Section B and C

Volume-22

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12. APPLIED BIOLOGY:

B. ANIMAL TISSUE CULTURE

1. INTRODUCTION:

Tissue culture was first devised at the beginning of the twentieth century as a method for studying the behaviour of animal cells free of systemic variations that might arise *in vivo* both during normal homeostasis and under the stress of an experiment. As the name implies, the technique was elaborated first with un-disaggregated fragments of tissue, and growth was restricted to the migration of cells from the tissue fragment, with occasional mitoses in the outgrowth. As culture of cells from such primary explants of tissue dominated the field for more than 50 years, it is not surprising that the name ‘‘tissue culture’’ has remained in use as a generic term despite the fact that most of the explosive expansion in this area in the second half of the twentieth century was made possible by the use of dispersed cell cultures. Disaggregation of explanted cells and subsequent plating out of the dispersed cells was first demonstrated by Rous, although passage was more often by surgical subdivision of the culture to generate what were then termed cell strains. 1929 was the first cloned cell strain, isolated by capillary cloning from mouse L-cells. It was not until the 1950s that trypsin became more generally used for subculture, following procedures described by Dulbecco (1952) to obtain passaged monolayer cultures for viral plaque assays, and the generation of a single cell suspension by trypsinization, which facilitated the further development of single cell cloning. Gey established the first continuous human cell line, HeLa; this was subsequently cloned by Puck when the concept of an X-irradiated feeder layer was introduced into cloning. Tissue culture became more widely used at this time because of the introduction of antibiotics, which facilitated long-term cell line propagation although many people were already warning against continuous use and the associated risk of harbouring cryptic, or antibiotic-resistant, contaminations. The 1950s were also the years of the development of defined media, which led ultimately to the development of serum-free media.

The term *tissue culture* is used as a generic term to include organ culture and cell culture. The term *organ culture* will always imply a three-dimensional culture of undisaggregated tissue retaining some or all of the histological features of the tissue *in vivo*. *Cell culture* refers to a culture derived from dispersed cells taken from original tissue, from a primary culture, or from a cell line or cell strain by enzymatic, mechanical, or chemical disaggregation. The term *histotypic culture* implies that cells have been reaggregated or grown to re-create a three-dimensional

structure with tissue like cell density, e.g., by cultivation at high density in a filter well, perfusion and overgrowth of a monolayer in a flask or dish, reaggregation in suspension over agar or in real or simulated zero gravity, or infiltration of a three-dimensional matrix such as collagen gel. *Organotypic* implies the same procedures but recombining cells of different lineages, e.g., epidermal keratinocytes in combined culture with dermal fibroblasts, in an attempt to generate a *tissue equivalent*.

2. ANIMAL TISSUE CULTURE LABORATORY

The major requirement that distinguishes tissue culture from most other laboratory techniques is the need to maintain asepsis. Although it is usually not economically viable to create large sterile areas, it is important that the tissue culture laboratory be dust free and have no through traffic. The introduction of laminar-flow hoods has greatly simplified the problem and allows the utilization of unspecialized laboratory accommodation, provided that the location is suitable.

Several considerations need to be taken into account in planning new accommodation. Is a new building to be constructed, or will an existing one be converted? A conversion limits you to the structural confines of the building; modifying ventilation and air-conditioning can be expensive, and structural modifications that involve load-bearing walls can be costly and difficult to make. When a new building is contemplated, there is more scope for integrated and innovative design, and facilities may be positioned for ergonomic and energy-saving reasons, rather than structural ones. The following items should be considered:

(1) **Ventilation**

a) *Pressure balance.* Ideally, a tissue culture laboratory should be at positive pressure relative to surrounding work areas, to avoid any influx of contaminated air from outside. However, if human material is being used, most safety regulations will require that the tissue culture laboratory be designated as Category I and this requires that it is at negative pressure relative to the surrounding areas. To satisfy both requirements it may be preferable to have a positive-pressure buffer zone outside the tissue culture laboratory, such as the Preparation Area and Microscope Room or the corridor.

b) *Laminar flow hoods.* Consider where air inlets and extracts must be placed. It is preferable to duct laminar-flow hoods to the exterior to improve air circulation and remove excess heat (300–500 W per hood) from the room. This also facilitates decontamination with formaldehyde, should it be required. Venting hoods to the outside will probably provide most of

the air extraction required for the room, and it remains only to ensure that the incoming air, from a central plant or an air conditioner, does not interfere with the integrity of the airflow in the hood. Laminar-flow hoods are better left to run continuously, but if they are to be switched off when not in use, then an alternative air extract must be provided and balanced with the extract via the hoods.

