University of Basrah

College of Science

Department of Pathological Analysis

Course title: Tissue Culture Techniques

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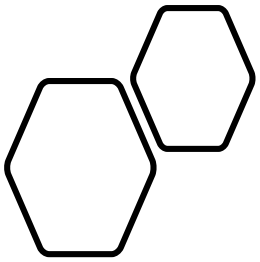

Animal Tissue Culture

Biology of animal Cell; basic concepts (media requirements for cell culture)

Culture media of animal cells: Serum and Serum Free Media

Cell Culture Basics

This Lecture provides information on the fundamentals of cell culture, including

1- the selection of the appropriate cell line for your experiments
2- media requirements for cell culture,
3- adherent versus suspension culture

Note that the following information is an introduction to the basics of cell culture, and it is intended as a starting point in your investigations.

1- Selecting the Appropriate Cell Line

Consider the following criteria for selecting the appropriate cell line for your experiments:

- **Species:** Non-human and non-primate cell lines usually have fewer biosafety restrictions, but ultimately your experiments will dictate whether to use speciesspecific cultures or not.

- **Functional characteristics:** What is the purpose of your experiments? For example, liver- and kidney-derived cell lines may be more suitable for toxicity testing.

- **Finite or continuous:** While choosing from finite cell lines may give you more options to express the correct functions, continuous cell lines are often easier to clone and maintain.

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- **Normal or transformed:** Transformed cell lines usually have an increased growth rate and higher plating efficiency, are continuous, and require less serum in media, but they have undergone a permanent change in their phenotype through a genetic transformation

- **Growth conditions and characteristics:** What are your requirements with respect to growth rate, saturation density, cloning efficiency, and the ability to grow in suspension? For example, to express a recombinant protein in high yields, you might want to choose a cell line with a fast growth rate and an ability to grow in suspension.

- **Other criteria:** If you are using a finite cell line, are there sufficient stocks available? Is the cell line well-characterized, or do you have to perform the validation yourself? If you are using an abnormal cell line, do you have an equivalent normal cell line that you can use as a control? Is the cell line stable? If not, how easy it is to clone it and generate sufficient frozen stocks for your experiments?

Acquiring Cell Lines

You may establish your own culture from primary cells, or you may choose to buy established cell cultures from commercial or non-profit suppliers (i.e., cell banks). Reputable suppliers provide high quality cell lines that are carefully tested for their integrity and to ensure that the culture is free from contaminants. We advise against borrowing cultures from other laboratories because they carry a high risk of contamination. Regardless of their source, make sure that all new cell lines are tested for mycoplasma contamination before you begin to use them. Invitrogen offers a variety of primary cultures and established cell lines, reagents, media, sera, and growth factors for your cell culture experiments.

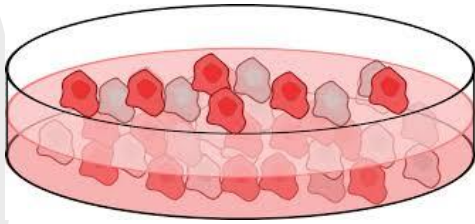
Culture Environment

One of the major advantages of cell culture is the ability to manipulate the physicochemical (i.e., temperature, pH, osmotic pressure, O2 and CO2 tension) and the physiological environment (i.e., hormone and nutrient concentrations) in which the cells propagate. With the exception of temperature, the culture environment is controlled by the growth media. While the physiological environment of the culture is not as well defined as its physicochemical environment, a better understanding of the components of serum, the identification of the growth factors necessary for proliferation, and a better appreciation of the microenvironment of cells in culture (i.e., cell-cell interactions, diffusion of gases, interactions with the matrix) now allow the culture of certain cell lines in serum-free media

Adherent vs Suspension

Culture There are two basic systems for growing cells in culture, as monolayers on an artificial substrate (i.e., **adherent culture**) or free-floating in the culture medium (**suspension culture**).

