Module:
Techniques in Neuroscience

Week 4
Tissue culture: Growing and studying neural cells in a dish

Topic 1
An introduction to tissue culture – Part 1 of 2

Dr Graham Cocks
Postdoctoral Research Worker, Basic and Clinical Neuroscience, King's College London

These lectures will cover the fundamentals of tissue culture. A historical perspective will be given, highlighting the major advances that have allowed for the successful growth and study of cells and tissues outside of the organism.

So, what is tissue culture? Tissue culture refers to the cultivation of eukaryotic tissues or dissociated cells outside of the organism in a growth media with a necessary nutrients, inorganic salts and pH required to function in a physiologically normal manner. You will also come across the term 'cell culture,' which refers to the culturing of dissociated cells, rather than pieces of tissue. For consistency, this lecture will use the term 'tissue culture' to refer to both tissue and dissociated cell culture.

So, why is tissue culture useful? It is proven to be, and continues to be, enormously useful as a model system for studying the basic processes of cell biology and also has many clinical applications. These clinical applications include the diagnosis of chromosomal disorders from the culture of blood or amniotic fluid samples, the generation of monoclonal antibodies for the production of vaccines as a result of the development of hybridoma cell lines by Kohler and Milstein (1975), in vitro fertilisation through techniques developed for the culture of the early embryo, and first achieved by Patrick Steptoe and Robert Edwards in 1977, and many more.

The short-term maintenance of tissue outside an organism goes back at least as far 1885, when Roux demonstrated that neural tissue from early chick embryos could be maintained for a short period of time in a saline solution. However, this is generally considered to constitute temporary survival rather than true tissue culture, as the media did not support longer term growth. The first successful demonstration of maintaining animal cells in a substrate supporting growth and long-term survival is generally credited to Harrison, who in 1907 removed small sections of frog embryos and embedded them in blood clots on the underside of coverslips to allow microscopic evaluation. By using good aseptic technique, he was able to observe the outgrowth of nerve cells over a period of weeks. Building upon work of Harrison, Burrows and Carrel were able to establish much longer-term cultures: by refining the growth media and by employing careful aseptic technique, they were able to generate the first indefinitely growing cell line from an embryonic chicken heart.

Cell lines have the capacity to grow indefinitely in culture. However, they typically acquire this capacity through genetic mutations and chromosomal abnormalities, a fact that was unknown at this time. The history of tissue culture from then on was the refinement of the method. The major steps in that history will be covered in this lecture.

The use of blood products as a means of maintaining tissue gave rise to problems with the reproducibility of experimental results, due to the poorly-defined nature of the material. Many researchers have subsequently sought to develop fully chemically-defined media formulations to overcome this problem. One of the first attempts to do this was published in 1911 when Lewis and Lewis demonstrated that they were able to grow embryonic chick tissue in a relatively simple, defined, liquid media. There have been many subsequent developments in the use of defined media for growing a wide range of different cell types, and new media formulations are still being developed.

However, even today there are cell types such as human fibroblasts that are still typically grown with media containing serum. Another important advance came in 1916 when Rous and Jones first demonstrated the use of the proteolytic enzyme, trypsin, to dissociate tissues into individual cells for culture. Furthermore, most cell types, with the exception of blood cells, grow attached to an extracellular matrix. The extracellular matrix is composed of a complex mixture of polysaccharides and proteins, such as collagens and laminin. Tissue culture vessels coated with purified or unpurified components of the ECM help to support attachment and normal functioning of many types of adherent cells. Cell adhesion molecules on the surface of many cells bind strongly to components of the ECM. These contacts have to be disrupted to detach cells without causing cell death. The use of trypsin, therefore, also allowed for the replating of cells grown attached to a substrate. This replating - or passaging, as it is generally called - is essential when culturing dividing cells, which would eventually grow to occupy all the space in a cell culture vessel.

For many cell types when they no longer have the space to grow, they undergo what is known as contact inhibition, which stops the cell from dividing further and can alter the characteristics of the cells under investigation. Trypsin is still in use today to enzymatically dissociate tissues into single cells. However, for passaging cells, trypsin can cause a degree of cell death and gentler enzyme formulations, such as Accutase, and non-enzymatic methods, such as EDTA solutions, are increasingly used. The non-enzymatic method, such as EDTA solutions, chelate ions such as calcium that are essential for the function of cell adhesion molecules.

Disruption of these contacts allows the cells to be rinsed from the flask with much lower levels of cell death. Dissociated cells can be frozen indefinitely in liquid nitrogen. When freezing down cells, they are detached using the same methods used for passaging cells and resuspended in a solution with a cryoprotectant such as DMSO. This reduces the formation of ice crystals, which will cause cell death. Cells are frozen down initially in a -80 freezer at a rate of 1° C per minute using a vessel filled with isopropanol. They can then be transferred to a liquid nitrogen vessel and stored indefinitely. Cells are revived from liquid nitrogen by rapidly thawing in a 37° C water bath, again to minimise ice crystal formation before replating in growth media.

A big step forward was taken in 1923 by Carrel and Baker in the design tissue culture vessels. The methods of tissue culture developed by Harrison relied upon tissue grown in a plasma clot suspended from a glass slide. This method, known as the hanging drop technique, created a number of problems, including the fact that the cells in the clot were difficult to view under the microscope, and were also unable to grow to a large size. To address these issues, Alexis Carrel developed a new vessel for tissue culture, the Carrel flask, which was manufactured with an angled neck to prevent airborne particles from settling into the flask when opened. The next design also allowed for sterilisation with a flame, further reducing the risk of airborne contaminants infecting the culture

Most modern tissue culture vessels are made of plastic, are sterile and are intended for single use. This reduces the risk of microbial contamination and cross-contamination of cell lines. Most tissue cultures are performed in plates or flasks, and these come in varying sizes. Plates range from single dishes up to 15 centimetres in diameter, to plates with 384 wells. Flasks typically range in size from 25 centimetres squared to 175 centimetres squared. Flasks often have vented lids to prevent airborne particles entering, whilst allowing the free exchange of gasses.