

Bone Scaffold Covers All Angles

Patients with osteoporosis stand to gain just as much from artificial bone as those with serious injuries and bone cancer. **Peter Fratzl, John Dunlop** and **Wolfgang Wagermaier** are researching the optimum conditions for generating bone tissue at the **Max Planck Institute of Colloids and Interfaces** in Potsdam-Golm.

TEXT **KLAUS WILHELM**

Bones are associated with hardness and stability, and yet, at least biologically speaking, they are hives of activity on the inside. When we think about their mechanical properties, we tend to envisage a lifeless, robust, simply structured material, but bones are actually incredibly complex and far more dynamic than all the artificial materials ever conceived by engineers. “This is a real challenge for materials scientists, but one that really excites us,” says Peter Fratzl, Director at the Max Planck Institute of Colloids and Interfaces in Potsdam-Golm in the German federal state of Brandenburg.

“We want to know exactly what goes on in the bone, particularly during the healing process following a fracture,” explains the Vienna-born scientist. The complexity and broad nature of this topic is already evident from the wide-ranging backgrounds of the team of physicists, biologists, chemists, materials scientists, mathematicians and computer scientists who are working on these details in the department of biomaterials at the Golm-based Max Planck Institute.

Through its basic research studies, the team is pursuing the practical objec-

tive of enabling “doctors to use our knowledge for the benefit of their patients in the medium to long term.” Here, Fratzl has in mind people with serious bone problems that exceed a certain scale and overtax the bone’s natural self-healing powers – for example, bone fractures arising from serious injury, bone cancer and chronic conditions like osteoporosis.

TISSUE ENGINEERING HAS YET TO MEET EXPECTATIONS

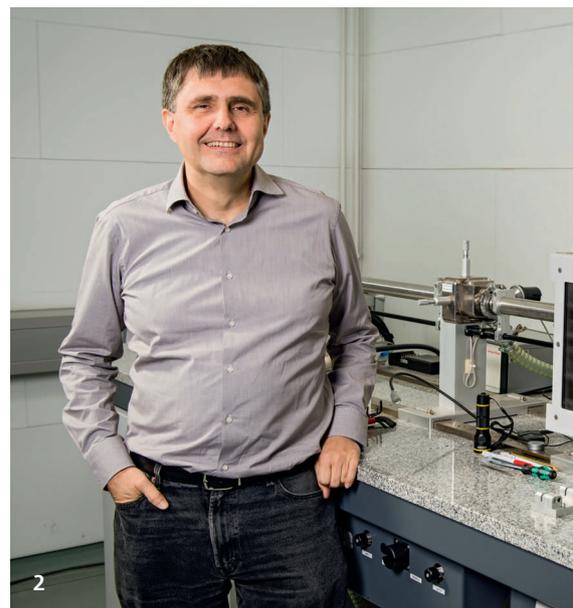
Apropos osteoporosis (see box on page 53): Peter Fratzl reports of many elderly people with femoral neck fractures whose bones are very slow to heal. This can cause further problems, such as long periods of bed time with the accompanying risk of chronic open wound development. As a result, a seemingly harmless hip fracture can eventually prove fatal. “The idea that we may one day be able to help these patients is a great source of motivation for us.” The same goes for the partners of the Golm scientists, particularly the experts at the Julius Wolff Institute of Biomechanics and Musculoskeletal Regeneration at the Charité – Universitätsmedizin Berlin. >



Photo: Bastian Ehl



At the natural limits of growth: With the help of these 3-D copies of human bone structure, materials scientists study how the curvature of the surface affects bone cell reproduction. As the researchers suspect, the sponge-like structure doesn't close up completely because its mean curvature is zero. There is therefore space left for the bone marrow.



- 1 Detailed analysis: Wolfgang Wagermaier examines the nanostructure of a sample using a small-angle X-ray scattering instrument. He wants to identify the conditions under which bone tissue with the natural microstructure can be generated.
- 2 Battle against osteoporosis: Director Peter Fratzl hopes that his research will help fracture-healing in people with brittle bones.
- 3 The scientists in Golm pour bone samples into plastic to stabilize it and process it. They use rat and sheep bones for their studies.
- 4 Cut to the bone: The researchers produce the samples with a bone saw.

