ORIGINAL ARTICLE

# Current Status and Thinking of Animal Cell Culture Technology

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***Abstract:*** Cell culture, an important technology in biomedicine, has been widely using, which is currently applied to plenty of fields, such as cell biology, biochemistry and clinical laboratory science. Animal cell culture technology is widely used in biology, medicine and other research fields. Animal cell culture methods include primary culture and subculture. By analyzing the progress of cell feeding technology, this article will discuss the existing problems of animal cell culture and its future research direction to establish and screen out suitable cell line culture methods. These methods will serve as theoretical reference for researchers in related fields to explore the establishment of other kinds of cell lines and screen suitable cell line culture methods.

***Keywords:*** Cell Culture; Screening; Subculture; Primary Culture

## Introduction

Tissue culture technology was performed at the end of 18th century. Harrison, an American biologist, had successfully cultivated frog embryo nerve tissue in a test tube under sterile conditions with lymph as culture medium in 1907. After that, tissue culture is gradually becoming an experimental technology of taking out cells from vivo tissues, simulating in vivo living environment, making them grow and reproduce and maintaining structure and function under sterile, proper temperature, pH and certain nutritional conditions. This technology has made important contributions to the research and application of cytology, genetics, virology and immunology.

With the rapid expansion of modern biological science in recent years, various molecular biology experiments, such as nuclear transplantation, cell hybridization, DNA-mediated gene transfer, etc., have been realized by means of cell culture technology. However, in a wide variety of animal worlds, the development of experimental technology of cell culture is uneven, and the research efficiency and effect of constructing animal cell culture system are also limited. Therefore, this article makes a systematic analysis and reflection on the research trends of animal cell culture technology at home and abroad, which provides reference for relevant researchers and is also the necessary preparation for our research group to carry out the research on the cell culture system of Eisenia foetida.

## 1. Basic concepts and characteristics of animal cell culture

### 1.1 Basic concepts

Cell culture refers to a kind of culture technology that takes out cells from internal tissues, provides them with a sterile environment with proper temperature and pH, and gives them sufficient nutrition to grow and reproduce and maintain their structure and function. Primary culture is the initial stage of cells taken out from


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the body, which is the essential step in primary cell culture. After the primary growth of cultured cells for a certain time, cells need to be transferred to another container due to the influence of the population environment. The process is called subculture. If the shape of the animal cells after passage is consistent with that of the original culture, it means that the passage is successful. These cells are called cell lines or cell strains.

### 1.2 Basic characteristics

In vitro culture of animal cells can be divided into two categories according to their dependence on growth substrates: One type is adhesion-dependent cells, which must be attached to the substrate surface to grow; The other type is non-attachment-dependent cells, which do not need to be attached to the substrate surface, and can be cultured in suspension similar to microbial cells. According to the different types and growth characteristics of animal cells, monolayer culture, suspension culture and immobilized culture can be used for large-scale animal cell culture. Animal cells have no cell wall, low mechanical strength, slow growth, high nutritional requirements, difficult control of growth conditions, and most of them have the characteristics of adherence dependence, which limits the size of bioreactor and affects the large-scale culture of animal cells.

## 2. Existing problems and prospects

In the past century, animal cell culture technology has made great progress through continuous development by researchers. However, the current technical level can not completely meet the requirements of the development and production of cell biological products.

(1) Because the techniques and methods of animal cell culture are not mature, the survival rate and utilization rate of cells are low, which leads to the phenomenon of low technical efficiency of animal cells.

(2) In the long-term and large-scale cell culture, the staff can't rule out the influence of cell metabolites on themselves, and the physiological function of cell population will also be affected in this process, generally showing the decrease or even loss of secretion and metabolism ability. In addition, the equipment and microcarriers used in animal cell culture technology are expensive, which leads to the increase of research cost and limits the large-scale animal cell culture project in China.

## 3. Current situation and application of cell culture in vertebrates and invertebrates

### 3.1 Vertebrates

Vertebrates are divided into fishes, amphibians, reptiles, birds and mammals from lower to higher levels. The research on vertebrate cell culture started earlier and the cell line was well established.

For example: 1. Reptiles Compared with mammals, reptiles are relatively low, belonging to temperature-changing animals. However, compared with other lower vertebrates, such as amphibians and fish, it is a real land animal, completely breaking away from the limitation of water. Therefore, when we carry out cell culture, we must consider the special living environment of reptiles, especially the temperature and osmotic pressure. Current domestic data. The primary culture and subculture of adult gecko spinal cord cells were explored for the first time, and some of them were identified.

### 3.2 Amphibians

The research on cell culture of amphibians is less than that of mammals. At present, it has been found that the oviduct epithelial cells of Rana chensinensis were primarily and subcultured according to domestic data, and their biological characteristics such as morphology, growth and proliferation ability were identified and studied. The method of in vitro culture of oviduct epithelial cells of Rana chensinensis was established, which has important practical significance for the development, application and protection of natural resources of Rana chensinensis.

