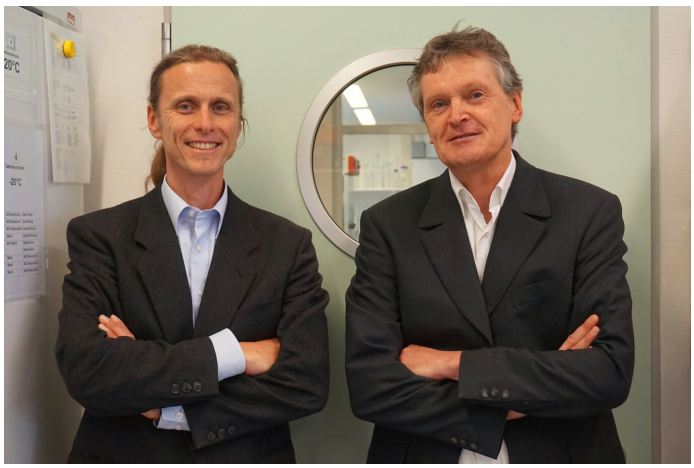
3D bioprinting: replicating the shape of bone parts

At present, 3D printers can only produce dead products such as plastic toys as well as bone and tooth substitutes. Researchers from Freiburg now want to take a decisive step forward in the field of individualised medicine by printing living tissue. Prof. Dr. Günter Finkenzeller, head of the Research and Tissue Engineering Laboratory in the Department of Plastic and Hand Surgery at the Freiburg University Medical Centre, and Dr. Peter Koltay from the Department of Microsystems Engineering (IMTEK) at the University of Freiburg, are being given German Research Foundation (DFG) funds for a period of three years to develop a 3D printing technique for functional bone tissue containing blood vessels. The production of living tissue is of great interest for organ replacement therapy and in pharmaceutical research where efforts are being made to reduce the number of animal experiments.

Tissue engineering is a discipline with huge future potential as it enables biological tissue to be produced in vitro. For some time now, researchers have been printing bone replacements. Rather than cellular components, the bone replacements contain synthetic substances that are absorbed by the surrounding tissue. Skin replacements that have long been used to treat burn victims and patients with chronic wounds also have a relatively simple structure. Skin tissue replacements of this kind are produced using tissue engineering methods. Tissue is primarily cultured in 2D cell cultures in the laboratory, and such tissue is unable to generate spatially controlled cell patterns. This means that the individual components of the cultures, i.e. cells, cannot be positioned at a specific location in the tissue, but are distributed totally at random. Researchers from Freiburg now want to rise to the challenge. 3D bioprinting, a special discipline and a continuation of tissue engineering, will allow the generation of spatially controlled cell patterns where the resulting construct contains a supporting scaffold as well as living cells.

Utopia and reality



Plenty of promises have been circulating on the Internet, intimating that patients will no longer have to wait several months for donor organs, as printing organs on demand will soon be possible. According to Dr. Peter Koltay, an engineer at the Department of Microsystems Engineering at the University of Freiburg (IMTEK), such visions of the future may be gaining a big following, but are completely

utopic. It has not yet been possible to print complex, functional structures.

Dr. Peter Koltay (left) and Prof. Dr. Günter Finkenzeller (right) would like to advance 3D bioprinting and print bone tissue that contains blood vessels.
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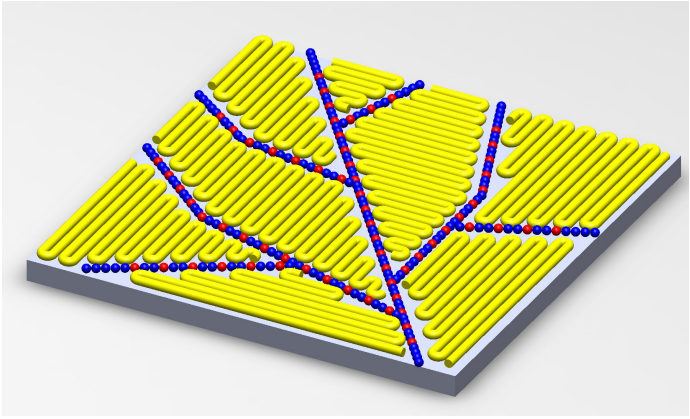
Initial attempts to do so are concentrated on bone, cartilage and skin tissue. The flights of imagination that take wing on the back of bioprinting soar very high indeed. However, we are a very long way off a printed beating heart. Organs consist of different tissues and highly specialised cell types. At some time in the future, it may be possible to produce simple tissue sections or rough cell aggregations that will then mature autonomously into organ-like structures. But there are many hurdles to overcome before that becomes likely.

3D bioprinting is in principle suitable for creating more complex, three-dimensional structures. "What is happening in medical research at the moment is that we are trying to create in vitro models that are more complex than before. These models will then be able to mirror the real situation far better than current ones," explains Prof. Dr. Günter Finkenzeller. Tissue engineering traditionally involves the use of scaffolds on which cells are grown. The researchers cannot influence the way cells are spatially distributed on the matrix. However, in order to organise several cell types to form a tissue substitute with a morphology that is comparable to real tissue, site-specific and interacting factors that play a huge role in vivo need to be taken into account.