Peter Fratzl and his colleagues have already achieved some initial results. They discovered, for instance, that cells can do incredible things: Sensing the curvature of a surface that is much bigger than they are. And that the geometry of surfaces alone can crucially accelerate the growth of bone-building cells.

Such insights can contribute to the optimization of bone generation in the context of tissue engineering – the simplistic term used to designate one of the great hopes of modern medicine in the 1990s. The promise made at the time was dramatic: in 20 years' time, at the latest, it would be possible to grow any kind of tissue in the laboratory. The technique appeared to offer a way of generating the ideal substitute for tissue that is lost in the course of various diseases – for example, heart tissue in patients who have suffered a heart attack, or bone tissue in individuals with osteoporosis and fractures that are difficult to heal.

The vision was further fuelled by research on stem cells from embryos and from adult tissue, as these versatile cells offer potentially ideal raw material for

tissue generation. Since then, doctors have administered to patients cells and tissue that have been produced using different processes. Especially cartilage is now replaced in this way. “However, the high hopes have not yet materialized,” says Peter Fratzl, and bemoans the fact that “the application of the methodology often advanced faster than the understanding of its underlying processes.”

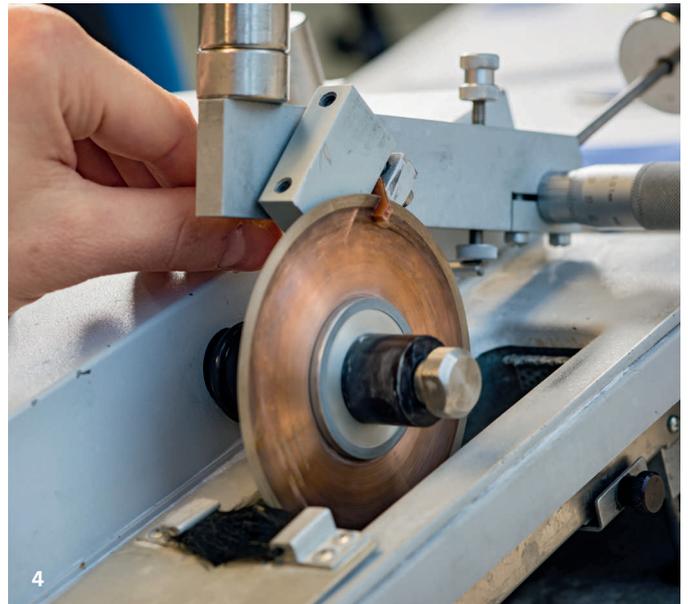
HOW BONE CELLS BEHAVE ON SURFACES

The successes have been modest also because researchers didn't understand the interaction between the cells involved and the intercellular spaces, the extracellular matrix. “This is a huge factor,” explains the Max Planck Director, and stresses that, for this reason, the experts in Golm started out by taking a step backward. Using cell culture experiments, physical measurement methods and computer simulations, they devote their attention to the basic principles of an important sub-area of tissue engineering: the behavior of cells on a sur-

face, or to put it more precisely, the scaffold on which the cells proliferate during artificial tissue generation.

The researchers also investigate the surface forms on which bone-building cells, or osteoblasts, can best be grown, and how the mechanical characteristics of bones change depending on the embedded mineral particles. A detailed understanding of exactly how bone heals following a fracture – another research focus at the Max Planck Institute in Golm – provides the essential basis for answering these questions. The ultimate aim of the research is to create optimum conditions for cells so that they rapidly form fully operational tissue for patients.

It has long been known that certain biochemical signaling substances stimulate cell reproduction. High doses of these growth factors are injected into the bodies of patients to stimulate bone healing. “This is the classic approach based on the use of an active chemical substance as a drug,” says John Dunlop. The cells register the biochemical message transmitted by the growth factors and prepare their machinery for divi-



sion. However, the long-term side effects of this process are potentially dangerous and unpredictable – and may lead to the formation of tumors, for example.

Researchers also administer growth factors to bone-building stem cells in the nutrient solutions to reproduce the cells and then allow them to proliferate three-dimensionally on a scaffold made of plastics or other materials. Somehow, the cells react with – and to – the surface of the scaffold material. “So the

physical conditions also influence the growth of bone tissue,” says Fratzl. For example, stem cells develop into bone cells only on hard surfaces; on soft surfaces, they become nerve cells.

