Basic techniques of working with tissue cultures

Research in medical biochemistry or in other biomedical fields is currently – and a change cannot be expected in the near future – impossible without experiments on living organisms. However, experiments on laboratory animals are expensive and demanding for many reasons (time, personnel, equipment requirements, they are complicated by interindividual differences between animals, as well as intraindividual variability of the whole organism - e.g. circadian and annual biological rhythms). Discussed ethical aspect of experiments with live animals cannot be ignored either. In some cases, it is also not advantageous to do an experiment on the whole organism, organ or tissue but it is necessary to examine the properties of only a certain type of cells. Therefore in recent decades, basic medical research cannot be imagined without another experimental tool: tissue cultures.

History

The history of tissue cultures begins at the end of the nineteenth century: around **1885 Roux** was able to maintain live explanted chicken embryonic cells in an artificially prepared medium for a certain period of time and several other authors achieved similar results at that time. However, the cells failed to divide as efficiently as under *in vivo* conditions. Even at the end of the 19th century it was also possible to freeze living cells and thaw them again which was used in cattle insemination. At the turn of the 19th and 20th century, **Ross Harrison** proved that newly formed axons can grow from nerve tissue taken from frog embryos and enclosed in a chamber with the frog's lymph. Finally, in the 40s of the 20th century, the first successful attempts to establish a cell culture appeared in which cells would multiply and survive for a longer, although limited time. Tissue culture techniques were being gradually improved and the results became more and more reliable. From the 1980s, tissue cultures became a common tool not only for research but also began to be used industrially for the production of antibodies, medicaments and chemicals. These methods are absolutely indispensable for molecular biology, the development of new drugs, etc.

Working with tissue cultures

Working with tissue cultures in some ways differs from other techniques used in biochemistry. First of all, it is necessary that all tools and chemicals which are used are sterile and that they do not contain some toxic substances that otherwise normally contaminate them in trace amounts. In addition to the usual equipment, some special devices and aids are also required. Therefore are these techniques relatively expensive and require specially built laboratories and trained personnel.

In principle, work with each cell line has **several phases** :

1. cell strain isolation
2. preservation of the cell line and its expansion
3. use of multiplied cells from the experiment.

Most cell lines have a **limited life span**, i.e. they undergo senescence and after a certain period of time they stop dividing. Only some tissue cultures are immortal - they are usually cells obtained from tumors or artificially immortalized by inserting suitable genes into their genome. In that case, we talk about **continuous cell lines**. Cells in these lines usually retain the ability to regulate cell division. If they lose it and divide uncontrollably, we are talking about a **transformed cell line**.

The vast majority of cell lines require a suitable surface (today most often polystyrene) for growth - **adherent cultures.** Only some types of cells can survive and divide in **suspension**. Cells are grown in **cultivation containers** (bottles, Petri dishes, etc.) in special **cultivation media**. These contain the necessary ions, buffers, energy sources, amino acids, vitamins, growth factors and other auxiliary substances. A mixture of the necessary growth factors, trace elements and other substances needed in low concentrations is usually secured by the addition of **fetal calf serum**. For optimal growth, tissue cultures must be grown in a sterile environment with a constant temperature of around 37°C and a humidified atmosphere with an increased carbon dioxide content.

The use of cells isolated from an experimental animal (or more rarely from a biopsy sample) results in a so-called **primary culture**. The cells gradually divide until they ideally form a confluent simple layer (monolayer) on the surface of cultivation media. If the cells are not transformed, the confluent culture stops growing at this point.

If we want to maintain cell line further, it is usually released from the surface of the culture container shortly before confluence is reached (mechanically, with the help of some enzymes - trypsin, collagenase, dispase, or with the help of divalent ion chelators - EDTA, citrate). The resulting suspension is diluted and put in new cultivation containers. The entire procedure is referred to as a **passage**, the new culture is called a **subculture**.

Links

Related articles

- Cell cultures
- Cultivation of cells and tissues in vitro, significance in medicine

Sources

Vejražka, M.: *Základní techniky práce s tkáňovými kulturami*. Prague, 2004