

Materials from the **Cell Factory**

Nature is a laboratory for particularly intelligent materials. This is what motivates **Joachim Spatz**, Director at the **Max Planck Institute for Metals Research**, to pursue his forward-looking vision of using living cells or their molecular components as a means of making innovative materials. Along the way, he anticipates new discoveries about the behavior of cells in tissues.

TEXT **ROLAND WENGENMAYR**



The rat-tissue cell on the screen searches around frantically for good areas to rest with tiny, fringe-like protrusions, morphs into angular shapes, and does not seem to be comfortable anywhere. The video recording compresses three hours of microscopic images into ten seconds, heightening the micro-drama even further. “Yes, this cell is very unhappy with its environment,” confirms Joachim Spatz, Director at the Max Planck Institute for Metals Research in Stuttgart: “After about 24 hours, these cells even activate their suicide program and die.”

The rat cell’s behavior is completely different in a second video recording. Calm, round in shape and visibly happy, it grows like a fried egg spreading out in a skillet. “It even begins to form tissue – in other words, it manufactures material,” explains the biophysicist and materials scientist. That is, in fact, the biological role of this type of cell. The embryonic fibroblasts form connective tissue while the fetus grows. But the cells do this only when external stimuli tell them they are in the right place to do so.

The reason behind the two cells’ very different behaviors is too small to be seen through the optical microscope in the videos. The cells sit on a kind of nanocarpet that knots together tiny gold particles into a very fine, regular pattern. A layer of peptides – short chains of amino acids – covers the gold particles and turns them into biocompatible contacts for the cells.

The coated gold nanoparticles act like tiny chemical hands: each hand can grasp a certain receptor in the cell membrane. Receptors are large proteins and form the “sensors” for the cells, transmitting information to them. The gold particles affect how adjacent receptors interact during the transmission of information and thus influence their vital functions. By varying the

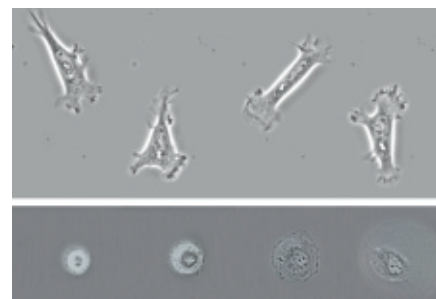
pattern on the nanocarpet, the Stuttgart-based scientists drastically change the behavior of the cells.

Exactly the same thing happens in living tissue – in principle. However, the factors that are especially important in this process are still largely unknown. At the institute in Stuttgart, the scientists want to help solve this mystery with their artificial contacts. Amazingly, the invisible nanocarpet in the two video recordings differ in just a single characteristic: the distance between the gold dots. Whereas 58 nanometers (millionths of a millimeter) make the rat-tissue cells grow happily, 73 nanometers cause them to perish. Just why the cells react so sensitively is what Joachim Spatz and his team want to find out.

Living tissues possess a wealth of properties that materials scientists find fascinating – for instance, their ability to heal injuries. “With the human body, biology created a prototype that can do much more, from a materials science perspective, than we can currently achieve in our synthetic world,” explains the biophysicist. It is thus one of his dreams to employ living cells to create new, “biohybrid” materials. Such materials may be capable of regeneration. Today, research into these kinds of self-healing materials is dominated by fairly non-biological strategies, such as synthetic materials containing liquid adhesive components for cementing microcracks.

ANTI-REFLECTION COATING LIKE A MOTH’S EYE

Yet living organisms are much smarter when they have to repair injuries or need to adapt autonomously to stress. With their complex, extremely variable genetic programs, they are able to react much more flexibly to their environment than dead matter can. If it were to become possible to exploit this spe-

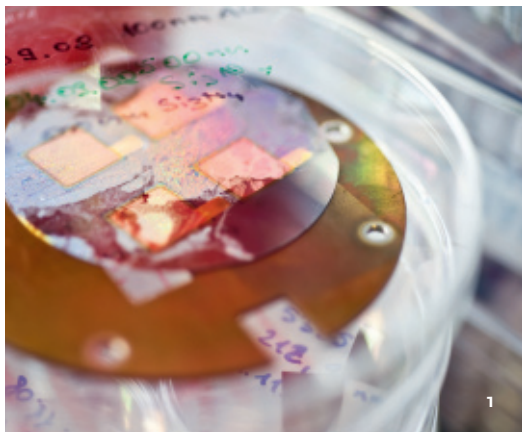


Stressed or feeling fine – the difference is a mere 15 nanometers for these cells: If the gold dots (too small to see) on the substrate are 73 nanometers apart, the cell feels around frantically (top). On a substrate where the distance between gold dots is 58 nanometers, the cell feels comfortable and grows happily (bottom).

cial biological quality to develop materials, such materials could be equipped with a completely new set of properties.