The mere fact that these multi-talented cells develop different functions on mechanically different surfaces shows that a cell can actually sense and detect the forces and mechanical properties in its environment – in other words, whether it is located on a hard

or soft surface. The cells thus respond solely to the mechanical information that is transmitted to their interior from their membrane via certain protein complexes.

Inside the cell, the cell “muscles,” known as actin filaments, which constantly change their structure to adapt to the external signals, react to this information. As a result, the shape of the cell changes in the space, and the cell control center in the nucleus is in-

THE SKELETON – A WORK IN CONSTANT PROGRESS

The human skeleton consists of more than 200 bones – the exact number is impossible to specify, as some bones fuse with each other over the course of life. Their structure is complex. From the perspective of a materials scientist, bone is first and foremost a composite material consisting of several different materials. Under the bone skin, or periosteum, lies a thick layer of dense bone tissue that becomes a sponge-like scaffold of fine cancellous bone or spongiosa in the interior. The structure is stable, yet light.

The actual substance of the bone consists of different types of bone cells (osteocytes). These are embedded in a matrix that consists of calcium-rich hydroxylapatite, proteins, such as elongated collagen molecules (fibrils), water, mineral particles and other substances. The mineral particles, in particular calcium apatite, make the bone hard and solid.

Bone tissue is never at a standstill. It rebuilds itself every day; worn substances are replaced with new ones. The never-ending building work is regulated by coactive hormones, vitamins and messenger substances. The actual work is done by the osteoblasts, which build the new bone, and the osteoclasts, which reabsorb the discarded bone material. The rebuilding processes are systematic, and their exact nature depends on how a bone is used. Richard Weinkamer from the Max Planck Institute of Colloids and Interfaces traced this process in a computer simulation. According to this, the bone cells (osteocytes) in the matrix sense the kind of mechanical stress at work and jointly transmit a signal, so that the new bone substance is tailored to the most common mechanical stress. Movement, by the way, is the best thing for bones. In general, during the first 30 years of life, bones produce more mass than they break down. Thereafter, with age, more bone is lost than gained.

» The growth of the osteoblasts depends on the curvature of the surface: the stronger the curvature in a pore, the quicker the cells build tissue.

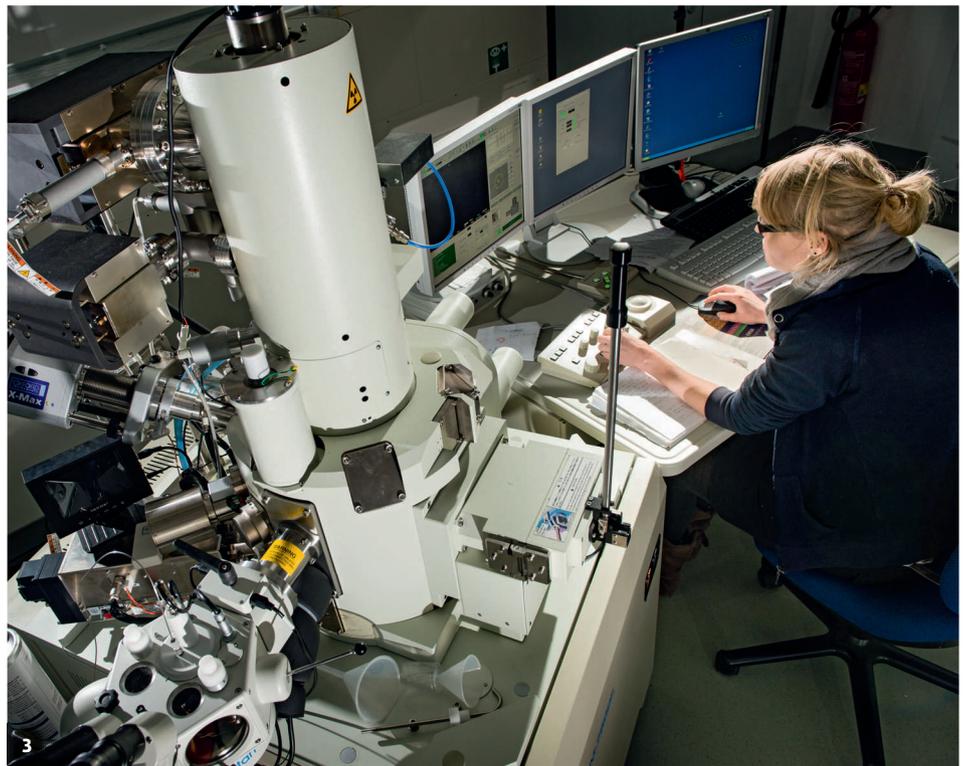
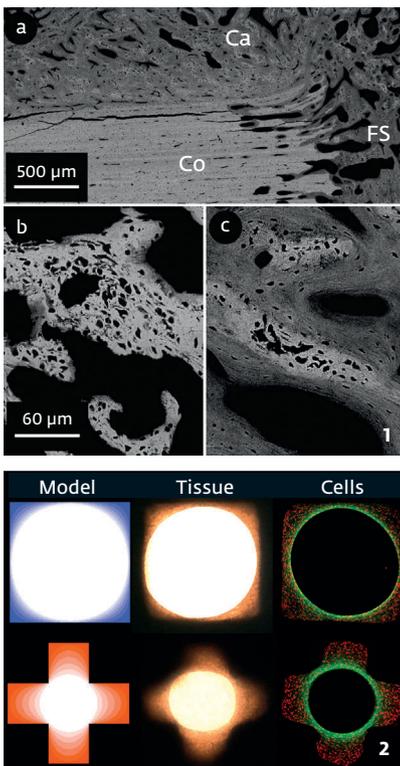
formed of this. Certain genes are then activated that tell the cell: “Divide and reproduce!” or “Leave everything as it is.” Or even: “Die!”

