Engineering 3D Biocompatible Scaffolds for Neuronal Network Growth

"There's a lot of interest these days in creating artificial structures that mimic the properties of human organs," saysDr. Angelo Accardo of Delft University of Technology's Department of Precision and Microsystems Engineering (PME) "which would be very useful as a model for both in vitro drug-screening and tissue-engineering, for restoring brain tissue damaged by Alzheimer's disease or stroke for example."

At PME, Accardo, who is Assistant Professor of Soft Micro- and Nano-system Technology for Life Sciences and Biology, is developing a way to create such structures - tiny three-dimensional (3D) constructions that provide both a support system and an attractive microenvironment for cell growth, specifically nerve cell growth: "We start with designs that mimic the porosity, geometry and stiffness of the brain tissue, then seed neural or stem cells on top of the 3D structures, and finally we see if these cells behave in a way similar to the one we expect from a normal human brain."
Building on this concept, Accardo has been able to create tiny 3D "scaffolds" which he has shown support the growth and interconnection of nerve cells: "Thanks to the porous nature of the scaffold geometry, we observed the growth of a ramified neuronal network throughout the 3D architecture."

3D Scaffolds and Biocompatible Hydrogels

Crucially these structures have to be 3D explains Accardo: "In the past, cell biologists cultivated cells as two-dimensional monolayers in petri dishes but organs are not 2D – they're 3D so we needed to provide a three-dimensional spatial configuration to guide the cell growth because cells in a tissue are arranged in complex architectures and these architectures play a fundamental role in how the tissue functions."

Also important is the material used to make the scaffolds as they need to satisfy a number of different criteria. Accardo: "For one, the material must be biocompatible, so safe for use with cells or in the human body, and in some cases, it also has to be biodegradable. If you imagine potential tissue engineering applications such as neuronal implants in the brain, you want these neuronal scaffolds to fade away once they've restored the brain tissue and reconnected neuronal networks - you don't want to have a piece of polymer in your brain forever!"
A third consideration is the degree of 'stiffness', which has to be comparable to that of brain, the softest tissue in the body. "Nowadays I'm working on 3D neuronal scaffolds made of biocompatible hydrogels, which are not yet as soft as the brain but going in the right direction. The aim is to use these biocompatible materials to promote the formation of biomimetic 3D neuronal networks able to follow the geometrical and mechanical cues imposed by the 3D structure in order to have a much more natural microenvironment."

Laser Assisted Fabrication Techniques

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In order to ‘shape’ the scaffolds, Accardo uses two types of laser-assisted fabrication techniques called ‘Two-photon Direct Laser Writing’ and ‘Stereolithography’, which compared to all other Additive Manufacturing or 3D printing techniques, can be used to create structures with the highest level of single feature resolution, so objects at the micrometric (10^{-6} m) or even the sub-micrometric scale.”
However using these laser-assisted techniques presented Accardo with another major problem: “In order to produce extremely precise structures like these scaffolds, you need to use materials that are sensitive to light. That means that you have to customise the hydrogel using photo-initiators so that, once the material is struck by the laser beam, it solidifies – photo-polymerises. The problem is that most of these photo-initiators are not biocompatible. So much of the initial work is searching for the right ingredients to customise the material in order to make it compatible with both the laser-assisted 3D fabrication technique and with the cells.”

Once the problems were solved, Accardo was able to create hydrogel scaffolds that support the growth of neurons and even promote neural connections: “Once we started using PEGDA (Polyethylene glycol diacrylate), the hydrogel that we had customised, we were able to obtain neuronal networks which were much more ramified and with more neuronal connections per cell body than the ones we’d obtained using earlier scaffold versions made from stiffer materials. So this showed how the presence of soft materials plays a major role in the creation of neuronal networks, which mimic the ones that we find in the brain.”

In practice, Accardo creates the neuronal scaffolds by using a laser beam to “write” a 3D structure within a photosensitive polymer solution: “The laser beam goes through the solution which then, at the focal point of this laser spot, becomes solidified in extremely confined regions called voxels, a 3D version of a pixel. In other words you’re “writing” the shape you want within the polymer. Then, once you have your solidified structure, you can use a solvent to flush away the unexposed material leaving the freestanding 3D structure behind. Finally you put this 3D structure into a cell culture environment and observe the cell colonisation mechanisms taking place within the engineered biomaterial.”

Observing the cell growth

And how can we do that? Because Accardo has also developed imaging protocols that allow direct observation of cell growth on, and even inside, these microscopic scaffolds: “Conventional optical, fluorescence and scanning electron microscopy techniques normally only let you see the morphology of the external shell of these scaffolds and not the inside. So we recently developed some protocols based on Light Sheet Fluorescence Microscopy and Two-photon Confocal Imaging that overcome this limitation. They allow us to literally “shed light” on the core of these architectures so that you can see the evolution of the nerve cells, not just around the 3D scaffold but also in its most inaccessible regions.”