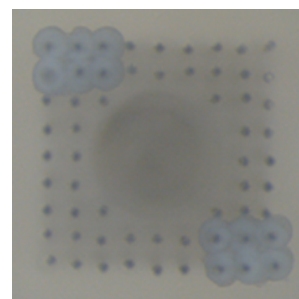


## Neural Organoid for Future Treatment of CNS and Spinal Cord Injuries

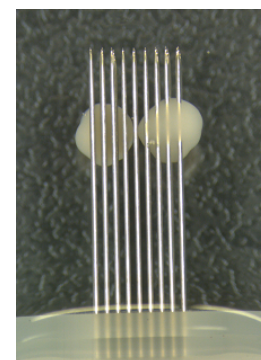
Currently there are no definitive therapies for CNS (central nervous system) and spinal cord injuries. The aim of a neural organoid is to overcome limitations with current therapies and provide better solutions for a damaged human CNS or a spinal cord injury. A neural organoid might also be useful in conducting a neurotoxicity test for chemical agents.

The steps for making a neural organoid using the **Bio 3D Printer Regenova (Cyfuse Biomedical K.K.)** are as follows.

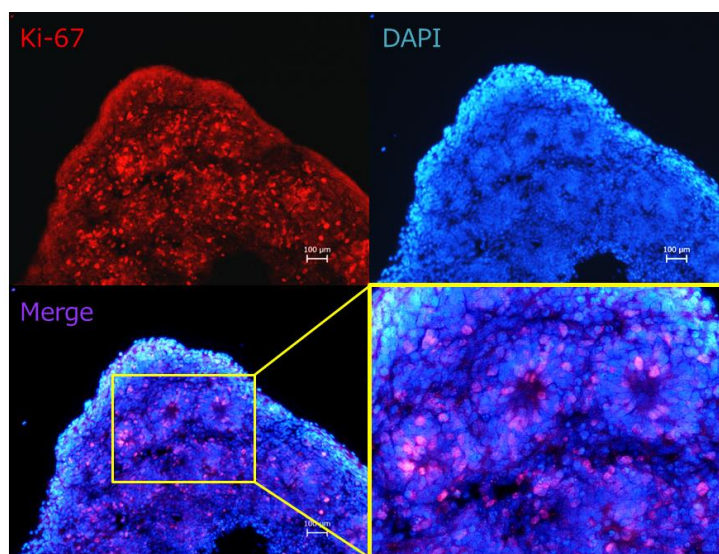
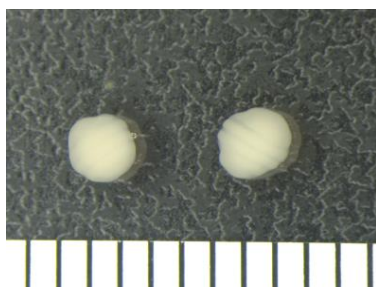
1. Prepare cell aggregations (spheroids) with **Human iPSC-Derived Neural Stem Cells (hNSCs - ax0015, Axol Bioscience)** in approximately half of the 96 well plates. The diameter should be ~ 500  $\mu\text{m}$ . For details, see the application note "Preparing Spheroids (Cell Aggregates)".
2. Perform 3D printing with **Regenova**  
Use 9X9 Kenzan and print two structures of 3x3x2 spheroids.
3. Mature printed tissue on Kenzan with **Neural Maintenance Medium (Axol Bioscience – ax0031/ax0032-500)** in a sterilized container until it spontaneously differentiates into a mixed population of mature neurons and glia. This process takes up to four weeks.
4. After nine days of maturation, a rosette formation and Ki-67 expression (proliferation marker) are observed in the neural organoid.



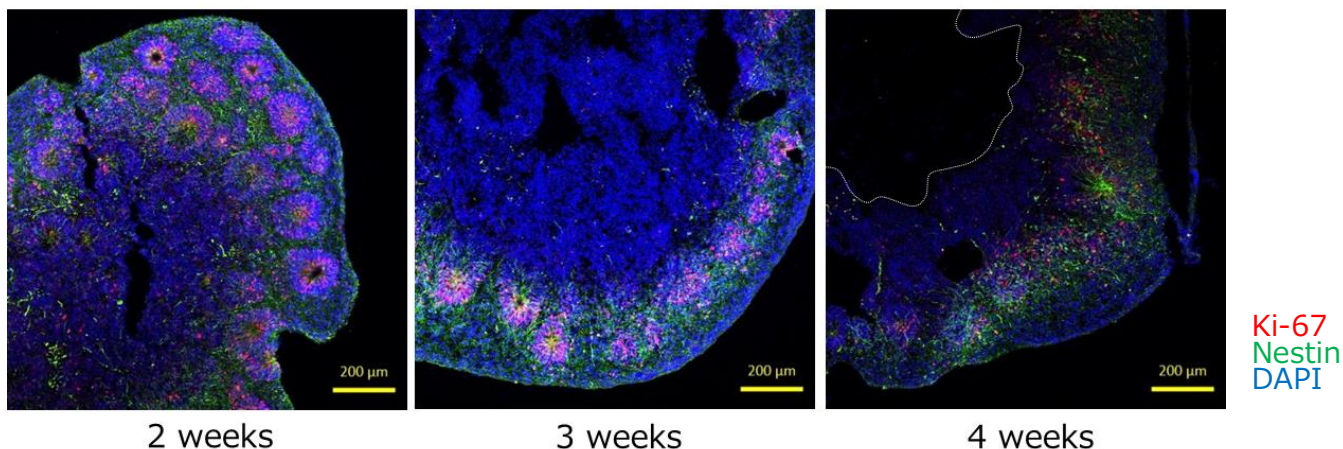
3x3x2 spheroids



After nine of days of maturation



5. After two to four weeks of maturation, on-going differentiation is observed. Proliferating or differentiated cells are seen more in the external layer.



6. About one month after the graft was transplanted to the brain of a mouse, a smooth surface between the host and graft is observed. Blood vessels are observed in the graft.