Because the cells in a tissue are connected to each other through the extracellular matrix, they can communicate mechanically with their neighbors and, as a result, coordinate their behavior over large length scales. The behavioral patterns of collections of cells are thought to arise in this way, and this is a specific point of interest for the Potsdam-based researchers.

Using fixed samples from rat and sheep bones, materials scientist Wolfgang Wagermaier studies how the bone tissue uses such processes during healing. After a fracture, a soft, fibrous tissue known as the callus grows around the site of the fracture. As Wagermaier explains, the callus is “a kind of natural splint that connects the two bone ends with each other.” The researcher demonstrated that the bone healing process unfolds in two stages in this callus – exactly like the everyday formation of new bone (see box on page 51).

During the first stage, osteoblasts migrate to the callus; despite the soft environment, they quickly form a simple, relatively disordered bone. “We also discovered that the osteoblasts use this initial scaffold to organize themselves,” explains Wagermaier. They colonize the surfaces, communicate with each other using mechanical signals, and form flat, three-dimensional structures. Even at this stage, they make a mechanically far superior bone than before: a lamellar bone. It is highly oriented and corre-

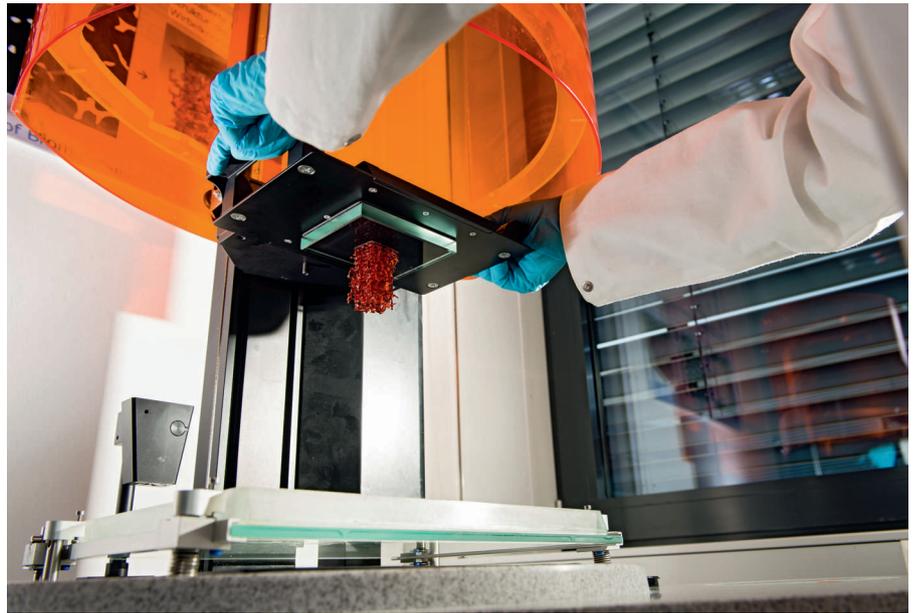
- 1 The stages of bone repair: During the healing of a fracture, the callus (Ca) forms around the site of the fracture (FS). This image, which was recorded using an electron microscope, shows the fracture at the outer cortex (Co) of the bone after nine weeks. In the first two weeks, (b) disordered bone develops in the callus; (c) bone lamellae that form later align themselves along the early disordered bone tissue. The dark gray areas show recently formed bone structures; the lighter areas represent tissue that formed a long time ago.
- 2 Comparing theory and practice: The predictions of the scientists’ model for two pore shapes (left) tally with the actual tissue growth of the cell cultures (center). Examination via a confocal microscope (right) shows that cells in cross-shaped pores form considerably more actin filaments (green) than those in square ones.
- 3 The scientists from the Max Planck Institute of Colloids and Interfaces uncover the secrets of bone generation with the help of an electron microscope, among other things.



