Organ Culture and Its Techniques | Animal Biotechnology

Organ culture means the explantation and growth in vitro of organs or part of organs in which the various tissue components, such as parenchyma and stroma, and their anatomical relationship and function, are preserved in culture, so that the explanted tissue closely resembles its parent tissue in vivo.

The outgrowth of isolated cells from the periphery of the explants is discouraged and minimized by suitable culture conditions, and the 'new' growth is composed of differentiated structures. Thus, in glands, new glandular structures are formed, or, in lung tissue, new small bronchi develop at the periphery of the explant. They consist of alveoli lined with secretory, cuboidal, or columnar glandular or bronchial epithelium.

In tissues lined with squamous epithelium, such as skin or oesophagus, or in bladder lined with transitional epithelium, the epithelium follows a similar pattern of differentiation as in the organs in vivo. Hormone-dependent tissues remain hormone sensitive and responsive, and endocrine organs continue to secrete specific hormones. Finally, in fetal tissues, morphogenesis in vitro closely resembles that seen in vivo.

Organ Culture Techniques:

i. Clotted Plasma Substrate:

Fell and Robison introduced the 'watchglass technique' by which organ rudiments or organs were grown on the surface of a clot consisting of chick plasma and chick embryo extract, contained in a watchglass. This became the classical standard technique for morphogenetic studies of embryonic organ rudiments.

The method has later been modified to investigate the action of hormones, vitamins, and carcinogens in adult mammalian tissues and will be described in detail below. Another type of culture vessel consisted of an embryological watchglass containing a plasma clot and closed with a glass lid sealed on with paraffin wax.

This was first introduced by Rudnick and later adopted by Gaillard, He used a clot consisting of two parts of human plasma, one part of human placental serum, and one part of human baby brain extract mixed with six parts of a saline solution.

The plasma clot, although it supported the growth and development of fetal and adult organs, had several disadvantages. It usually became liquefied in the neighborhood of the explants so that they came to' lie in a pool of medium. Moreover, because of the complexity of the medium no biochemical investigation was possible.

ii. Agar Substrate:

The problems encountered using plasma clots could be eliminated by the use of agar gels. The agar gel technique was first introduced by Spratt. Wolff and Haffen modified Gaillard's technique and used an agar gel contained in an embryological watchglass.

The agar method has been successfully used for developmental and morphogenetic studies and will, like the watchglass technique, be described in detail below. Although the agar does not liquefy, it cannot be added or analysed without transplanting the cultures. This disadvantage was overcome by the use of fluid media combined with a support which prevented the cultures being immersed.

iii. Raft Methods:

Chen found that lens paper used for cleaning microscope lenses (Gurr) is non-wettable and will float on fluid medium. He explanted 4-5 cultures on a 25 X 25 mm raft of lens paper which floated on serum in a watchglass. Richter improved on this by treating the lens paper with silicone which enhanced its flotation properties. Lash et al. combined the lens paper with Millipore filters.

They punched a small hole in the centre of the lens paper raft and covered this with a strip of Millipore filter. Different types of tissues were cultured on either side of the filter and their interaction with each other studied. Shaffer replaced the lens paper with rayon acetate.

The rayon acetate strips were made to float on the fluid medium by treating the four corners with silicone. The rayon acetate has the advantage over lens paper that it is acetone soluble and can be dissolved during the histological procedures by immersing it in acetone.

iv. Grid Method:

The use of rafts floating on a fluid medium did not provide ideal conditions. The rafts often sank and the tissues became frequently, and to different depths, immersed into the medium. This difficulty was overcome by the grid technique devised by Trowell. He introduced metal grids, made at first of tantalum wire gauze.

This was replaced later by the more rigid expanded metal, obtainable as a continuous sheet of stainless steel, or by titanium. The grids were square with a surface of 25 X 25 mm, with the edges bent over to form four legs, and about 4 mm high. Skeletal tissues could be cultured directly on the grid, but softer tissues such as glands or skin were first explanted on strips of lens paper and these deposited on the grids. The grids with their explants were placed in the culture chamber filled with medium up to the level of the grid.

The original Trowell technique was aimed at maintaining adult mammalian tissues which have a higher requirement for oxygen than fetal organs. To achieve this, the culture chambers were enclosed in containers which were perfused with a mixture of carbon dioxide and oxygen.

The method succeeded in preserving the viability and histological structure of the adult tissues, such as prostate glands, kidney, thyroid, and pituitary. The technique, particularly the application of the gas phase, has since been simplified and in this modified form has been and still is widely used.

v. Intermittent Exposure to Medium and Gas Phase:

More recently, a method which provides intermittent exposure to medium and gas phase has been successfully used for the long-term culture of human adult tissues, including bronchial and mammary epithelium, oesophagus, and uterine endocervix.

