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A Method for Isolation and Culture of Rat Astrocytes

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Abstract: *The in vitro isolation of astrocytes is a key factor in establishing cell-based models for studying animal central nervous system (CNS) diseases. In this study, cerebral cortices from newborn rats were cultured in vitro, and cultures enriched with astrocytes were obtained, providing an experimental model for investigating the biological functions of astrocytes. Newborn rats (within 24 hours after birth) were used as experimental subjects. Bilateral cerebral cortices were harvested under sterile conditions, and cell suspensions were prepared by mechanical pipetting to disperse the cells. Differential adhesion treatment was performed to remove fibroblast components. The cell suspensions were subjected to primary culture for 8–10 days. When the cells fused into a confluent monolayer and covered the bottom of the culture flask, trypsinization was carried out for subculture. Astrocytes were identified using glial fibrillary acidic protein (GFAP) immunohistochemical staining. This improved astrocyte culture method successfully isolated and cultured astrocytes with a purification rate of over 95%. The rat astrocytes cultured by the method described in this experiment exhibited good growth and high purity, laying a solid experimental foundation for subsequent studies on the biological functions of astrocytes.*

Keywords: *Astrocytes, Cell Culture, Identification, SD Rat*

1. Introduction

Glial cells are one of the two major types of cells that make up neural tissue, and their biological functions have received increasing attention. Astrocytes (AS) are the main part of them and play very important roles in various normal physiological activities of the central nervous system such as nervous system development, synaptic transmission, stability and metabolism of the nervous system internal environment, as well as the pathological mechanisms of neurological diseases [1]. In vivo, due to the mixed existence of astrocytes with other cell components, it is necessary to obtain purified astrocytes first to study their biological functions and the effects of drugs on them. In this paper, the cerebral cortices of neonatal rats were cultured in vitro, and cultures rich in astrocytes were obtained, providing materials for the further establishment of cell action models.

2. Experimental Materials

2.1 Experimental animals

Nine SPF-grade adult SD rats (provided by Guangdong Weitong Lihua Experimental Animal Co., Ltd., with a male-female ratio of 2:1) were caged together and successfully bred offspring. Four neonatal SD rats of either sex were selected within 24 hours after birth.

2.2 Reagents

High-glucose DMEM medium (Gibco), fetal bovine serum (Wuhan Punosai Life Science & Technology Co., Ltd.), trypsin-EDTA (0.25%) digestive solution (Beijing Solarbio Science & Technology Co., Ltd.), 0.5 g/mL polylysine (PLL), rabbit anti-human glial fibrillary acidic protein (GFAP) antibody, biotinylated goat anti-rabbit antibody (Sigma), 1 ×PBS buffer, TritonX-100.

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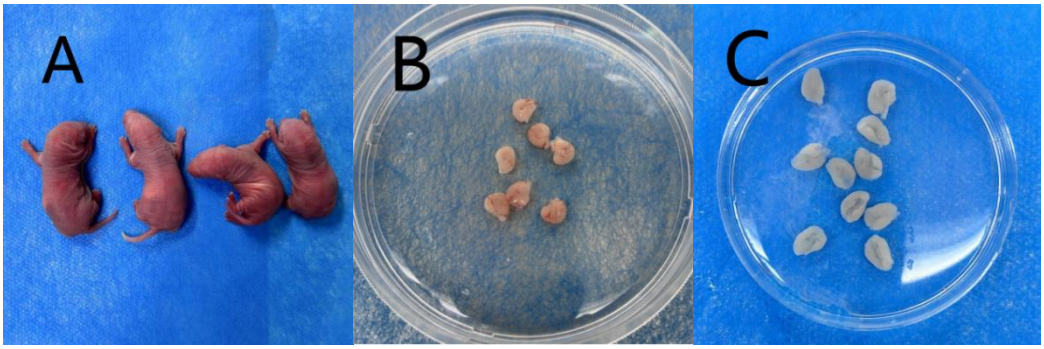
3. Methods

