General applications of tissue cultures

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The important applications of animal cell cultures are:

1- Investigation the normal physiology and biochemistry of cells.

2- Study the effect of various chemicals or drugs on specific cell types (cytotoxicity tests).

3- Large scale production of valuable biologicals "vaccines and antibodies".

4- Use of tissue cultures to generate artificial tissues “biotechnology or tissue engineering".

Investigation of the normal physiology and biochemistry of cells

• The primary impetus for the development of cell culture was to study, under the microscope, normal physiological events of cells.

• **Haberlandt (1902)** stated that the in vitro-culture techniques for plants were developed primarily to facilitate basic physiological research.

• **Harrison (1907)** developed his culture to study the development of nerve fibers.

• Animal or plant cell, when removed from tissues and supplied with the appropriate nutrients and conditions, grows and acts as independent unit, much like a microorganisms such as a bacterium or fungus.

Use of tissue cultures in toxicity testing

• Mammalian cell cultures can be a suitable alternative for the use of whole animal tests to establish the potential toxicity of compounds.

This due to many reasons:

1- They can overcome the disadvantages of the whole animal tests including:

- High costs.
- Variability of results.

2- Growing moral objections to the use of animals in toxicity testing (See, Directive 76.768 by European Parliament).

3- Cell culture tests are rapid, allow more efficient screening of novel compounds and sometimes can allow the identification of metabolic targets of inhibition.

• Cell culture tests can be designed to evaluate various effects:

- Reduced growth rate.
- Breakdown of membrane permeability
- Tissue specifiicty of response.
- Ability to metabolize toxic compounds.
- Genetic effects/mutagenicity.

Use of tissue cultures for production of biological products

A) Production of vaccines:

• Two factors stimulated the use of tissue cultures for vaccine production:

- The ability to grow viruses in cell cultures.
- Current egg-vaccine production requires long time (9 months) that hinder the response to unanticipated demands.

• In (1949), **Enders** discovered that the poliomyelitis virus could be grown from primary monkey cells in culture.

• The polio vaccine, produced in 1954, was the first human vaccine to be produced using large-scale cell culture tchniques.

• Animal cell technology is considerably developed for the production of a range of human and veterinary viral vaccines against a variety of diseases (see, Table 1).

B) Production of antibodies:

• Also, the in vitro methods for production of mABs are the methods of choice because of:

▫ The ease of culture for production.

▫ Less economic consideration compared with the use of animals.

• These advantages make the in vitro methods meet more than 90% of the needs for mABs.

• The ability to generate hybridomas has been stimulated the use of the in vitro methods for mABs production (see, B lymphocyte-myeloma cell hybrid).

• Practical uses of the in vitro produced mABs:

▫ Diagnostic tests for the identification of small quantities of specific antigens.

▫ mABs also are used therapeutically: OKT3 recognizes a surface antigen (CD3) on T cell and

is one of the most effective agents in preventing immunological rejection of transplanted kidneys.

▫ Various mAbs designed to destruct tunor cells by targeting a membrane bound protein antigens specifically expressed by these cells.

▫ The conjugation of radiactive or toxic compounds to the antibody can result in a localized high concentration resulting in cytotoxicity to the target cells.

C) Recombinant proteins:

• This idea based on the ability to transfect cells with isolated genes and amplify it to allow high level of expression of the crossponding proteins.

• Proteins extracted from biological sources have been important for the substitution therapy since the 1920s when **Best** and **Banting** used insulin to treat diabetes.

Some examples for these biological products:

1- Interferone:

Discovered when **Isaacs and Lindenmann** (1957) found that culture medium taken from cells that had supported viral growth could protect non-infected cells from a subsequent viral infection.

2- Tissue plasminogen activator (t-PA):

t-PA was produced in large scale by Genenteck

from transfected CHO-K1 cells. It is used to prevent undesirable formation of fibrin clots in the bloodstream.

3- Blood clotting factors:

For example, factor VIII is produced in large scale by Bayer through transfection of the mammalian kidney cell line (BHK) with an appropriate gene.

Tissue engineering

This means the re-constitution of human tissues from the combinations of cell types grown in culture. This is an important prospect for future therapeutic treatment with organ failure. This include:

1- Artificial tissues:

• The re-constitution of skin following severe

burns is considered the most successful application of tissue engineering.

• Artificial skin can be formed from two layers derived from cultured human cells:

▫ A dermal-equivalent formed from fibroblasts.

▫ An epidermal-equivalent which is layered on the dermal surface (see, Artificial skin).

2- Artificial organs:

• Construction of organs in in vitro have met technical difficulties:

▫ Multiple cell types require complex scafolds and an extracellualr matrix to support the functional relationship between cells.

▫ Multiple cell layer require a nutrient supply equivalent to blood capillaries in vivo.

Cell therapy

Literally, cell therapy means treatment with cells, i.e. replacing diseased or dysfunctional cells with healthy functioning ones.

For example:

• When hematopoietic cells are vulnerable to destruction by any cytotoxic drugs used in chemotherapy to eradicate residual tumor cells.

• Bone marrow pluripotent stem cells can be isolated and expanded prior to chemotherapy to provide a source of mature hematopoeitic cells following chemotherapy.

Gene therapy

• The concept of gene therapy is that a missing or faulty gene is replaced by a normal working gene.

• The process involves the transfection of a specific gene into cells of patient with an identified and well-characterized genetic disease.

• The gene can be introduced into inside the patient (*in vivo*) or outside the patient (*ex vivo*).

• For example, severe combined immunodeficiency (SCID) is associated with a defective copy of a gene, required for the expression of the enzyme adenosine deaminase (ADA).

• Treatment by gene therapy involves:

▫ Isolation of bone marrow stem cells from the patient.

▫ Infection of the cells with a retrovirus constructed

to carry the ADA gene.

- The trasduced stem cells are then introduced into the bone marrow of the patient where they can proliferate and differentiate into immunocompetent cell.

Thank you for your interest