Spatz sketches out his future dream of using synthetic moth-eye structures. His team came up with this invention together with Robert Brunner from Carl Zeiss AG in Jena. One of the things that enable moths to see in the dark is a clever anti-reflection coating. On the surfaces of their multi-faceted eyes, tiny protuberances are lined up side by side like pillars. When the light hits them, they transmit the electromagnetic waves almost without loss from the optically thin air to the optically denser chitin of the lenses. On a completely smooth surface, however, such as window glass, the light comes up against an abrupt change in the material’s properties. As a result, it is partially reflected, with about 4 percent of the light being lost in the case of window panes. Moths’ eyes, on the other hand, collect almost all of the light that hits them.

The scientists in Stuttgart are experts at making these kinds of nanostructures. They developed a method



- 1 | The scientists in Stuttgart use tiny plates with a gold structure as their sensors, here decorated with a layer of miniscule plastic beads.
- 2 | A clean catch: Lisa Maus, doctoral student in Joachim Spatz's department, purifies gold nanoparticles.
- 3 | Discussing how to realize their ambitious concept: Joachim Spatz (right) and Ralf Kemkemer, head of the Central Scientific Facility for Biomaterials (ZWE Biomaterials).

that creates artificial nanopillars on glass surfaces. These surfaces work like moths' eyes and are more efficient than the established anti-reflection coatings on glasses, lenses and monitors. From a technical perspective, the synthetic structure of the material is in many ways superior to a real moth's eye. "Glass can withstand higher temperatures, for example," says Spatz. But as soon as the finely structured surface is damaged, the glass cannot repair itself. This is where bionics or biomimetics – that is, mimicking nature's tricks through technology – reaches its limits. Hence, Joachim Spatz's dream for the future goes much further than this. The question he wants to answer is: "Can we make living cells deposit these kinds of structures onto synthetic surfaces such as glass, and even repair specific damage there?"

This bold vision is one of the central themes in this biophysicist's research work: "It's all about integrating biological functions into synthetic

materials." The conceivable areas in which this concept could be applied are incredibly diverse. They range from synthetic yet biologically compatible "spare parts" for the body in a medical setting to biominerals that are as hard as tooth enamel or as tough as bones, and even to brand new magnetic materials. It may also be possible to make regenerative batteries that generate electricity with the help of microorganisms and a nutrient solution, without ever running out of power. What all applications have in common is their origin: scientists plan to make them in the biofactories of real, living cells. From magnetobacteria to mammalian cells, the researchers have a truly infinite choice of nature's potential material-makers at their fingertips.

If you want to make cells work for you, you have to be able to communicate with them. That's the challenge. However, the language that tissue cells use to keep in touch with their environment is still largely indecipherable, so

the scientists in Stuttgart are concentrating on trying to decipher it. Cells communicate with their immediate environment through many subtle contacts, which they can actively switch on and off. In highly developed multicellular organisms like mammals, the environment we're talking about here consists of a complex network of stabilizing fibers in a basic aqueous substance. The latter contains all of the messenger substances necessary for life, such as hormones and molecular energy suppliers. Biologists call this protective zone around the cells the extracellular matrix.

Researchers claim, with a bit of a wink, that there are four different scientific dialects in which to speak to cells: biochemical, electrical, mechanical and structural. Of course, the importance of biochemistry has long been apparent to researchers. After all, every vital function in a cell is based on biochemical processes. The effect of electrical stimuli has also been the subject of investigations for quite some time.

»» The Stuttgart-based scientists trigger different genetic programs in the rat-tissue cells by varying the distances between the gold nanoparticles.

However, the cells' enormous sensitivity to the subtlest of mechanical and structural stimuli on the nanometer scale is something scientists discovered only recently. And the interdisciplinary team of biologists, chemists and physicists brought together by Joachim Spatz at the Max Planck Institute for Metals Research made a key contribution to this discovery. That's how the two video recordings with the relaxed and the stressed rat cell came about.