3.1 Primary culture of rat cortical astrocytes

Newborn SD rat pups within 24 hours after birth were selected (see Figure 1 A). First, surface disinfection was performed with 75% ethanol solution for 15 minutes. After disinfection, the experimental animals were transferred to a laminar flow hood and placed on a pre-cooled operating platform for dissection. After quickly decapitating the rats, the whole brain tissue was taken out by cutting the skull; the cerebral hemispheres were separated, and the cerebellum and midbrain parts were carefully removed (Figure 1 B); the bilateral cerebral cortical tissues were obtained and placed in pre-cooled DMEM basal medium(Figure 1 C); under a dissecting microscope, the meninges and vascular tissues were carefully dissected, and the tissue samples were rinsed twice with DMEM basal solution to ensure the cleanliness and sterility of the samples. The rinsed cortical tissues were transferred to a sterile culture dish, an appropriate amount of DMEM basal solution was added, and the tissues were cut into pieces about size in 1 mm^3 with ophthalmic scissors; 0.25% trypsin digestive solution was added at a ratio of 1:8 by tissue volume, and enzymatic digestion was carried out for 15 minutes at condition in 37˚C, with gentle oscillation every 5 minutes; after digestion, DMEM complete culture medium containing 10% fetal bovine serum was immediately added to terminate the reaction; centrifugation was performed at 1000 r/min for 5 minutes, the supernatant was removed, and rinsing was repeated three times; a dispersed cell suspension was obtained by gentle pipetting and filtered through a pore size in 70μm filter mesh. After adjusting the cell concentration to cell concentration in 1.5×10^6 cells/mL, the cells were inoculated into a culture container of appropriate size. Primary culture was carried out in a constant temperature incubator at temperature in 37˚C and carbon dioxide concentration in 5%, and the fresh culture medium was changed every 48 hours.



A:24 h Newborn rats B: Dissect the brain tissue C: Cerebral cortex without vascular membrane

Figure 1: Lysis Process of SD Rats

3.2 Subculture of Rat Cortical Astrocytes

After 8-10 days of primary culture, when the cell confluence reaches 80%-90%, purification is carried out: Seal the culture flask mouth, place it in a 37℃ constant temperature shaker, and shake culture at 180 rpm for 20 hours; Discard the culture medium and wash three times with pre-warmed PBS buffer. Subculture operation: Use 0.25% trypsin solution, 37˚C digest for about 3 minutes; Add complete culture medium to terminate digestion, collect the cell suspension; After centrifugation, resuspend again and perform subsequent inoculation or identification experiments according to experimental requirements.

3.3 Immunofluorescence Identification of Rat Cortical Astrocytes

GFAP is specific to AS cells and is the cytoskeletal protein and marker protein of AS. Immunofluorescence staining is used to identify GFAP as follows: Inoculate the prepared cell suspension into a six-well culture plate with a sterile coverslip, and the inoculation density is 2×10^5 cells/well. When the cells grow to 80% - 90% confluence, first wash three times with PBS buffer (5 minutes each time), then add 4% paraformaldehyde to fix for 20 minutes (room temperature). After fixation, repeat the PBS washing step. Cell permeabilization and blocking: Treat the cells with 0.5% Triton X-100 solution for 20 minutes (room temperature) to enhance cell membrane permeability, and then wash with PBS again. To reduce non-specific binding, use 10% goat serum blocking solution to

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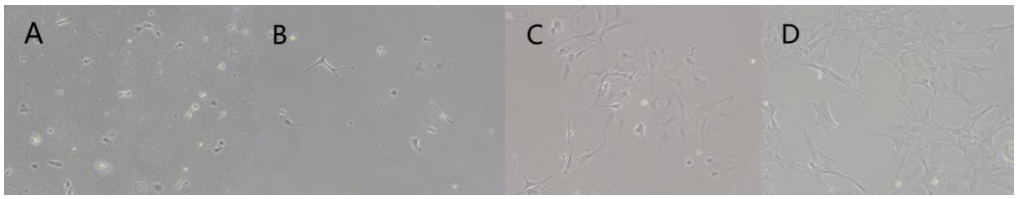
block at room temperature for 1 hour. Antibody incubation: Add specific primary antibody, that is, GFAP antibody (1:200,800μl/ wells) for labeling, 4˚C incubate overnight under the condition, and then wash three times with PBS. Secondary antibody treatment and observation: Add the corresponding secondary antibody (goat anti-rabbit IgG, 1:200) and incubate in the dark for 1 hour (room temperature), wash with PBS and then mount with DAPI. Finally, observe and record the specific labeling of each cell type through a fluorescence microscope.

4. Results

4.1 Microscopic Morphological Observation

After 24 hours of primary culture, it was observed under the microscope that the cells had successfully adhered to the wall (see Figure 2 A), indicating the initial success of primary culture. After 36 hours of culture, the cell morphology gradually became clear, with fine protrusions visible, and some cells began to migrate out from the edge (Figure 2 B). After 5 days of culture, the cell proliferation rate increased, the cell bodies enlarged, grew radially and intertwined into a reticular structure (Figure 2 C). After purification and subculture, astrocytes formed a monolayer, with abundant cytoplasm, Star-shaped or polygonal, irregular in shape, and most of the cytoplasmic processes extended in long cord-like shapes (Figure 2 D).