The majority of the cells derived from vertebrates, with the exception of hematopoietic cell lines and a few others, are anchorage-dependent and have to be cultured on a suitable substrate that is specifically treated to allow cell adhesion and spreading (i.e., tissue-culture treated).



However, many cell lines can also be adapted for suspension culture. Similarly, most of the commercially available insect cell lines grow well in monolayer or suspension culture. Cells that are cultured in suspension can be maintained in culture flasks that are not tissue-culture treated, but as the culture volume to surface area is increased beyond which adequate gas exchange is hindered (usually 0.2–0.5 mL/cm2), the medium requires agitation.

Adherent Culture	Suspension Culture
Appropriate for most cell types, including primary cultures.	Appropriate for cells adapted to suspension culture and a few other cell lines that are nonadhesive (e.g., hematopoietic).
Requires periodic passaging, but allows easy visual inspection under inverted microscope.	Easier to passage, but requires daily cell counts and viability determination to follow growth patterns; culture can be diluted to stimulate growth.
Cells are dissociated enzymatically (e.g., TrypLE™ Express, trypsin) or mechanically.	Does not require enzymatic or mechanical dissociation.
Growth is limited by surface area, which may limit product yields.	Growth is limited by concentration of cells in the medium, which allows easy scale-up.
Requires tissue-culture treated vessel.	Can be maintained in culture vessels that are not tissue-culture treated, but requires agitation (i.e., shaking or stirring) for adequate gas exchange.
Used for cytology, harvesting products continuously, and many research applications.	Used for bulk protein production, batch harvesting, and many research applications.

Media

The culture medium is the most important component of the culture environment, because it provides the necessary nutrients, growth factors, and hormones for cell growth, as well as regulating the pH and the osmotic pressure of the culture. Although initial cell culture experiments were performed using natural media obtained from tissue extracts and body fluids, the need for standardization, media quality, and increased demand led to the development of defined media. The three basic classes of media are basal media, reduced-serum media, and serum-free media, which differ in their requirement for supplementation with serum.

Serum

is vitally important as a source of growth and adhesion factors, hormones, lipids and minerals for the culture of cells in basal media. In addition, serum also regulates cell membrane permeability and serves as a carrier for lipids, enzymes, micronutrients, and trace elements into the cell. However, using serum in media has a number of disadvantages including high cost, problems with standardization, specificity, variability, and unwanted effects such as stimulation or inhibition of growth and/or cellular function on certain cell cultures. If the serum is not obtained from reputable source, contamination can also pose a serious threat to successful cell culture experiments.

Basal Media

The majority of cell lines grow well in basal media, which contain amino acids, vitamins, inorganic salts, and a carbon source such as glucose, but these basal media formulations must be further supplemented with serum.

Reduced-Serum

Media Another strategy to reduce the undesired effects of serum in cell culture experiments is to use reduced-serum media. Reduced-serum media are basal media formulations enriched with nutrients and animal-derived factors, which reduce the amount of serum that is needed.

Serum-Free Media

Serum-free media (SFM) circumvents issues with using animal sera by replacing the serum with appropriate nutritional and hormonal formulations. Serum-free media formulations exist for many primary cultures and cell lines,

including recombinant protein producing lines of Chinese Hamster Ovary (CHO), various hybridoma cell lines, the insect lines Sf9 and Sf21 (Spodoptera frugiperda), and for cell lines that act as hosts for viral production (e.g., 293, VERO, MDCK, MDBK), and others.

