Cell and Tissue in vitro Cultivation

First of all, in vitro refers to the biological processes that are made in a such a way as to occur outside the living body, in specialized laboratory apparatus ('in glass').

Cultivation of Tissues and Cells

Cultivation or culture refers to the growth of tissues or cells in a suitable selected culture medium. A culture medium is defined as a nutrient material that is either solid (agar) or liquid (physiological saline), and is used to promote and help microorganisms to grow and reproduce. Conditions that should be controlled during a cultivation:

- nutrients;
- temperature;
- pH;
- O2 levels;
- H2O content;
- osmotic factors;
- light;
- pressure.

→ Levels of each factor are selected according to the type of cell or tissue under cultivation.

Scientific Uses

Cell or tissue cultivation is a way of chromosomal analysis, thus enabling a wide spectrum of scientific uses:

- regeneration of complete plants for commercial propagation (e.g. orchids);
- production of virus-free crops;
- manufacture of viral vaccines (e.g. vaccines for polio, measles, mumps, rubella, and chickenpox);
- manufacture of enzymes, synthetic hormones, monoclonal antibodies and anti-cancer drugs.

Types and Examples

Cells for chromosome analysis must be capable of growth and rapid division in culture. There are two types of cultures, the short-term and long-term cultures.

Short Term Cultures

In such cultures, T-lymphocytes are used. White blood cells are the most readily accessible cells that meet all the specified requirements for culturing. They are ideal for rapid clinical analyses. The only problem is that cultures prepared from peripheral blood don't last long; only 3-4 days. Technique:

1. draw sample of blood;
2. mix with heparin (anti-coagulant);
3. centrifuge;
4. discard supernatant and gather “buffy coat” (WBC’s layer);
5. place in tissue culture medium;
6. add mitogenic agent (stimulates mitosis) e.g. PHA;
7. incubate for about 72 hours;
8. add colchicine (prevents completion of cell division by inhibiting spindle formation), doing so arrests cells in metaphase;
9. add hypotonic KCl solution to swell up WBC’s and release chromosomes from cells
10. add fixative;
11. place culture on freezing slide (cryostat);
12. dry and stain, ready for chromosome analysis.

Long Term Cultures

For long-term analyses, three kinds of cells can be used: fibroblasts, bone marrow cells, or fetal cells.

1. Fibroblasts: A sample of fibroblasts is obtained from a skin biopsy. These are spindle-shaped cells that are capable of continuous growth in culture for many generations. They are used for biochemical or molecular studies and chromosomal analysis.
2. Bone marrow cells are obtained by an invasive procedure (marrow biopsy). Such a sample contains a high proportion of dividing cells, therefore very little culturing is needed. They are used in the diagnosis of hematological malignancies. Their disadvantage is that chromosomes are short and fuzzy and therefore hard to bond.
3. Fetal Cells are obtained from amniocytes in amniotic fluid or chorionic villus biopsy. They are used for cytogenetic, biochemical or molecular analysis.

Links

Related articles

- Karyotype

Sources

- PANZAK, . *Human Karyotype* [lecture for subject Biology and Genetics, specialization Biology and Genetics, 1LF Charles University in Prague]. Prague. 2008.

Bibliography