A: Astrocytes with medium changed after 24 hours of culture B: Astrocytes after 36 hours of culture C: Astrocytes after 5 days of culture D: Astrocytes after purification and passage

Figure 2: Morphology of primary cultured cortical astrocytes at different stages observed under an inverted microscope (*10×10*)

4.2 Results of immunofluorescence identification

In this study, immunofluorescence staining technology was used to identify the cultured astrocytes. The specific operation process was as follows: First, target cells were labeled with GFAP-specific antibodies, and then a Cy3 fluorescent dye was used for the color reaction. Under an inverted fluorescence microscope, it was observed that GFAP-positive cells showed obvious red fluorescence signals. Through systematic counting and analysis, it was shown that the proportion of cells expressing GFAP in the culture system exceeded 99%. This result fully confirmed the type purity of the cultured cells, which fully met the quality control requirements for in vitro culture of astrocytes (Figure 3).

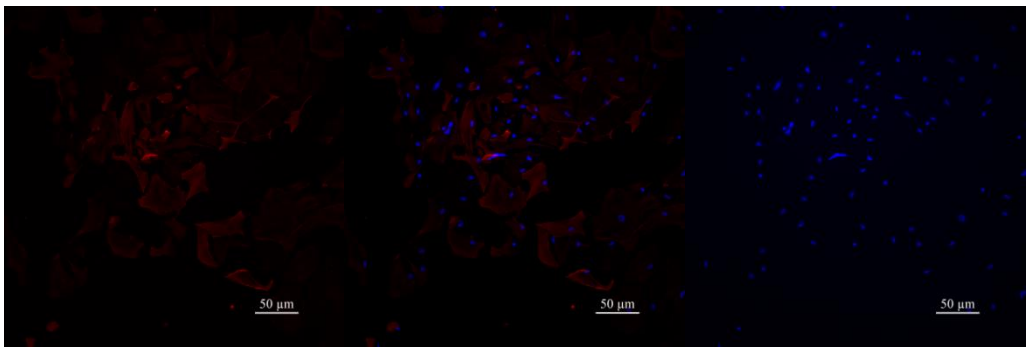


Figure 3: GFAP immunofluorescence of primary cortical astrocytes (*20×10*)

5. Discussion

This improved method for culturing astrocytes was established on the basis of referring to domestic and foreign culturing methods. Through many years of experimental research, we obtained astrocytes

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with a purity of over 95%, which fully met the experimental requirements and provided a basic experimental model for the research on the regenerative function of nerve cells [2] and the research on the pathological occurrence and molecular mechanism of action of various nervous system diseases in animals [3].

The main key points of the technical operations we summarized are as follows: During primary culture, for the selection of rat age, we used neonatal rats at 1 day old after birth as materials instead of rats at 1 week old [4]. Because during early development, the peak proliferation periods of neurons and glial cells are different. Neurons complete proliferation during the embryonic period, while the massive proliferation of glial cells occurs in the late embryonic period and after birth [5]. Using 1-day-old rats can obtain cell suspensions containing more glial cells and their precursor cells. Operations such as removing the olfactory bulb, hippocampus from the brain tissue and stripping the pia mater and large blood vessels are carried out on an ice plate, which is more conducive to improving the survival rate of cells after inoculation. Fibroblasts are mainly located in the pia mater, so the pia mater should be removed as completely as possible to facilitate subsequent cell purification. Sterilization of tissues before culture is the key to successful cell culture. Therefore, strict aseptic operation should be carried out and corresponding methods should be used to control cell contamination. During the culture process of astrocytes, purification is an important method to improve cell purity. Currently, purification methods are generally divided into two types: differential attachment method and constant temperature shaking method. Since the growth rate of fibroblasts is relatively fast, if the meninges and blood vessels in the brain tissue are not removed completely during the extraction of astrocytes, a large number of fibroblasts will be mixed in during the culture process. Therefore, it is better to use the differential attachment method to remove fibroblast components in the culture medium. Because astrocytes grow at the bottom and have strong adhesion ability, and other glial cells grow on top of them. After primary and passage cultures with constant temperature shaking, microglia, oligodendrocytes and other non-adherent miscellaneous cells are removed as much as possible [6-7]. For cell passage, EDTA-trypsin is used, which has a milder effect than ordinary trypsin, reducing cell damage and over-digestion resulting in insufficient passage density. Digest the extracted mouse cerebral cortex with EDTA-trypsin and observe the cells in the culture medium under a microscope. It is found that the length of digestion time will affect the purity and viability of astrocytes. Obvious damage will occur after 40 minutes of cell digestion. In this experiment, the digestion time of trypsin is strictly controlled within 15 minutes.

In summary, the in vitro culture model of rat brain astrocytes conducted in this experiment is more convenient and feasible than previous methods in terms of both isolation and purification and identification after purification. It has strong operability, is easy to popularize, and at the same time, the purity after purification can also meet the requirements of subsequent experiments.

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