Photos: Bastian Ehl (large image), MPI of Colloids and Interfaces (small images, 2)



Model on request: Using rapid prototyping (right), a bone structure can be printed in 3-D. The scaffolds on which the researchers generate bone cells are formed using the same process (above).



spondingly stable in structure. “The organization and the cooperation decide,” adds Fratzl. “It isn’t a matter of indifference for the osteoblasts whether they find themselves in a pure hematoma or in a 3-D situation in which they detect solid surfaces produced by their predecessors.”

Wagermaier uses every conceivable resource, such as high-tech microscopy and spectroscopy and big machinery, to cajole the bones into revealing the secrets behind their dynamics. He is currently preparing another experi-

ment in the synchrotron storage ring in Berlin-Adlershof, where he analyzes the mineral particles in bone samples. These are the smallest structural elements in the bone tissue. The materials scientist tracks the different healing stages and sees how the orientation and size of the mineral particles shape the mechanical quality of the bone, and how the internal structure of the bone and the bone cells changes if they are pulled or if they anchor themselves to a surface and exert force. One day, the knowledge gained from this experi-

ment will also be used to optimize the regeneration of bones.

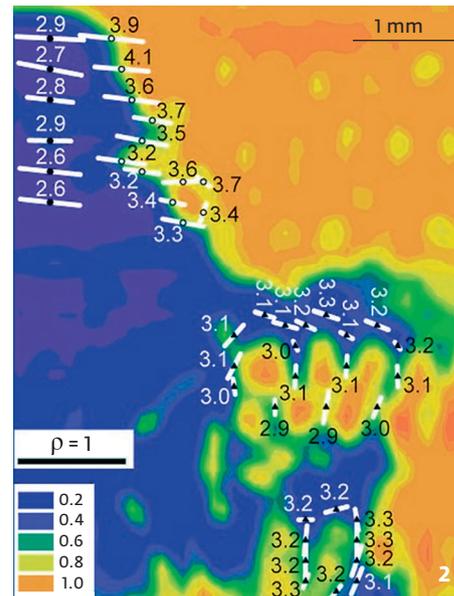
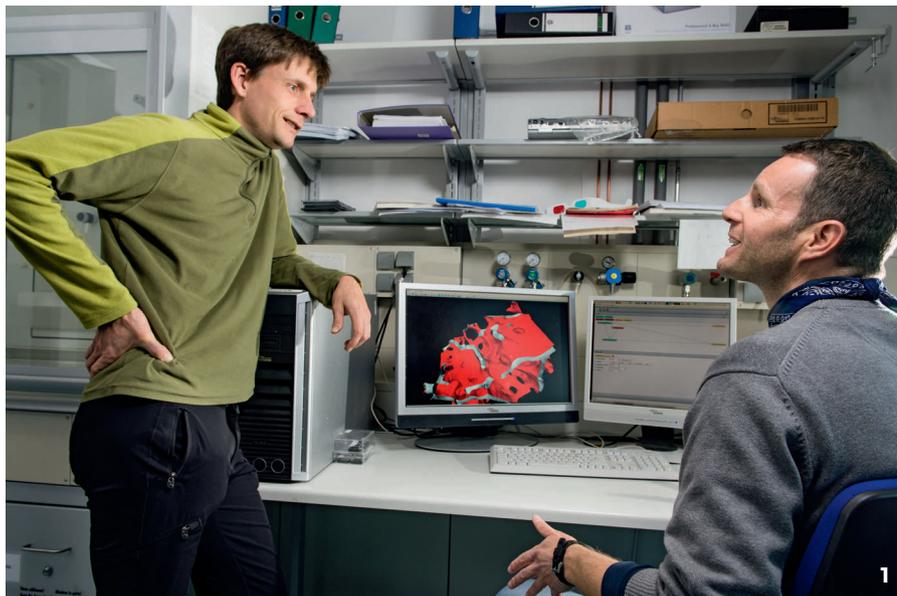
John Dunlop is already using the results to design and refine his experiments. At the Max Planck Institute in Golm, the Australian scientist investigates how the fine structure of an artificial scaffold for tissue engineering should look so that mechanically flawless bone tissue forms on it as quickly as possible. Dunlop’s working group uses rapid prototyping for this purpose. It enables them, for example, to design scaffolds from plastics or other

