Development of iPSC-derived 3D human brain micro-tissues for drug discovery applications

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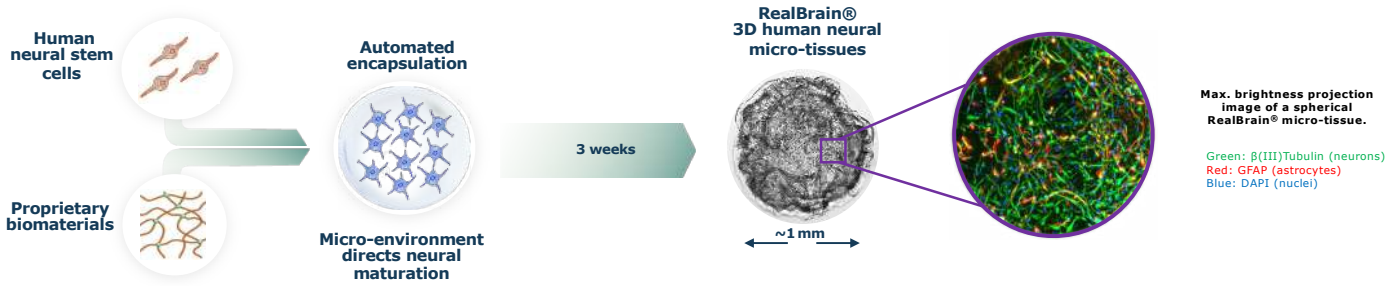
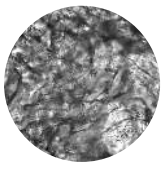
1. Summary

Recent advances in 3D cell culture systems provide a more physiologically relevant alternative to current approaches for high throughput compound screening. RealBrain® 3D neuronal micro-tissues (Tessara Therapeutics) demonstrate several advantages over other 3D models, including automated casting, facile maintenance, rapid maturation for use within weeks, optical clarity for simplified imaging, broad assay compatibility, utility for acute and chronic studies (>4 months in vitro) and they are cost effective. Robotic manufacturing has also been optimised to ensure reproducible and scalable production of RealBrain® micro-tissues (>15,000 micro-tissues/day from a single instrument). Industry collaborations demonstrate the utility of our screening platform for interrogating and filtering drug libraries and also highlight the capacity to develop bespoke assays to investigate pathways involved in the pathogenesis of multiple neurodegenerative conditions. To further develop our technology and to take advantage of the large number of available patient-derived cell banks, we have engaged with Axol Bioscience to develop the first iPSC-derived micro-tissues in our platform. iPSC technology enables the creation of pluripotent stem cells from primary materials (blood or fibroblasts) donated by AD patients. These stem cells can be differentiated into functional neuronal and neuro-inflammatory subtypes for use *in vitro* assay systems. Axol Bioscience manufactures these cells and supporting media and supplements at scale under ISO9001 accredited conditions. We have now demonstrated proof-of-concept for the generation of iPSC-derived human 3D micro-tissues using cells sourced from both healthy donors and patients with familial Alzheimer's disease.

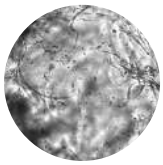
2. Automated RealBrain® production



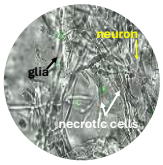
3. ADBrain™ - a 3D model of Alzheimer's disease



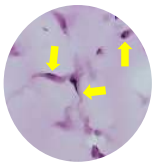
Healthy RealBrain® (4x obj.)



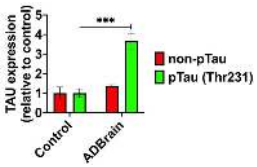
ADBrain™ showing loss of neural networks (4x obj.)



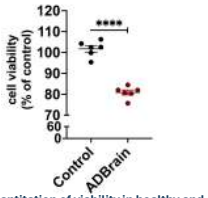
ADBrain™ showing fluoro-tagged Aβ42 in neurons, glia and necrotic cells (20x obj.)