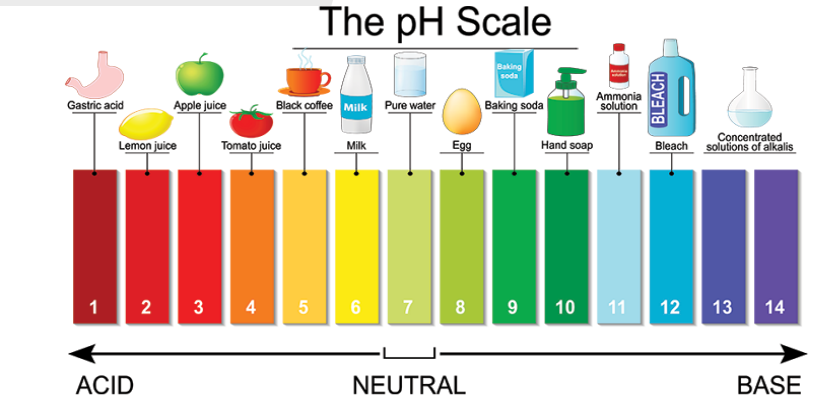
One of the major advantages of using serum-free media is the ability to make the medium selective for specific cell types by choosing the appropriate combination of growth factors.

The table below lists the advantages and disadvantages of serum-free media.

Advantages	Disadvantages
• Increased definition • More consistent performance • Easier purification and downstream processing • Precise evaluation of cellular functions • Increased productivity • Better control over physiological response • Enhanced detection of cellular mediators	• Requirement for cell type-specific media formulations • Need for higher degree of reagent purity • Slower growth

pH

Most normal mammalian cell lines grow well at pH 7.4, and there is very little variability among different cell strains. However, some transformed cell lines have been shown to grow better at slightly more acidic environments (pH 7.0–7.4), and some normal fibroblast cell lines prefer slightly more basic environments (pH 7.4–7.7). Insect cell lines such as Sf9 and Sf21 grow optimally at pH 6.2



CO2

The growth medium controls the pH of the culture and buffers the cells in culture against changes in the pH. Usually, this buffering is achieved by including an organic (e.g., HEPES) or CO2-bicarbonate based buffer.

Because the pH of the medium is dependent on the delicate balance of dissolved carbon dioxide (CO2) and bicarbonate (HCO3 –), changes in the atmospheric CO2 can alter the pH of the medium. Therefore, it is necessary to use exogenous CO2 when using media buffered with a CO2-bicarbonate based buffer, especially if the cells are cultured in open dishes or transformed cell lines are cultured at high concentrations.

While most researchers usually use 5–7% CO2 in air, 4–10% CO2 is common for most cell culture experiments. However, each medium has a recommended CO2 tension and bicarbonate concentration to achieve the correct pH and osmolality; refer to the media manufacturer's instructions for more information.

Temperature

The optimal temperature for cell culture largely depends on the body temperature of the host from which the cells were isolated, and to a lesser degree on the anatomical variation in temperature (e.g., temperature of the skin may be lower than the temperature of skeletal muscle). Overheating is a more serious problem than under heating for cell cultures; therefore, often the temperature in the incubator is set slightly lower than the optimal temperature

Temperature

- Most human and mammalian cell lines are maintained at 36°C to 37°C for optimal growth.

