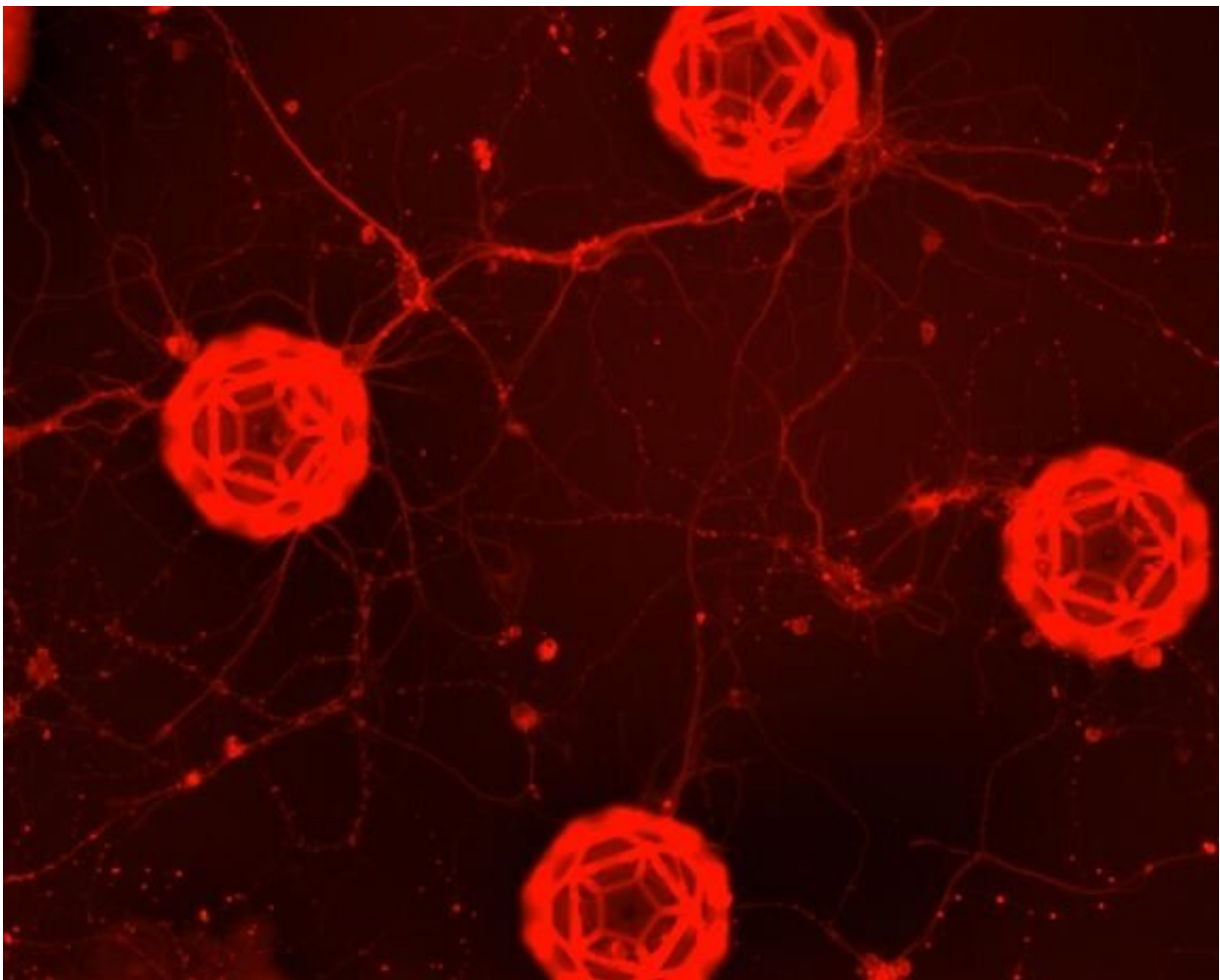


# Biofabrication: 3-D printing, sonic tweezers, and the creation of neurons in microscopic cages

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Connections are growing between the neurons inside different buckyballs.  
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Microscopically small cages have been produced at TU Wien (Vienna) with grid openings only a few micrometers in size, making them ideal for holding cells and allowing living tissue to grow in a very specific shape. This new field of research is called biofabrication.

In a collaboration with Stanford University, the researchers introduced nerve cells into spherical cage structures using acoustic bioprinting technology to allow multicellular nerve tissue to develop there. The researchers also created nerve connections between the cages. To control the nerve cells, [sound waves](#) were used as acoustic tweezers.

## **Football-shaped cages**

"If you present living cells with a certain framework, you can strongly influence their behavior," explains Prof. Aleksandr Ovsianikov, head of the 3-D-Printing and Biofabrication research group at the Institute for Materials Science and Materials Technology at TU Wien. "Three-dimensional printing enables the high-precision production of scaffolding structures, which can then be colonized with cells to study how living tissue grows and how it reacts."

In order to grow large numbers of nerve cells in a small space, the research team decided to use so-called "buckyballs"—geometric shapes made of pentagons and hexagons that resemble a microscopic football.

"The openings of the buckyballs are large enough to allow cells to migrate into the cage, but when the cells coalesce, they can no longer leave the [cage](#)," explains Dr. Wolfgang Steiger, who worked on high-precision 3-D printing for biofabrication applications as part of his dissertation.

The tiny [buckyball](#) cages were manufactured using a process known as two-photon polymerization: a focused laser beam is used to start a

[chemical process](#) at specific points in a liquid, which causes the material to harden at precisely these points. By steering the focal point of the laser beam through the liquid in a well-controlled way, three-dimensional objects can be produced with extremely high precision.

## **Acoustic waves as tweezers**

Assembling cells into the buckyballs through microscale openings is very challenging. An innovative 3-D acoustic bioprinting technology developed at the Stanford School of Medicine successfully addressed this challenge. Prof. Utkan Demirci co-directs the Canary Center at Stanford for Early Cancer Detection and his research group, the Biosensing and Acoustic MEMS in Medicine (BAMM Lab), uses acoustic waves in biomedical applications including sensing cancer biomarkers and bioprinting 3-D tissue models to sensing.

"We generate acoustic oscillations in the solution in which the cells are located. The cells follow the sounds waves like rats follow the Pied Piper of Hamelin as in the legend In the process, nodes of oscillation form at certain points—similar to a vibrating string," says Prof. Demirci. At these nodal points, the liquid is comparatively static. If cells are located at these points, they remain there; everywhere else they are moved away by the acoustic wave. The cells therefore move to the spots where they are not whirled around—and that is where the buckyballs were placed. The sound wave can thus be used in a very well-controlled way, almost like tweezers, to direct the [cells](#) to the desired location.

"The [acoustic waves](#) enabled us to fill the scaffold structures much more densely and efficiently than would have been possible with conventional methods of cell colonization," reports Tanchen Ren, Ph.D., from Prof. Demirci's research group.

Once the buckyballs had been successfully colonized with [nerve cells](#) in

this way, they formed connections with neurons of neighboring buckyballs. "We see enormous potential here for using 3-D printing to create and study neural networks in a targeted manner," says Aleksandr Ovsianikov. "In this way, important biological questions can be investigated to which one would otherwise have no direct experimental access."

**More information:** Tanchen Ren et al. Enhancing cell packing in buckyballs by acoustofluidic activation, *Biofabrication* (2020). [DOI: 10.1088/1758-5090/ab76d9](https://doi.org/10.1088/1758-5090/ab76d9)

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