RAFT CULTURES

Submitted by

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I. INTRODUCTION

The technique of organ culture involves the cultivation of fragments of organs or entire embryonic organs in vitro. One endeavors to maintain differentiated tissues in their normal tissue relationships as they exist in vivo.

Explants for organ culture should be obtained aseptically and as rapidly as possible, preferably from an animal killed by mechanical means. In general, organs from younger animals are more easily cultivated. The maximum size that may be maintained successfully is on the order of 8 mm^3. Considerable care should be taken in dissecting such explants to avoid tearing or crushing of the tissues. Moreover, recognition of the normal architecture and histogenesis of the organ should be taken into consideration before dissection.

II. MATERIALS

Organ culture dishes, sterile plastic, No. 3010 Falcon[1]
Fine forceps sterile, watch-maker No. 5 Miltex[2]
Coarse scissors sterile, 4 1/2-inch, No. MX5-12 Miltex[2]
Coarse forceps sterile, 4 1/2-inch, No. MX6-4 Miltex[2]
Fine scissors sterile, 4 1/2-inch, No. MX5-304 Miltex[2]
Scalpels, sterile, blades No. 10 size, No. 1110 Bard-Parker[3] and handles No. 3 size, No. 1030 Bard-Parker[3]
Capillary pipettes, sterile cotton-plugged
Serological pipettes, sterile, 10 ml and 1 ml
Rubber bulbs, sterile latex, 2ml, No. 56311-049 VWR[4]
Sterile rafts of stainless steel wire mesh, No. 3014 Falcon[1]
Trowell medium T8, No. 149 GIBCO[5] (Biggers' BGJB or Waymouth's 752/1 may be substituted).
Three-week-old mouse
Balanced salt solution (BSS), Hanks' No. 402 GIBCO[5]
Petri dishes, sterile, 100 mm, Pyrex, No. 3160 Fisher[6]

[1] Falcon Plastics, Division of Bioquest, Cockeysville, MD.
[2] Roboz Surgical Instruments Co., Inc., Washington DC.
[3] Bard-Parker, Division of Becton Dickinson and Co., Rutherford, NJ.
[4] VWR Scientific, Rochester, NY.
[5] Grand Island Biological Co., Grand Island, NY.
[6] Fisher Scientific Co., Rochester, NY.

III. PROCEDURE

A. Preparation of tissue

1. Moisten the absorbent disc in the sterile plastic culture vessel with 5ml BSS.

2. Kill animal by cervical dislocation and stretch on a cork board.

3. Swab surface of animal completely with 80% alcohol or an alcohol-iodine solution to thoroughly moisten fur.

4. Incise the skin to produce a flap which may be moved out and away from the area using sterile (flamed) coarse scissors and forceps.

TCA Manual, Vol. 2, No. 3, 1976

Procedure No. 4 3 1 2 2

5. Flame instruments and incise the underlying musculature (abdominal musculature, rib cage, etc.) to produce a similar flap.

6. Remove the target organ (lymph nodes, ovaries, thyroid, etc.) and place in sterile balanced salt solution using sterile fine forceps (watch-makers) and fine scissors.

7. Cut away extraneous tissues (fat, connective tissue, etc.) by careful dissection, with scalpels. Be sure to cut, not tear.

8. When necessary, cut explants into 1 to 2mm pieces in all three dimensions such that the explant is not larger than 8 mm^3. Incisions must be made with a pair of sharp scalpels used in an opposing, scissor-like fashion.

B. Planting and cultivation of tissue

1. Pipette explants (usually 4) aseptically onto a stainless-steel raft in a sterile Petri dish using as small a droplet of BSS as possible with a capillary pipette attached to a sterile rubber bulb. Remove excess fluid from the explant. Pick up raft with sterile forceps and place carefully over center well of an organ-culture dish.

2. Add approximately 0.9ml of medium to center well. Be sure that the raft and tissue are in contact with the medium but not submerged.

3. Incubate at 35°C. Gas with 5% CO_2 to maintain correct pH. Depending upon the organ, the oxygen tension required may vary from 50% to 95% (e.g. embryonic and neoplastic tissues are adversely affected by high oxygen tensions).

4. Refeed every third day by aspirating medium from below raft. (Medium may be kept for assay.) Replace with 0.9ml fresh medium.

5. After 6 to 7 days, fix explants in either Bouin's, Zenker's or formalin-acetic acid-alcohol, depending upon the staining procedure to be used. Tissues can then be sectioned and stained for evaluation.

IV. DISCUSSION

Historically, the technique for the maintenance of organs in vitro was developed by Honor B. Fell (1) to study bone development. During its early history, the method was utilized extensively as a purely morphological tool (2-6). However, in recent times, the technique has been adopted for many physiologic studies including the effects of vitamins, hormones, carcinogens and embryonic inductors.

Organ explants may be successfully maintained for periods of up to two weeks in vitro (5,6). Unfortunately, many of the physical and chemical limitations such as gaseous requirements, pH, osmolality and supplementary factors have not been established for every organ type. Such conditions must be defined for each specific target organ, taking into consideration the age of the donor tissue.

A histological evaluation of the explants should be made in order to assess the architecture of the tissue at the end of the culture period. This evaluation will provide you with information on the morphological integrity of the explant and the cell-to-cell relationships.

The major advantage of organ culture is that it bridges the gap between cell culture and the in vivo condition because structural relationships of the original tissue can be recognized.

V. REFERENCES

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