The tissue needs to be supplied effectively

If you want to generate bone tissue, you have to find ways to solve the problem of blood supply to the tissue. Blood vessels are crucial building blocks of artificial tissue, especially large three-dimensional objects that are to be implanted. As far as artificial implants are concerned, blood vessels must grow into the tissue from surrounding tissue. And as this process takes around two weeks, many cells die off due to lack of oxygen. Above a certain size, nutrient transport must also be guaranteed during the in vitro production of the tissue.



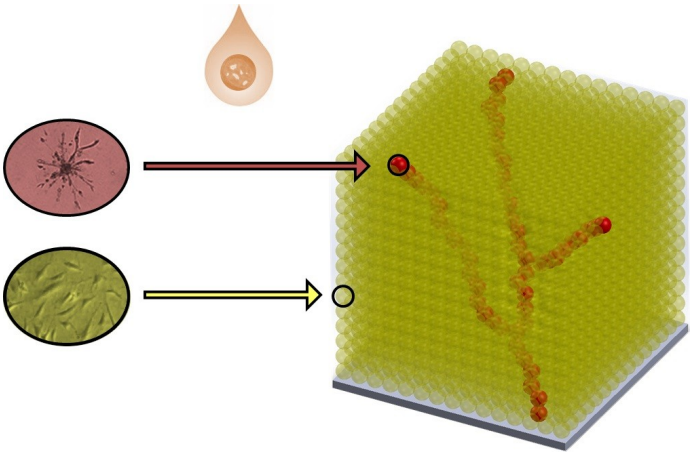
The idea is as follows: hydrogel spheroids (blue) and endothelial cells (red) are expected to form blood vessels within the printed paste of mesenchymal stem cells, which will later develop into bone substance.
© Dr. Peter Koltay, IMTEK, University of Freiburg

"How can cells in the tissue be supplied with nutrients? This is a core topic in our project," says Koltay. It leads to the issue of how to generate blood vessels in artificial tissue and how to connect these blood vessels to the natural blood vessels in adjacent healthy tissue after implantation. The researchers have decided to start their experiments with bone tissue as bone has been shown to be a very rewarding material for bioprinting. According to Finkenzeller, bone is the only tissue that is able to heal without scars. A broken bone regenerates completely, and, unlike other tissue, does not fill the defect with connective tissue.

Koltay and Finkenzeller are now developing a 3D printing method that they hope to use to create functional bone complete with blood vessels. They hope that the print-outs will have a major time advantage. The printed vessels only need to connect with other blood vessels directly bordering on the implant and the natural tissue in order to ensure a proper blood supply to the implanted

tissue.

Mix of control and self-organisation



The result is blood vessels (red) in the bone substance (yellow-green) that is made up of living osteoblasts.
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The scientists want to develop a printer that uses small hydrogel beads (spheroids) loaded with living human cells for printing three-dimensional bone tissue. They can choose between non-contact and contact printing. In the case of non-contact printing, a cell-containing spheroid is catapulted into the air and hardens when it hits the working area. In the case of contact printing, a paste is pressed through a very thin needle, generating a line that contains the cells. "We are also trying to combine both methods in one object in order to find out which method is best suited for which tissue," says Koltay. Koltay and Finkenzeller want to explore all possible combinations. When printing bone tissue, the researchers first apply a material that contains mesenchymal stem cells and growth factors. The stem cells later differentiate into osteoblasts, providing the bone substance with firming structure and the matrix where the cells exchange substances with each other. In the next step, spheres or pastes with endothelial cells will be printed at the exact location in the tissue where the blood vessels are supposed to form. Finkenzeller and Koltay hope that the cells' ability to organise themselves will be advantageous in this venture. They know from preliminary experiments involving the implantation of human endothelial cells into immunodeficient mice that the cells turn into vascular structures within a week or so. "What happens here cannot be done in vitro," says Finkenzeller. "The endothelial cells only do this when they are in the body." In the mouse, a human blood vessel system forms at the implant site and fuses with the murine blood vessels.

With regard to the time required for producing a functional replacement tissue, it can be assumed that it will be of advantage when the cells are assigned a specific place. "We thus eliminate the randomness of the procedure as we already have a preliminary vascular network," says Finkenzeller. This level of control is currently only possible with bioprinting.

A broad range of random factors

Koltay and Finkenzeller are now working on the development of bone printing methods and there is a lot that still needs to be addressed: The cells must proliferate and differentiate in biocompatible printing material. Stem cells can grow uncontrollably and differentiated cells can, under certain conditions, dedifferentiate again. In both cases, this would lead to a loss of function. "We have many degrees of freedom, but little stability," says Koltay. "Bioprinting is associated with the problem that the cells might change in dynamic systems."

Article

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