Gallyas silver stained ADBrain™ showing argyrophilic deposits

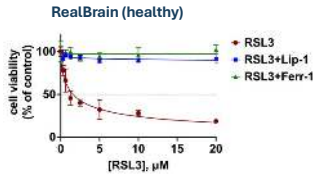


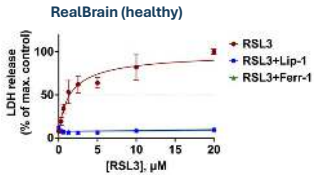
Quantitation of tau in healthy and ADBrain™ micro-tissues

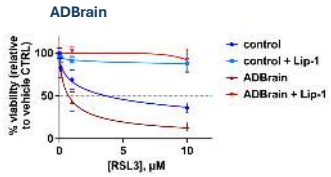


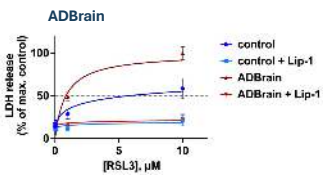
Quantitation of viability in healthy and ADBrain™ micro-tissues

- Tessara's ADBrain micro-tissues are generated following exposure of cells (primary human cells in this panel) to a proprietary Aβ formulation that results in neurodegeneration consistent with that seen in Alzheimer's disease.
- This models key pathological features of the AD brain including; Aβ42 deposition, loss of neural networks, hyperphosphorylated Tau, dystrophic axons and senile plaques.
- This ADBrain model is more susceptible to cell death as compared to the healthy brain, as shown below.





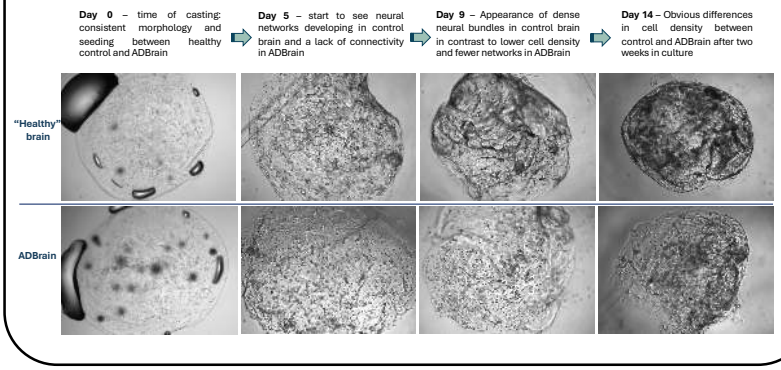




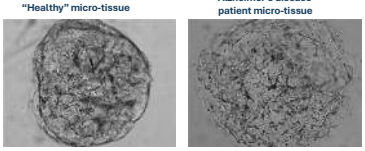
- Ferroptosis is a distinct mode of cell death characterized by iron-dependent accumulation of lipid hydroperoxides.
- It is implicated as a mechanism of neurodegeneration in Alzheimer's disease and other indications (e.g. Parkinson's disease and traumatic brain injury).
- RSL3 can induce ferroptosis and is rescued by liproxstatin-1 (Lip-1) and ferrostatin-1 (Ferr-1).
- We used a cell viability (A,C) and lactate dehydrogenase (LDH) release cytotoxicity assay (B,D) to show that the ADBrain™ has increased susceptibility to ferroptosis-induced cell death as compared to healthy control brain.
- This is one example highlighting the physiological relevance and utility of this platform

4. iPSC-derived RealBrain® micro-tissues

- Selected Axol Bioscience NSCs were subject to Tessara's expansion protocol prior to exposure to Aβ and encapsulation into micro-tissues
- Micro-tissues display characteristic Aβ42 deposition, cell death and loss of neural networks. Further characterization is underway.



5. Patient-derived RealBrain® micro-tissues



- Selected patient-derived cells from Axol Bioscience were used, including from an AD patient with a presenilin-1 (PS1) mutation (shown here).
- PS1 mutant micro-tissues were less dense with a reduced network connectivity.
- Further characterization is underway

6. Conclusions

We have successfully demonstrated proof-of-concept for the development of iPSC- and patient-derived 3D human brain micro-tissues. This work will pave the way for the development of protocols for "clinical trial in a dish" studies to help accelerate personalised medicine approaches for neurological conditions and will help drive innovation and knowledge translation. It will also significantly de-risk drug discovery pipelines by reducing cost and providing earlier insights into drug neurotoxicity and therapeutic efficacy. With current changes in the regulatory landscape, this work will also foster the adoption of non-animal-based screening approaches across the sector.