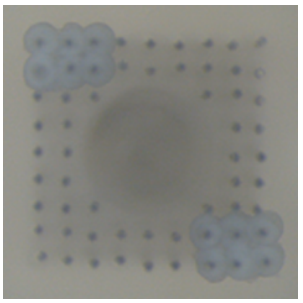
Neural Organoid for Future Treatment of CNS and Spinal Cord Injuries

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.

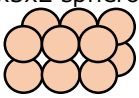
The steps for making a neural organoid using the **Bio 3D Printer Regenova** (**Cyfuse Biomedical K.K.**) are as follows.

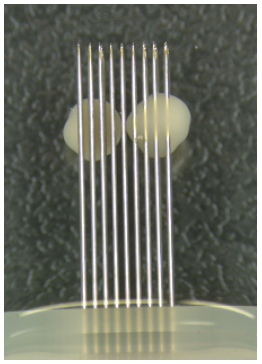
1. Prepare cell aggregations (spheroids) with **Human iPSC-Derived Neural Stem Cells** (**hNSCs - ax0015**, **Axol Bioscience**) in approximately half of the 96 well plates. The diameter should be ~ 500 μm. For details, see the application note "Preparing Spheroids (Cell Aggregates)".



2. Perform 3D printing with **Regenova**
   Use 9X9 Kenzan and print two structures of 3x3x2 spheroids.

3x3x2 spheroids

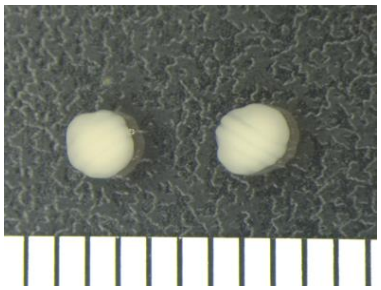


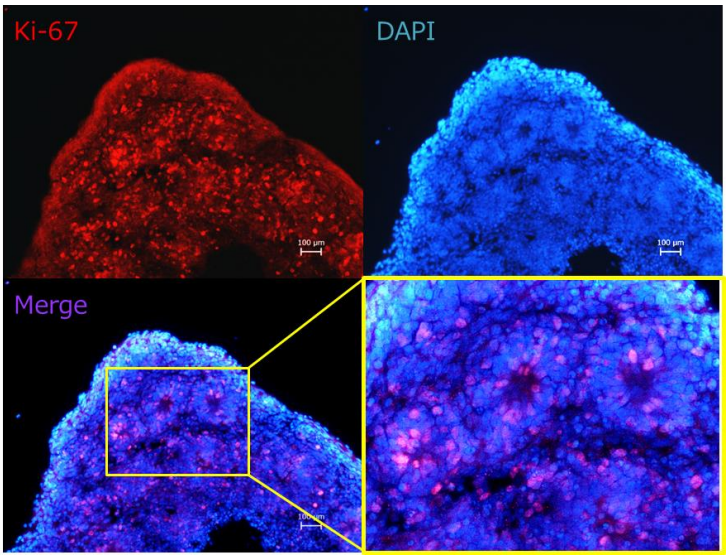


3. Mature printed tissue on Kenzan with **Neural Maintenance Medium** (**Axol Bioscience** – **ax0031/ax0032-500**) in a sterilized container until it spontaneously differentiates into a mixed population of mature neurons and glia. This process takes up to four weeks.

4. After nine days of maturation, a rosette formation and Ki-67 expression (proliferation marker) are observed in the neural organoid.

