacteria think the nanocapsules, shown here as circles, are human cells and infect them. As soon as the pathogens break through the shell, either dyes (on the left) or antibacterial substances, indicated with α , are released.

to trigger a two-stage reaction to an infection. Thus, a dye contained in the external capsule could be released first, followed by an antibiotic contained in the inner capsule.

However, the first task is to look for suitable nanocapsules. "After 18 months' work on the project we have already developed 13 systems, some of which appear to be promising," says project leader Förch. "And the issue of practicality is always at the back of our minds." For example, the scientists use only substances that have been authorized by the US Food and Drug Administration (FDA) for the shells.

The know-how relating to coating using the plasma process is also proving essential for the development of dressings with nanocapsules, as the minute spheres must be firmly attached to the dressing. "To this end, we coat the dressing material in such a way that individual chemical groups protrude like little trees from the earth," explains Renate Förch. The nanocapsules can also be made in such a way that reactive groups protrude from them like anchors. These chemical an-

chors bond with the surface functional groups and fuse the capsule with the surface of the dressing.

"The materials and processes that we select for this coating are suitable for production on an industrial scale," says Förch. "Our aim is to achieve functioning demonstrators." The chemist freely admits that striking this balance between basic and applied research is not always easy. A requirement of EU-funded research projects is that they generate added value for society, so they must have potential feasibility.

On the one hand, the scientists in Mainz are generating basic knowledge

about innovative intelligent dressings and implants; on the other hand, they are ensuring that the results of their research will not be left collecting dust in a drawer. Förch's success with research applications for the EU, which is particularly critical in this regard, is evidence of the fact that their concepts are suitable for achieving this balance: "On average, 90 to 95 percent of applications fail the evaluation. We have been successful with all three of the applications we have submitted to date." Thus, the chances that Amber Young's wish for an intelligent burn dressing will be fulfilled are looking good. ◀

TO THE POINT

- Multi-resistant pathogens often cause fatal infections in operation wounds, healing injuries and implants.
- In the EU projects *Embek1* and *BacterioSafe*, scientists are developing intelligent medical dressings. These are aimed at indicating the presence of infection in a wound by releasing dyes, and killing bacteria through the targeted release of antimicrobial substances.
- The scientists use the plasma process to develop thin coatings for implants, or they package their weapons for fighting the pathogens in nanocapsules.