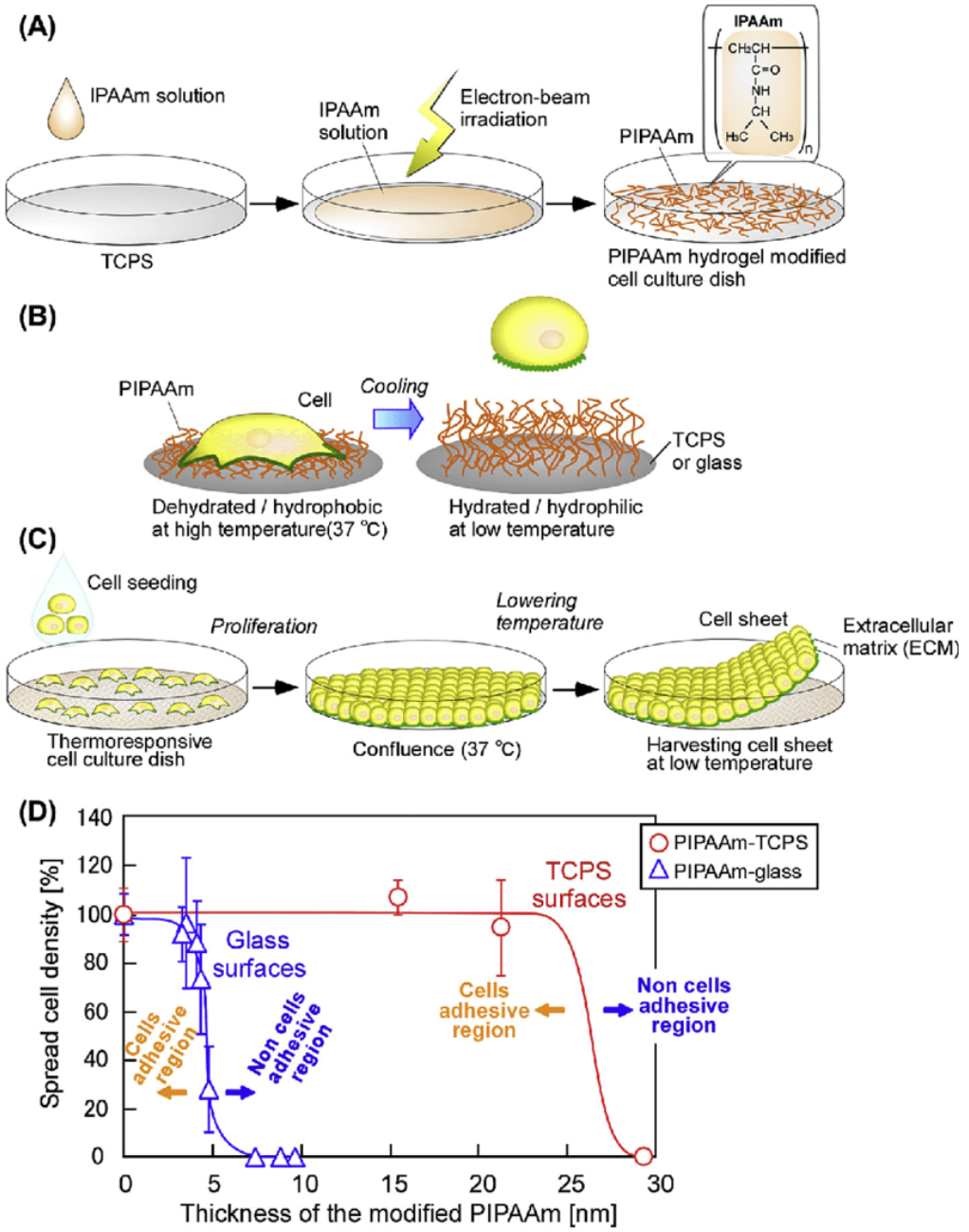
- Insect cells are cultured at 27°C for optimal growth; they grow more slowly at lower temperatures and at temperatures between 27°C and 30°C. Above 30°C, the viability of insect cells decreases, and the cells do not recover even after they are returned to 27°C.

- Avian cell lines require 38.5°C for maximum growth. Although these cells can also be maintained at 37°C, they will grow more slowly.

- Cell lines derived from cold-blooded animals (e.g., amphibians, cold-water fish) tolerate a wide temperature range between 15°C and 26°C.

Thermoresponsive cell culture dish for thermally modulated cell adhesion and detachment and cell sheet fabrication: (A) preparation of thermoresponsive cell culture dish by electron beam-induced polymerization, (B) temperature-dependent cell adhesion and detachment, and (C) cell sheet fabrication using thermoresponsive cell culture dish. (D) Relationship between the spread cell density and thickness of the PIPAAm hydrogel layer on substrate.

https://www.researchgate.net/figure/Thermoresponsive-cell-culture-dish-for-thermally-modulated-cell-adhesion-and-detachment_fig7_320653783



Note that cell culture conditions vary for each cell type

Thank you