A MOLECULAR LADDER FOR A MOTOR PROTEIN

If cells are to move, they require components that act like muscles. The cell's skeleton (the cytoskeleton) takes on this function. Like our own muscles, it contains relatively stiff, strong fibers made of the protein actin. These fibers serve as a molecular ladder for a so-called motor protein, a myosin, to climb. Depending on the direction in which it climbs, the myosin-actin motor pulls an area of the cell together in a certain direction, or stretches it. It is precisely this molecular mechanism that enables our muscle cells to contract particularly strongly (see *MAXPLANCK-RESEARCH* 4/2004, p. 20 ff.).

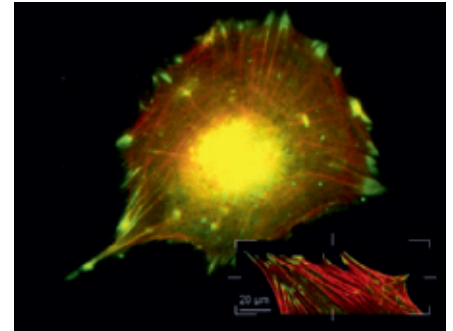
The cytoskeleton becomes active as soon as the cell pushes out parts of its membrane to search the environment for suitable contact points, as in the video recording. In an electron microscopic image with a resolution in the nanometer scale, Joachim Spatz shows what the cell does on the synthetic surfaces made by the Stuttgart-based research group. It puts out tiny little feet, known as filopodia, that reach down to the peptide-coated gold nanoparticles.

If the cell likes its synthetic environment, it acts almost as it would if it were in its natural environment, the ex-

tracellular matrix. It begins with the job it performs in the living organism, producing the building blocks for tissue, for example. But if the environment does not suit it, then it initiates its suicide program. In our body, programmed cell death, known as apoptosis, prevents such things as uncontrolled tissue growth.

The scientists from Stuttgart activate different genetic programs in the rat-tissue cells by changing the distances between the gold nanoparticles. The act of switching between 58 and 73 nanometers apparently sets a mechanism in motion that also occurs in the natural environment of the extracellular matrix. Scientists believe this is caused by the collagen fibers that run through the connective tissue and lend it stability. This framework of fibers is made up of sections that are 67 nanometers long. "That's almost exactly the same as what we're doing," says Spatz. However, they have as yet been unable to furnish conclusive proof that it really is the 67-nanometer-long structure of the collagen fibers that causes a cell to exchange signals with the extracellular matrix. "It's very difficult to prove in the living environment," explains Spatz, "because it's so complex."

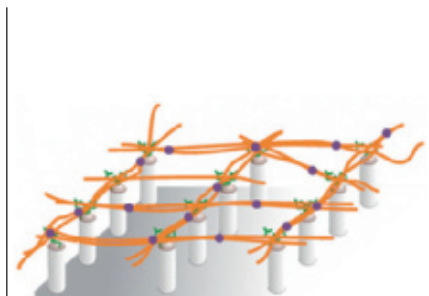
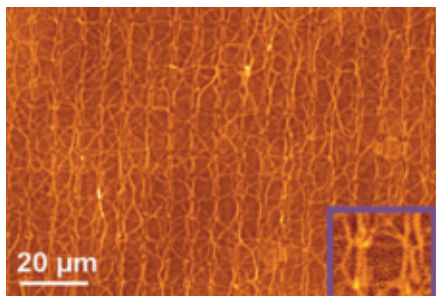
The Stuttgart-based scientists' synthetic pattern structure, on the other hand, has considerably more transparent properties. It could help them find an answer to this question. But to Spatz, even more important is the fact that the structure apparently holds the key to communicating with the cells – what you might call cell whispering through nanocontacts. "If we offer a cell a certain material property in a very precise manner, the cell reads it, processes it and responds to it," says Spatz, "and always in exactly the same way." We are still in the very early days of programming cells in a targeted manner



A network of actin fibers (red) stretches between the green fluorescent contact points in this cell. The detail (bottom right) shows a magnified view of some of the actin fibers and their contact points.

to be harnessed for materials science. That gives scientists working in basic research the freedom – and indeed the duty – to do a great deal of playing around – intelligently, of course! The Stuttgart scientists systematically vary the patterns, as well as other conditions, and observe how the cells react. To speed things up substantially, they test several variants in parallel on biochips in a process known as high-throughput screening.