After nine of days of maturation





Ki-67
DAPI

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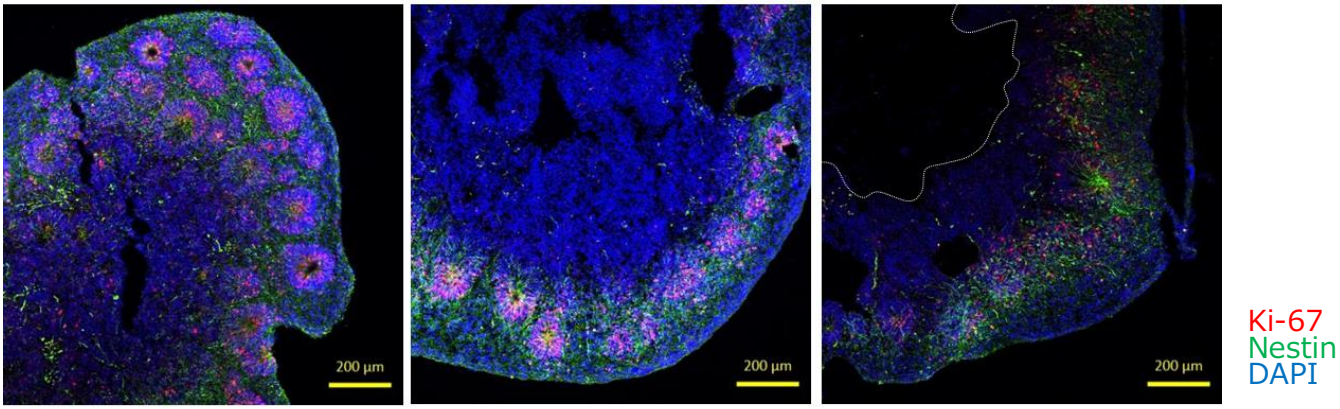
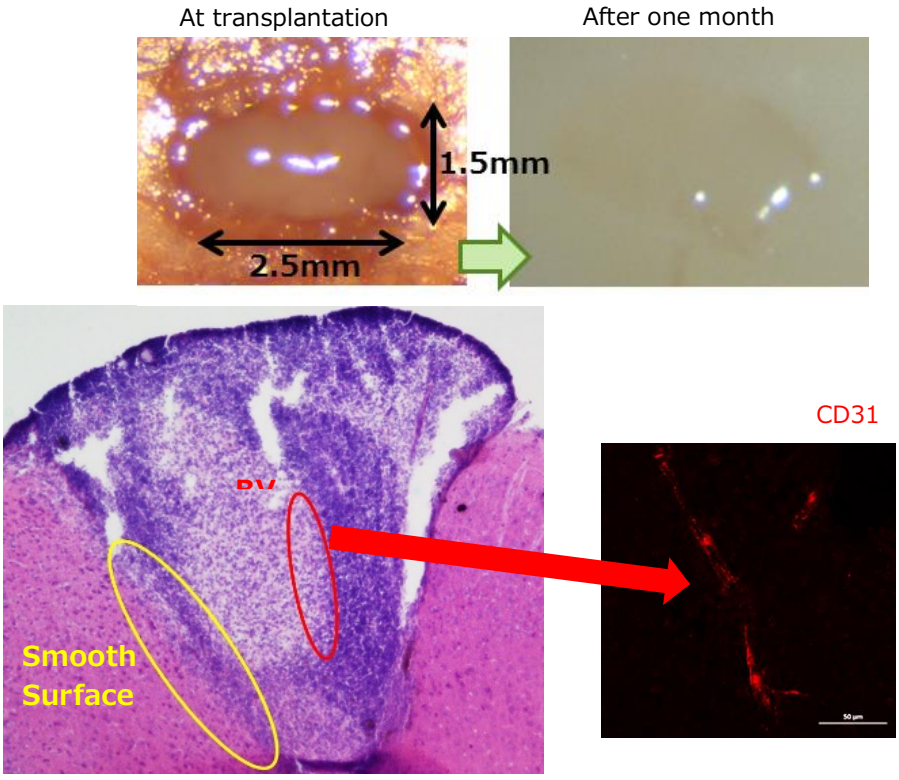
5. After two to four weeks of maturation, on-going differentiation is observed. Proliferating or differentiated cells are seen more in the external layer.



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.



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