### 3.3 Invertebrates

Invertebrates include Porphyra, Coelenterata, Annelida, Arthropoda, Mollusca and Echinoderma. The research on cell culture of invertebrates started later than that of mammals. Because of the particularity of invertebrates' cells in morphology, structure, function and nutritional requirements, their cell culture mostly stays at the level of primary culture and limited cell lines[2-3]. Up to now, immortal cell lines have not been established, but with the development of natural active products of some lower invertebrates, such as sponges, and the need of research on prevention and control of diseases of farmed

shrimp and shellfish, invertebrate cell culture will be paid attention to.

# 4. Animal cell culture technology

## 4.1 Biological characteristics of animal cells cultured in vitro

Animal cells have no cell wall structure, low mechanical strength, and are sensitive to shear force, so they can not adapt well to the external environment. In addition, the cells cultured in vitro have lost their neurohumoral regulation and interaction between cells. They not only lose their original tissue structure, but also are difficult to maintain their original cell morphology. They are in a living environment lacking dynamic balance, and their differentiation ability decreases until they develop into malignant cells. Therefore, animal cell culture in vitro should pay close attention to the state of cells in vitro, whether the conditions provided by the growth environment are suitable for survival, and whether they are infected by microorganisms.

## 4.2 Environment needed for cell culture

### 4.2.1 Sterile and nontoxic environment

Aseptic and nontoxic operating environment is the prerequisite to ensure the success of animal cell culture in vitro. In vitro cultured cells are faced with the problem of being infected by microorganisms or affected by their own metabolites. Therefore, in vitro cell culture, it is necessary to remove the metabolic wastes produced by cells in time to ensure a sterile and nontoxic living environment for the cells cultured in vitro.

### 4.2.2 Gas environment

Gases are mainly O2 and CO2. O2 can provide energy for cells, while CO2 is not only a necessary substance for cell proliferation, but also a metabolite of itself. In addition, CO2 can regulate the pH of culture medium.

### 4.2.3 Temperature

Appropriate temperature can keep the cells growing vigorously. If the temperature exceeds the appropriate temperature range, it will not only affect the normal metabolism of cells, damage cells, and even kill them.

### 4.2.4 Buffer environment

The function of buffer environment is to provide cells with a culture solution with pH within the physiological range of cultured cells, provide water and inorganic salts, and maintain the normal metabolism of cells.

### 4.2.5 Culture medium

The culture medium is divided into natural culture medium and synthetic culture medium, which is the direct environment for cell growth and reproduction, and provides cells with the necessary nutrition. Natural culture medium is isolated and extracted from animal body fluids or tissues. Plasma, serum and lymph can be used as natural culture medium. Animal cell culture mainly uses synthetic medium. The synthetic medium contains inorganic salts, saccharides, vitamins, amino acids and other basic substances needed for cell growth, and a proper amount of serum will be added if there are special requirements.

## 4.3 Methods for cell culture

### 4.3.1 Monolayer culture

For those cells with adherence dependence, monolayer culture is generally used. Generally, such cells need to grow on the surface of non-chemical substances, and finally grow to a monolayer on the surface. When the whole plane is covered, the cells will have contact inhibition.

### 4.3.2 Suspension culture

Suspended adaptive cells, tumor cells and other non-attachment-dependent cells do not need a supporting surface for growth, and the cells can be suspended in liquid medium and proliferate in large quantities. Therefore, suspension culture is an ideal way for large-scale cell culture. Although there are various difficulties in animal cell culture, researchers will continue to improve the research level of molecular biology and expand the research scope, because animal cell culture technology is an important tool in many disciplines.

### 4.3.3 Immobilization culture

Both anchorage-dependent cells and non-anchorage-dependent cells can be cultured in vitro by immobilized culture. Immobilization culture belongs to embedding culture, and the existing immobilization culture includes microcarrier culture, hollow fiber culture and microcapsule culture. Immobilized culture not only has lower shear force and better transfer effect, but also is easy to collect, separate and purify cell products. Cells cultured in this way also have strong anti-pollution ability, with concentrated cell growth and high growth density.

# 5. Conclusion

Although there are various difficulties, it can't stop people from pursuing the development and utilization of animal cells. With the development of molecular biology research, animal cell culture technology will continue to be an important tool for multi-disciplinary research. More and more animal cells will also become important biological experimental materials and the basis of industrial production and utilization. In the future, the development direction of animal cell culture technology should focus on optimizing cell environment and improving cell characteristics, so as to improve the yield and output of biological products and expand the production scale. Although there are various difficulties in animal cell culture, researchers will continue to improve the research level of molecular biology and expand the research scope, because animal cell culture technology is an important tool in many disciplines. In the near future, many biological experimental materials and industrial production need animal cell culture technology to carry out their own research and development work.

# References


1. Zhou Y, Yang M, Wang B, *et al*. Research progress of animal cell culture bioreactor (in Chinese). Guizhou Journal of Animal Husbandry & Veterinary Medicine 2019; 43(4): 27–30.
2. Wei Q, Sun H. Research status and thinking of animal cell culture technology (in Chinese). Chinese Journal of Animal Husbandry and Veterinary Medicine 2014; (8): 10–11.
3. Hou X, Lin X, Li W. Animal cell culture technology research status and thinking. Journal of Liaoning University of Traditional Chinese Medicine 2010; 12(11): 37–39.