The scientists from Stuttgart may have already identified the first words in the programming language for living cells. For example, in their experiments with certain tissue cells, fibroblasts, they discovered that they were, in fact, able to switch their protein-making factory between two production modes by varying the contact distance. In a 58-nanometer gold pattern, the cells produce a different kind of tissue adhesive from the extensive family of fibronectin proteins compared with what



Cell scaffolding without a cell: The Stuttgart-based scientists have stretched actin fibers from a chicken heart cell across flexible micro-pillars (left in an image from a microscope, right in diagram form). If the motor protein myosin sticks to the fibers, the scaffolding contracts.

they produce when the distance between contacts is 73 nanometers. “So we really can tell the cell to make this fibronectin or the other one,” says Spatz, clearly thrilled with the discovery. This is a promising start. “In the foreseeable future, perhaps in the next ten years, scientists will be able to make cells deposit certain structures on surfaces,” declares Spatz, looking to the future, “I really do believe that.”

WHAT CHANGES IN DISTANCE CAN A CELL FEEL?

It’s partly because the Stuttgart-based researchers are such experts at making nanostructured surfaces that these are their most important tools. “We can manipulate surfaces in almost any way we want,” confirms Spatz: “That forms the platform for many of our experiments.” And so it was a fairly obvious step to send the cells off along a track studded with gold contacts at distances that change in miniscule nanometer steps. Just how minute a change can the single-celled organisms still feel? It’s like the princess and the pea.

And so the scientists made surfaces with different subtle pattern variations and placed the cells on top. The smallest

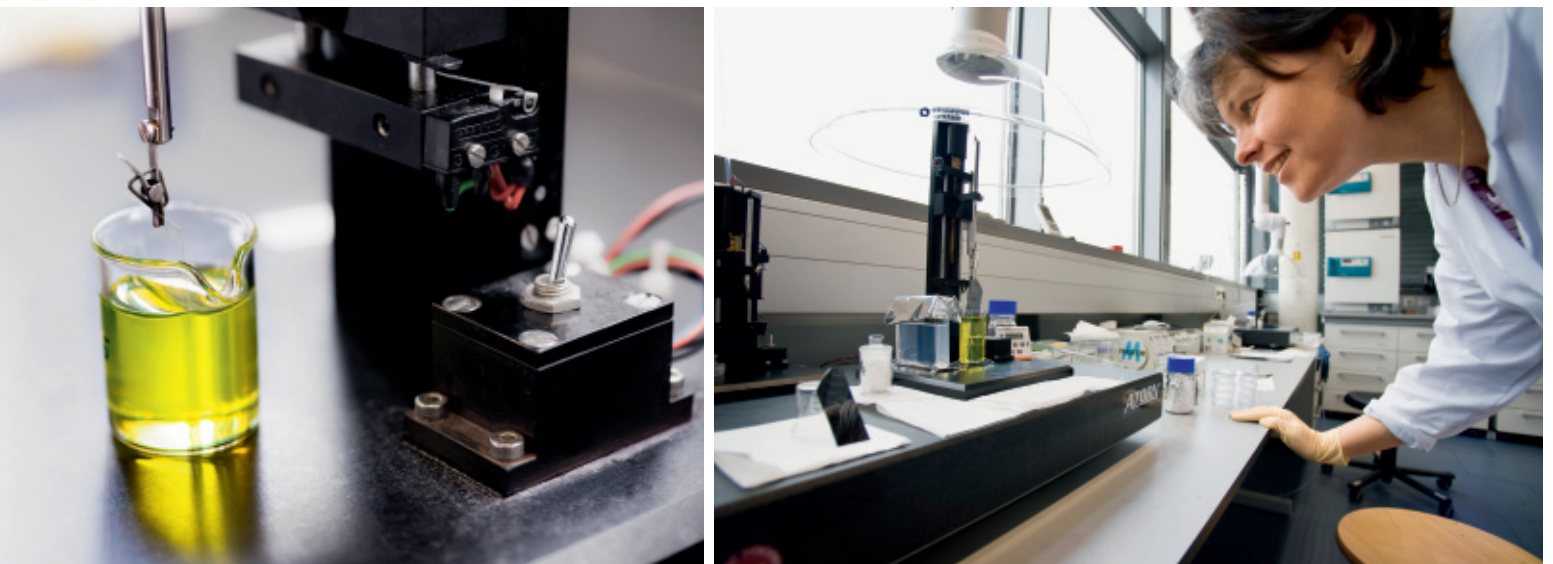
variation in contact distance measured a mere 15 millionths of a millimeter within the space of a single millimeter. Even here, the cells could still feel where they had to go to find a more accommodating pattern distance. “This represents a change of just one nanometer from one end of the cell to the other,” explains Spatz, visibly fascinated: “Thus, in comparison, the cells are much more sensitive than even such highly sensitive measuring instruments as an atomic force microscope.”