(2) **Accommodation**

a) *Staff numbers.* How many people will work in the facility, how long will they work each week, and what kinds of culture will they perform? These considerations determine how many laminar-flow hoods will be required (based on whether people can share hoods or whether they will require a hood for most of the day) and whether a large area will be needed to handle bioreactors, animal tissue dissections, or large numbers of cultures. As a rough guide, 12 laminar-flow hoods in a communal facility can accommodate 50 people with different, but not continuous, requirements.

b) *Space.* The largest area should be given to the culture operation, which has to accommodate laminar-flow hoods, cell counters, centrifuges, incubators, microscopes, and some stocks of reagents, media, glassware, and plastics. The second largest is for washup, preparation, and sterilization, third is storage, and fourth is incubation. A reasonable estimate is 4:2:1:1, in the order just presented.

c) *Aseptic area.* Ensure that tissue culture is reasonably accessible to, but not contiguous with, the animal facility. Windows can be a disadvantage in a tissue culture laboratory, leading to heat gain, ultraviolet (UV) denaturation of the medium, and the incursion of microorganisms if they are not properly sealed.

d) *Hoods.* The space between hoods should be approximately 500 mm (2 ft), to allow access for maintenance and to minimize interference in airflow between hoods. This space is best filled with a removable cart or trolley, which allows space for bottles, flasks, reagents, and a notebook.

e) *Incubation.* What type of incubation will be required in terms of size, temperature, gas phase, and proximity to the work space? Will regular, nongassed incubators or a hot room suffice, or are CO_2 and a humid atmosphere required? Generally, large numbers of flasks or large-volume flasks that are sealed are best incubated in a hot room, whereas open plates and dishes will require a humid CO_2 incubator.

f) *Preparation area.* Facilities for washing up and for sterilization should be located (i) close to the aseptic area that they service and (ii) on an outside wall to allow for the possibility of heat extraction from ovens and steam vents from autoclaves. Give your washup, sterilization, and preparation staff a reasonable visual outlook; they usually perform fairly repetitive duties, whereas the scientific and technical staff look into a laminar-flow hood and do not need a view.

g) *Servicing aseptic areas.* Will an elevator be required, or will a ramp suffice? If a ramp will do, what will be the gradient and the maximum load that you can expect to be carried up that gradient without mechanical help?

h) *Storage.* What is the scale of the work contemplated and how much storage space will this require for disposable plastics, etc? What proportion of the work will be cell line work, with its requirement for storage in liquid nitrogen?

(3) **Renovations.** If a conversion of existing facilities is contemplated, then there will be significant structural limitations; choose the location carefully, to avoid space constraints and awkward projections into the room that will limit flexibility.

(4) **Access.** Make sure that doorways are both wide enough and high enough and that ceilings have sufficient clearance to allow the installation of equipment such as laminar-flow hoods (which may need additional space for ductwork), incubators, and autoclaves. Make sure that doorways and spacing between equipment provide access for maintenance.

(5) **Quarantine.** Newly introduced cell lines and biopsies need to be screened for mycoplasma before being handled in the same room as general stocks, and some human and primate biopsies and cell lines may carry a biohazard risk that requires containment. These questions will enable you to decide what size of facility you require and what type of accommodation—one or two small rooms, or a suite of rooms incorporating washup, sterilization, one or more aseptic areas, an incubation room, a dark room for fluorescence microscopy and photomicrography, a refrigeration room, and storage. It is better to have a dedicated tissue culture laboratory with an adjacent preparation area, or a number of smaller ones with a common preparation area, rather than to have tissue culture performed alongside regular laboratory work with only a laminar-flow hood for protection. A separate facility gives better contamination protection, allows tissue culture stocks to be kept separate from regular laboratory reagents and glassware, and will, in any case, be required if human or other primate cells are handled, and in some other cases.

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