In this technique, the explants are attached to the bottom of a plastic culture dish and covered with medium. The dishes are enclosed in an atmosphere-controlled chamber which is filled with an appropriate gas mixture. The chamber is placed on a rocker platform and rocked at several cycles per minute during cultivation.

vi. Watchglass Technique:

The technique was introduced by Fell and Robison, originally to study the development of avian limb bone rudiments, but was extended to investigate growth and differentiation of other avian and mammalian tissues. By this method, avian tissues are placed on a clot, consisting of chick plasma and chick embryo extract in equal proportions, which is contained in a watchglass.

One or two such watchglasses are enclosed in a Petri dish carpeted with moist cotton wool or filter paper (Figure 3) to prevent evaporation of the clot and explants. The Petri dishes are transferred to an incubator and incubated, usually at 37.5°C

Method:

1. Before removing the embryos, wipe the blunt end of the eggs with cotton wool soaked in 70% alcohol to sterilise the surface of the shell. Remove the upper end of the shell carefully, by tapping the shell to crack it and peeling outwards, and tear open the inner shell membrane covering the embryo with a pair of straight forceps.

Lift embryos out by their heads using a pair of curved forceps and drop them gently into a Petri dish. Wash them thoroughly with HBSS, remove the eyes, and mince the embryos by hand with a pair of curved scissors.

2. Transfer the minced tissue with a wide-mouthed pipette to a homogenizerand grind by hand for a few minutes.

3. Mix the resultant homogenate with an equal amount of HBSS and centrifuge the mixture at 20 g for 10 min at 4°C. Remove the supernatant and if necessary re-centrifuge to remove suspended cell debris. The final supernatant should be free of cells and appear opalescent.

4. The extract can be used immediately or transferred to vials in 1 ml aliquots for storage at – 20°C. Use within 3 months.

Setting-up of Cultures:

1. Remove the organs or organ rudiments from the embryos with the aid of a dissecting microscope. During dissection, keep the embryos moist with HBSS containing 2% serum.

2. Transfer the tissues to be cultured and wash them in HBSS contained in a cavity slide.

3. Transfer them to the agar gel, either with the aid of two cataract knives or by gently sucking them up in a wide mouthed pipette with HBSS, and deposit them on the agar.

4. Orientate the explants on the agar with two needles and suck off the excess fluid with a fine pipette. Usually, one explant is accommodated on each gel. The optimum size of the explant is 1.5 x 1.5 x 10 mm, the maximum size 2 x 2 x 1.5 mm. However, if the tissue is very thin, say less than 0.5ram thick, the other dimensions can be larger.

5. Using the small paint brush, seal the glass lids on to the chambers with atleast two coats of warm paraffin wax (60 °C). Transfer the chambers to the incubator and incubate at 37 °C or any other desired temperature. It is advisable not to place them directly on the metal shelves but to insulate from the metal with a polystyrene foam tile.

Many experiments involving studies of morphogenetic changes and cyto-differentiation do not require a lengthy culture period and the answer to the question posed may be available within 3-7 days. In this case, a change of medium is not necessary. If the experiment requires a longer period of cultivation, the explants have to be moved to a fresh agar gel every 5-7 days. A fresh gel is made in a fresh chamber, prepared with the same proportion of agar, serum, and defined medium as before.

Remove the lids from the old embryological watchglasses with a warmed razor or scalpel. Lift the explant off the old gel with the aid of two cataract knives or one cataract knife and a dissecting needle, wash in HBSS contained in a cavity slide, transfer to the fresh gel, and seal the watchglasses with a fresh sterile glass lid, using a small paint brush and paraffin wax.

The viability of the explants and macroscopic changes can be monitored during cultivation by viewing them by daylight or with the aid of a light source from the dissecting binocular. Healthy and growing tissues usually appear translucent with a shiny surface; opacity suggests loss of viability or the beginning of necrosis of the explanted tissue.

This modification, in which the saline solution was replaced by defined medium, has been found suitable for studying the induction by testosterone of the prostate gland from its anlage, the urogenital sinus, from mouse or rat embryos, and the role of mesenchyme in this process. The agar supported the development of the prostate gland from the whole sinus, as well as that of its components.

Agar is also used for the preparation of combination cultures to study the interaction of various tissue components, in particular that of mesenchyme and epithelium during the development of fetal organs.

Using this method it has been found that the mesenchyme plays an essential role in the differentiation of the epithelium, and that in hormone-dependent organs it mediates the effect of the hormone on the epithelium.

The result was obtained by using cultures in which the mesenchyme and epithelium of fetal organs were separated and re- associated in various recombinations. The method can be used in developmental studies of many organs, but here its application by Mizuno to the study of prostatic development is described.

vii. Autoradiography:

Measurements of isotope incorporation of labelled tissues by extraction and counting give the total radioactivity, but its distribution between different tissue components or cells is uncertain. Autoradiography provides this information and pinpoints the incorporation in different tissue components or individual cells, their cytoplasm or nucleus. This label can be recognised in suitably treated sections by light microscopy.