The scientists now want to describe – in newly developed theoretical models – how the cells are able to sense changes in their substrate with such an enormous degree of sensitivity. Spatz suspects that, once again, the cytoskeleton is involved. When a cell forms contacts using its filopodia, a network of actin fibers radiates out through the entire cell from these contacts. What the cell does next is a bit like climbers testing whether the next handhold can take their weight: they give it a careful pull. “The cell is constantly tugging at the actin filaments with its molecular motors,” postulates Spatz. In doing so, the cell braces all of the contacts against each other and tests their mechanical stability. The imbalance of forces is

what enables the cell to feel, to an extremely high degree of sensitivity, the direction in which the contacts are positioned more or less densely.

Playing this game of contacts is what led the Stuttgart scientists to a spectacular discovery. Cells taken from older people react to mechanical stress differently than cells from younger people. The discovery was made by Ralf Kemkemer, a member of Spatz’s team, when he subjected human skin cells to different kinds of mechanical stress. Some of the cells were from donors who were younger than 30, and the rest were from people over the age of 50. It’s important to note here that the cells themselves did not differ in age. New cells are constantly being formed in our bodies through the process of cell division, and older cells die off.

In some of the stress tests, Kemkemer places the cells on a kind of rubber cloth. As the experiment progresses, the cloth is permanently being pulled in a certain direction and then relaxed. The frequency of this change can be increased or decreased. At slower frequencies, the cells are still able to respond actively: they take up the fight and turn in the direction of the stress. In the human body, this is



Dipping in the service of science: A glass plate is dipped in a solution (left) and Maria Sycha, institute technician, checks whether the glass plate is centered in the beaker.

what cells do in an attempt to strengthen tissue against mechanical stress. However, if the frequency of periodic stretching drives the stress rhythm so high that the complex molecular ballet within the cell can no longer keep up, the cells prefer to avoid the stress. They position themselves perpendicular to the direction of stretching.

The Stuttgart-based scientists observed that the cells from older donors avoid the stress at much lower frequencies than cells from younger donors. Other stress tests where the cells had to orient themselves on a nanogrooved substrate also strongly confirm this behavior. Between the ages of about 30 and 50, human cells lose some of their “mechanosensitive” ability to adapt to stress.

Joachim Spatz was particularly astounded by this aging effect. “You can see it in your skin every morning when you look in the mirror,” says

Spatz with a smile. But the effect does not depend on the donor, as the scientists have discovered. “It seems there are genes in the cells that have some effect on this,” says the biophysicist: “And it’s these genes we want to identify with our experiments.” At any rate, the scientists in Stuttgart could even use their cell stress tests to test the effectiveness of today’s anti-aging creams, as Spatz points out.

Also interesting are the chicken heart cells in another video recording: this time, they sit on elastic micro-pillars, happily pulsating away. The pillars beneath the cells sway in sync. Spatz and his team succeeded in cutting the network of actin fibers out of the cells and stretching it loosely across one of these pillar structures.

“If we now add myosin motors and the energy supplier adenosine triphosphate, the fibers actually contract,” reports Spatz. However, the pulsating is

missing. That’s why the scientists want to embed the fibers in a vesicle. A vesicle is a little bubble with a closed membrane, which replicates real cells – a “synthetic” cell, in other words. The third component these cells need are certain receptor molecules in the membrane, and then they should be able to pulsate. This dramatically simplified model system of a heart cell fits perfectly into Joachim Spatz’s research strategy. “We want to understand how biological systems work,” he says in closing, “and then mimic it, sort of half biologically, half synthetically.” ◀

GLOSSARY

Extracellular matrix

The cell environment, made up of stabilizing fibers; it contains nutrient and messenger substances.

Actin fibers

Strings of proteins that make up the cytoskeleton; they form the “scaffolding” for the locomotor system within the cell.

Myosin

A motor protein that moves across the actin fibers.

Filopodia

Projections used by a cell to search for contact with its environment.