DENSE BONE IS NOT ALWAYS A GOOD THING

If the constant building and removal of bone becomes increasingly out of kilter in advanced age, the body is at risk of developing brittle bone disease, or osteoporosis. This is mainly a disorder of the bone remodeling process. Almost eight million people in Germany are affected by this condition – the majority of them post-menopausal women. Because their bone mass is less dense, there is a greater risk of fracture even with low strain.

The density of the bone – its quantity – is easy to measure. The same does not apply to its quality, which can be determined only through biopsy. The Golm-based scientists are experts in the area of bone quality measurement. In clinical studies of new drugs for osteoporosis, mainly carried out with colleagues from the Ludwig Boltzmann Institute of Osteology in Vienna, they have tested whether these drugs reduce the quality of the newly formed bone.

The quality of a bone is good if the mineral plates, which are only 3 nanometers thick, run parallel to the collagen fibrils and, moreover, within and on the surface of the collagen fibrils. Furthermore, minerals account for 30 to 40 percent of the volume of a normal bone. The study results show that, in most cases, the biophosphonates that are frequently prescribed in the treatment of osteoporosis today don’t damage the bone quality even after ten years of treatment. Fluoride-based treatments, which are no longer used today, increase the density of the bone, but impair its quality. In the case of treatment using drugs containing strontium, the calcium-like element is deposited in the bone mineral and stored there. It doesn’t influence the mechanical quality of the bone material.



- 1 No bones about it: John Dunlop (left) and Richard Weinkamer discuss the 3-D computer simulation of a bone.
- 2 The image produced by the small-angle X-ray scattering instrument reveals how strongly mineralized the bone tissue is after three months. In the false color image, highly mineralized areas are shown in blue: the mineralization diminishes in the direction of the orange coloration. The direction of a line reflects the dominant orientation of the mineral particles in this location, and the line length shows how high the proportion of particles with this orientation is. (A line length of $\rho = 1$ indicates that all particles are parallel.) The numbers in the image indicate the thickness of the mineral particles in nanometers.

materials on the computer and print them in 3-D in exactly the required shapes. “This enables us to vary the surface of the material exactly as we want it, down to the last detail,” explains Dunlop.

His team has therefore had scaffolds built with differently shaped pores, each of which is around one millimeter in diameter. The pore sections varied in shape from triangles to hexagons and circles. Because all of the pore openings had the same perimeter, a complete revolution of 360 degrees arose in the different shapes on the same section. Thus, all of the pores had the same mean curvature.

In a series of experiments, the scientists repeatedly seeded osteoblasts on the scaffolds and waited for a few weeks. “The results are astonishing,” says Dunlop. As he and his team discovered, the osteoblasts, which are just one micrometer in size, somehow detect the curvature of a surface that is around a thousand times bigger than they are. It is the equivalent of us being

able to figure out whether and how strongly an area the size of a soccer field is curved using just our sense of touch and our feet. “And the cells can even measure angles,” adds Dunlop, still marveling at the discovery.

