Developing human dish models of neurological pathology

Chris Adams, Jon Sen, Jacqueline Tickle, Maria Bushra, Nikolaos Tzerakis, Divya Chari

The use of live animals in medical research has been an emotive and controversial issue for decades. Doubts have been raised surrounding the predictive utility of current, widely used pre-clinical animal models for human disease. At the polar end, many will remember the violent campaigns run by anti-vivisectionist groups in the 90's, which led to special laws being drawn up in the UK and a nationwide crackdown on extremist animal rights activity. High profile agencies such as People for the Ethical Use of Animals (PETA) maintain that "*ANIMALS ARE NOT OURS to eat, wear, experiment on, use for entertainment or abuse in any other way*" (www.peta.org.uk/). The opposing case for animal testing has had high profile and articulate proponents also. Organisations such as Understanding Animal Research outline compelling reasons why animals are needed in research (www.understandinganimalresearch.org.uk/). They point out that animal research has played a vital part in nearly every medical breakthrough over the last decade. Diseases such as cancer and AIDS are no longer the death sentence they once were thanks to animal research. Drugs for asthma, TB, malaria, meningitis, and diabetes have all relied on animal experiments. The future development of modern vaccines for deadly diseases that threaten the human population will rely on animal testing.

In terms of public opinion, a 2014 UK survey by the Department for Business, Innovation and Skills revealed that the majority of the British public (68%) accepts the use of animals in scientific (medical) research '*where there is no alternative'*. This is an incontrovertible position, and such a widely held view has led to a major global drive to Replace, Reduce and Refine animal experimentation, known as the 3R's. It should be noted that the central nervous system (CNS), consisting of the brain and spinal cord poses a unique challenge for biological modelling in given its intricate cellular architecture. This is further compounded in the dysregulated environment of pathology sites. However, recent progress in the development of regenerative therapies for CNS disorders means there is a critical requirement to develop high-throughput, yet biologically and clinically relevant experimental models within which to test new candidate therapies. Widely used experimental models such as *in vitro* co-cultures or microfluidic bio-networks are useful for reductionism, but are generally limited in their capacity to mimic the complexities of neurological pathology.

In this context, it has been suggested that "*the best predictability is achieved with human organotypic models that mimic the microenvironment of human tissues*" (Heinonen, 2015). Organotypic slices are 3-D tissue explants which can be maintained in a dish and show maturation features comparable to the donor tissue from which these are derived. These are

high-throughput, low cost, technically simple and reproducible, and have been proven to be valuable experimental model in basic neurophysiology/neuropharmacology. This is largely because they take advantage of the precisely controllable environment of *in vitro* systems yet retain complex neural architecture and cellular inter-relationships. This provides a powerful 'interface' between high throughput *in vitro* screening and pre-clinical animal models.

Despite these key advantages, surprisingly few groups across the world have attempted to use the organotypic paradigm to mimic neurological injury. To address this gap, in 2014, the Chari Laboratory developed a rodent, ‘dish’ organotypic model of traumatic spinal cord injury where nerve cells are severed (mimicking for example, gunshot or stab wounds to the spine) (Weightman et al, 2014). Subsequent characterisation of pathological responses in these model injuries proved that the slices are capable of replicating complex pathological responses that are seen in the intact nervous system, such as the formation of a cellular scar and infiltration by immune cells. The work was featured by the influential charity Fund for The Replacement of Animals in Medical Experiments (FRAME) (www.frame.org.uk/), which campaigns for replacement of animals in laboratories by application of “better science” in the award winning journal ATLA (Alternatives to Laboratory Animals). In ongoing studies, the Chari group has also proved that is possible to grow slices of the rodent cerebellum for extended periods (more than six weeks) in culture, with high cell survival and striking maintenance of many features of intrinsic cerebellar cytoarchitecture (for example, see Jenkins et al, 2011).

Given the initial findings, such organotypic models can be predicted to be of high value in the screening of novel therapies for human neural pathologies. However, the critical step next stage of evolution of such replacement paradigms is to **move towards the development of experimental systems using human derived tissue**. From 2017, the Neural Tissue Engineering group from Keele University will work with clinicians at the Department of Neurosurgery, University Hospital of North Midlands for the first time, to take a major step towards achieving this goal (www.uhnm.nhs.uk/research/ResearchNews/Pages/First-of-Its-Kind-Neuroscience-Research-at-UHNM.aspx). Made possible by funding from the North Staffordshire Medical Institute, the vision for the project is to prove the feasibility of growing organotypic slices of brain (removed during elective decompression surgery from patients with Chiari malformation- a condition where the cerebellum herniates through the foramen magnum towards the spinal canal) *in vitro*. The project will study the survival of various cell types in the brain slices and evaluate how long these can be maintained *in vitro*. Once this baseline data is obtained, the next step will be to introduce an injury paradigm, and study pathological responses to the induced injuries for detailed comparison with well documented

in vivo responses. If successful, we are hopeful that such human tissue models can have a significant impact in reducing animal usage and providing a medically relevant system for testing novel therapies in a dish, in order to identify the most promising therapeutic interventions for neurological disease and injury.

References

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