Module:
Techniques in Neuroscience

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

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

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A further significant step to counter the problem of microbial contamination of cultures came from the widespread use of the antibiotics penicillin and streptomycin in the 1940s. However, penicillin and streptomycin are ineffective against certain common strains of bacteria such as mycoplasma. Mycoplasma are the smallest known bacterial cells, making them very difficult to observe under conventional light microscopes. They can be a significant problem in long-term culture, affecting the growth and normal functioning of cells. There are antibiotics which are effective against microplasmas such as Ciprofloxacin. However, best practice is to prevent contamination in the first place by employing good aseptic technique. One of the most important developments in improving aseptic technique, and thereby reduce the risk of microbial contamination in cultures, was the development of biological safety cabinets.

The first biological safety cabinets were designed to protect the user from hazardous microbes. The earliest of this kind of safety cabinet reported dates back to 1909, and was a simple ventilated hood used at the WK Mulford Pharmaceuticals company for the preparation of tuberculin for mycobacterium tuberculosis. These cabinets are now classified as class one cabinets, and give some protection to both the user and the environment from the sample. The sample, however, is not protected from the airborne particles from the environment. However, for the manipulation of biological materials, such as eukaryotic cells, to subsequently be grown in culture for extended periods of time without microbial contamination, the development of an effective biological safety cabinet that also protected the sample from outside contamination was needed. These so- called Class II cabinets were developed in the 1960s. Class II cabinets rely on a continuous uniform flow of clean filtered air traveling down over the sample. The air flow then splits and flows through grills at both the front and the back of the cabinet. Unfiltered air entering the front of the cabinet is drawn directly into the grill of the front of the cabinet and out through a filtered exhaust without passing over the sample. For samples which require an even greater level of containment, Class III hoods can be used, which completely enclose the sample and can only be accessed through gloves integrated into the cabinet. For most routine tissue culture, Class II hoods are used and are very effective at reducing microbial contamination from airborne particles when manipulating the sample.

Tissue culture incubators have also been developed to maintain a number of critical parameters to allow optimal growth and survival. These include constant temperature, humidity and the levels of gases in the incubator, such as CO2 and oxygen. Most media currently in use for tissue culture use buffers that require an atmosphere of 5 per cent CO2 to maintain a physiological pH.

Some cell types, such as pluripotent stem cells, which will be discussed later, grow better under low oxygen conditions, and incubators are available that can reduce oxygen levels with displacement by nitrogen. In 1961, Hayflick and Moorhead derived the first strains of human fibroblasts WI-38. They made the distinction between primary cells, cell lines and cell strains. Primary cells are derived from normal tissue and grown without passaging. Cell strains are derived from primary calls which have a limited capacity for growth and division, but which retain a normal carrier type. Cell lines have the capacity to grow indefinitely and invariably have abnormal carrier types.

The most famous cell line was derived earlier in 1951 when Gey cultured cells from and an individual called Henrietta Lacks who had cervical cancer. He found that cells derived from the cervical tumour could grow and divided indefinitely, and generated a cell line from a single cell which he named HeLa cells after the donor. These cells have been used extensively in research, including in the development of the first vaccine against polio, and are still used in research to this day more than 65 years later.

A major advance in the development of cell lines that can be grown indefinitely and yet retain a normal karyotype and also the capacity to differentiate into specific cell types, was the isolation of embryonic stem cells from mouse blastocysts by Martin Evans in 1982. These cells can, in principle, generate any cell type of the body in a cell culture dish. In 1998, this was also achieved by Jamie Thompson with human embryonic stems cells, and allowed for the generation of inaccessible cell types, such as neurons, in large numbers for the first time. There are ethical issues surrounding the generation of human embryonic stem cells, however, as they require the use and destruction of human embryos.

In a major breakthrough by Yamanaka, however, it became possible to directly generate this type of cell by directly manipulating somatic cells, such as fibroblasts, in a process called reprogramming to produce so-called induced pluripotent stem cells, iPSCs. These iPSCs have become a powerful tool to study genetic diseases and inaccessible cell types, such as neurons, without the ethical issues surrounding embryonic stem cells.

Following on from the development of human iPSCs is the exciting prospect of using these cells for personalised regenerative medicine. In principle, an individual who is suffering from a disease, such as Parkinson's, could give a small sample of blood, which could be reprogrammed into iPS cells and then differentiated into dopaminergic neurons. These dopaminergic neurons could then be transplanted back into that individual to replace the ones that have been lost. The major advantage of this approach is that the transplanted cells will be genetically identical to that individual, eliminating the risk of rejection and the use of immunosuppressive drugs.

Clinical trials have already started to try and treat age-related macular degeneration using iPSCs, and this is undoubtedly a technology that will be refined and increasingly used in the future for treating a wide range of conditions requiring cell or tissue replacement.