The cells’ geometry sensing is apparently based on the fact that their actin filaments orient themselves precisely according to the mechanical loads to which the cell is subjected. Therefore, in the experiments carried out by the Max Planck researchers, they align themselves along the surfaces of the pores. Groups of cells anchor themselves there and, with the help of their muscles, exert forces – they pull on the surface and on their neighbors. In this way, they can measure their distance from each other and use this data to figure out the curvature of the surface. Yet as Dunlop discovered in some experiments, not every cell has such amazing mechanical skills. Fat cells differentiated from stem cells, for example, don’t pull on their surroundings, but connective tissue cells do.

John Dunlop’s group also discovered how the growth of the osteoblasts depends on the curvature of the surface. They don’t grow at all on convex – that is, outwardly domed – surfaces, and when it comes to concave surfaces: “The stronger the curvature in a pore, the quicker the cells build tissue,” explains the chemist. The cells scarcely reproduce at all on an even surface, and the tissue also grows much more slowly in the corners of hexagonal pores than in the angles of triangular or rectangular cavities. Because all of the pores in the Golm experiment had the same mean curvature, they essentially filled with the bone cells at the same speed.

Mesenchymal stem cells, which develop in the direction of bone cells, behave in exactly the same way as the osteoblasts. The principles for bone cells are probably universal.

“The simple relationship between the mean curvature and the growth rate of the bone cells is known from other contexts, for example the formation of soap bubbles,” says John Dunlop. “Es-

establishing that such a simple law also applies in a completely different area was a major highlight in my research to date." Based on their findings, the researchers were able to develop scaffolds with optimum shapes for generating artificial bone tissue. They succeeded in doubling the growth rate achieved using standard scaffolds – without having to use growth factor. Moreover, the researchers in Golm can already provide some indicators regarding the optimum properties of the scaffolds. The scaffold pores should be between 50 and 100 thousandths of a millimeter in size and have as many angles as possible. In terms of shape, cross-shaped pores could be a good starting point.

THE SURFACE IS IMPORTANT ONLY AT THE START

The researchers, however, haven't yet been able to explain in detail the biological reasons for which strong curvatures, in particular, encourage the bone cells to divide. "The surfaces of the scaffolds that form in the body after a fracture are very rough and, as a result, have a lot of curves," explains John Dunlop. Up to now, the research-

ers knew little else about this: it's difficult to study the exact geometric conditions under which bone cells grow in organisms. Consequently, there is no satisfactory data available on this yet.

However, as John Dunlop discovered, the surface of the scaffold is important only at the start of the bone-generation process. Once the first layers of cells have formed, the material from which the scaffold is constructed and its topography are of little to no importance.

Based on the insights gained to date, the researchers have developed a mathematical model with which they repeatedly test new pore shapes in scaffolds, so that they will someday be able to present an optimized prototype. "We want to rationalize the entire process," says Peter Fratzl. "We are in the middle of everything and are making good progress." Up to now, tissue engineering didn't take geometric factors into account in the production of scaffolds; however, the Max Planck Director is confident: "Our partners will be testing the results on animal models in the foreseeable future." And on humans, too, at some point. ◀

TO THE POINT

- By studying the factors that influence bone growth, researchers are trying to find the optimum conditions for generating artificial bone tissue.
- Bone cells register the hardness and geometry of a surface using actin filaments.
- How well osteoblasts reproduce in the early stage of bone growth depends, among other things, on the geometry of the surface – the more strongly curved it is, the faster the cells divide.

GLOSSARY

Actin filaments: Thread-like structures formed from the structural protein actin. As a component of the cytoskeleton, they stabilize the cells and are involved in both material transport in the cell and the contraction of muscles.

Fibrin: A protein that polymerizes during blood clotting and closes a wound.

Mesenchymal stem cells: Precursor cells of connective tissue that can differentiate into many different cell types. Osteoblasts can form from them, for example.

Osteoblasts: Bone-building cells that constantly form bone and play an important role in the healing of bone fractures.

Osteoporosis: A disease that causes bones in the elderly to become more prone to fractures. It arises when bone substance diminishes too quickly, which is why the bone density declines and the structure of the bones changes.



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