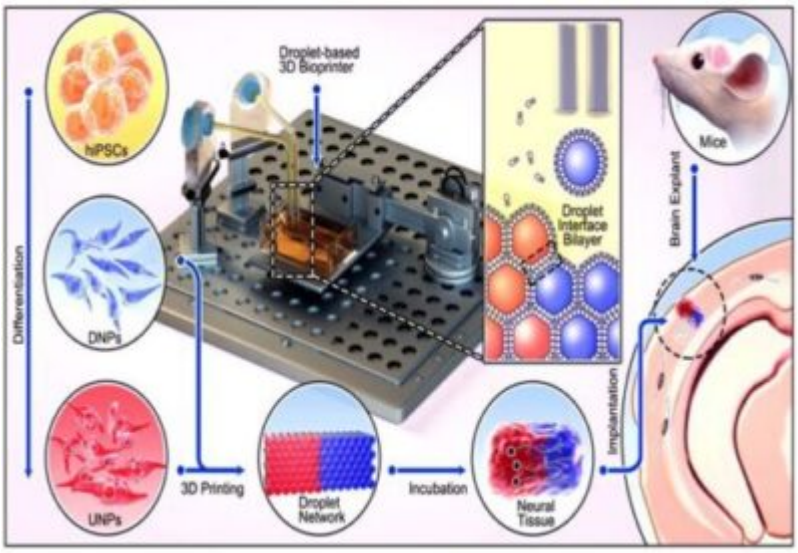
Brain injuries, such as traumatic brain injury, stroke, or surgical resection for cancer and epilepsy can cause significant damage to the cerebral cortex, resulting in severe disabilities. In this research, the authors from the United Kingdom fabricated the two-layer cerebral cortical tissue containing simplified cortical columns with a droplet-based 3D printing technique with human-induced pluripotent stem cells. They also investigated the integration of 3D-printed two-layer cerebral cortical tissue into *ex vivo* lesioned brain slices.

The cerebral cortex consists of six layer-specific neurons organized into vertical columns, but, current tissue engineering techniques cannot produce such structures. The authors, therefore, focused on generating the two-layer cortical tissue for potential applications involving implantation.




*The original illustration from the article of Jin, Y., et al. Nat Commun 14, 5986 (2023).*

### *About the study*

The essential first step towards fabrication of the two-layer cortical tissue was the generation of layer-specific cerebral cortical progenitor cells from human induced pluripotent stem cells which have the potential to generate cell types that make up all human tissues. The cerebral cortical tissue with a two-layer organization was formed using the 3D droplet printing technique, which enables the production of structurally defined tissues composed of cells and extracellular matrix. The authors explained that they used progenitors instead of mature neurons because progenitors were less sensitive to the dissociation procedure from 2D cultures than mature neurons and were compact for

printing.

Two neural progenitor subtypes, upper- and deep-layer neural progenitors were differentiated from human induced pluripotent stem cells. The printed progenitor cells underwent maturation, including terminal differentiation, neuronal process outgrowth, and migration.

Deep-layer cortical neurons, the early product of cortical neurogenesis, were first fabricated from deep-layer neural progenitors. This deep-layer cortical tissue was sectioned and immunostained to examine its tissue structure and cellular composition, which were subsequently visualized with neural markers.

Later, produced neurons migrated radially into the cortical plate and passed through the deep-layer neurons to become upper-layer neurons. In cortical neurogenesis, neuronal process outgrowth and migration are two important developmental phenomena. A magnified view showed that the upper-layer neurons were projected toward the deep layer. A real-time quantitative polymerase chain reaction for gene expression analysis confirmed the identities of the deep-layer and upper-layer neurons.

After two weeks of culture, the printed cortical tissues remained in the desired two-layer architecture.

The printed tissues were then implanted in *ex vivo* lesioned mice brain slices to assess the capacity of 3D-printed cerebral cortical tissue for tissue repair. Over a week, the cellular morphology, structural integration, and calcium (Ca2+) ion activity were monitored. Implantation of printed cortical tissues into *ex vivo* mouse brain explants resulted in substantial structural implant-host integration across the tissue boundaries. Confocal fluorescence imaging confirmed neuronal process outgrowth and migration of neurons from the implant towards the host, showing the integration of printed tissues into the brain explant. Individual neurons migrated across the implant-host boundary, and cells of the brain slices were 86 % viable five days after implantation.

The activity of the implanted cortical tissue was assessed by imaging with a fluorescent Ca2+ indicator, which demonstrated correlated Ca2+ oscillations between the implant and the host.

*Conclusion*

This study has shown that a simplified two-layer cerebral cortical tissue can be produced

using droplet-based 3D printing. During *in vitro* culture after printing, neuronal process outgrowth, migration of neurons, and maturation were observed. Implantation of printed cortical tissues into mouse *ex vivo* brain explants demonstrated the formation of structural connections and correlated Ca2+ oscillations between the implant and the host.

The authors concluded that this technique represents a significant advance in tissue engineering, particularly for future individualized implantation treatments that use 3D tissues made from a patient's own induced pluripotent stem cells. They stated that by employing droplet-printing technology, an implant can be designed to mimic the dimensions, orientation, cellular composition, and structure of the lost tissue.

This article was published in Nature Communications.

### *Journal Reference*

Jin, Y., *et al.* Integration of 3D-printed cerebral cortical tissue into an ex vivo lesioned brain slice. *Nat Commun* 14, 5986 (2023). https://www.nature.com/articles/s41467-023-41356-w