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Culture of Rat Lymphatic Smooth Muscle Cells

Zhang Yu, Wang Jing

Hebei North University, 075000, Zhangjiakou, China

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Abstract: Lymphatic smooth muscle cells are the basis of the study of lymphatic microcirculation. Traditional lymphatic culture often uses patching and enzymatic hydrolysis. However, because the traditional enzymatic hydrolysis method has a low success rate in culturing cells, this article introduces the use of trypsin digestion to treat rat thoracic ducts. Culture the smooth muscle cells of the lymphatic vessels, and compare the advantages and disadvantages of trypsin digestion and other smooth muscle cell culture methods. Experimental operation: select 4 fasting male wistar rats, anesthetize the rats by intraperitoneal injection of 1% sodium pentobarbital, and soak them in 75% alcohol for 5 minutes for disinfection. Place the rat on a sterile table for thoracotomy, take out the thoracic duct and surrounding tissues, quickly put in PSS cold solution for later use, separate the rat's thoracic lymphatic vessels under a microscope, rinse with PSS 3 times in the cold night to remove the floating liquid . Add warm DMEM to 37°C water bath and incubate for 30min, discard DMEM, add 2ml Trypsin and put it in 37°C water bath for digestion for 10min (vibrate once every 2min). Aspirate the digestion solution and wash it with DPBS for 3 times. Take out the thoracic duct and cut it into pieces. Add 4ml collagenase and put it in a 37°C water bath for 30min (vibrate once every 2min). After digestion, add 25%D/F2 4ml Terminate the digestion, and then perform centrifugation (rotating speed 1100r/min centrifugation for 3 minutes). Finally, the cells were resuspended in 2ml of 25% special culture medium, and 5% CO was injected into the cells. They were cultured in a 37°C incubator and regularly observed under the microscope.

1. Introduction

Hemorrhagic shock, severe burns and other injuries are often accompanied by the appearance of lymphatic microcirculation disorders caused by changes in the contractility of lymphatic smooth muscle cells. At the same time, lymphatic contractility is studied in the pathogenesis of lymphatic circulatory dysfunction during severe shock. Play an important role. Therefore, lymphatic smooth muscle cells are often regarded as the experimental basis for the study of lymphatic system microcirculation. The cultivation method of smooth muscle cells is roughly divided into enzymatic hydrolysis and patch method. At present, the patch method is commonly used in domestic laboratories, but the patch method requires a longer cell cycle for culturing smooth muscle cells, from cell digestion to smooth muscle cells. Adherent growth takes about two weeks. In addition, this method is more susceptible to external factors, and the survival rate of cell culture is low. In addition to the above two traditional methods, enzymatic hydrolysis can also be used to cultivate lymphatic cells. Traditional

enzymatic hydrolysis methods mostly use synergistic elastase or collagenase. In order to improve its culture efficiency, the method introduced in this article uses trypsin digestive enzymes to digest lymphatic vessel cells.

2. Materials and Methods

2.1 Reagents and Animal

Trypsin digestion solution, type I collagenase, bovine serum albumin, and PSS solution are all commercially available analytically pure, DMEM medium, 0.25% Trypsin (without EDTA) digestion solution, DPBS, FBS , PIS, sodium pyruvate, sodium pentobarbital powder, male wistar rats (4, weighing 300+-20g).

2.2 Preparation Before the Experiment

Prepare four wistar male rats (with a weight of 300 plus or minus 200g) that have been fasted for 12 hours, and irradiate the ultra-clean table and sterile cell culture room used in the experiment with ultraviolet light for one hour for disinfection. All the equipment used in the experiment is required Sterilize at a high temperature of 160°C for two hours, prepare the PSS culture medium required for the experiment, and treat it with oxygen for 30 minutes, and place it in a refrigerator at 4°C for later use. DMEM solution was used to make constant volume configuration of 2mg/mL type 1 collagenase solution and 2mg/mL fetal bovine serum protein solution. Dissolve 0.1g Trypsin reagent into 40mLDPBS solution to configure 0.25% Trypsin solution (without EDTA), 12.5mL FBS, 37.5mL primary smooth muscle cell special medium, 0.5mLp/s, 0.5mL 50mL of 25% special medium.

3. Experimental Operation

3.1 Collection of Lymphatic Vessels At the Thoracic Duct of Rats

Male wistar rats were selected and fasted for 12 hours before the experiment to exclude the contents of the digestive system for subsequent operations. After fasting, the rats were anesthetized by intraperitoneal injection of 1% sodium pentobarbital, and then immersed in 75% alcohol for 5 minutes for sacrifice and disinfection. Take out the rat, drain the excess alcohol, and transfer to a sterile table to open the rat's chest. Take out the rat's thoracic duct and surrounding tissues, and quickly put in PSS cold solution for later use.

3.2 Extraction of Lymphatic Smooth Muscle Cells

The rat thoracic lymphatic vessels were separated under a microscope, and the floating fluid was washed off with PSS for 3 times in the cold night. Then add warm DMEM and incubate in 37°C water bath for 30min, discard DMEM, add 2ml Trypsin and put it in 37°C water bath for digestion for 10min (vibrate once every 2min). Pipette to aspirate the digestion solution and wash it with DPBS 3 times. Take out the thoracic duct and cut it into pieces. Add 4ml Type I collagenase and put it in a 37°C water bath for 30min (vibration once every 2min). After digestion, add 25%D. /F2 4ml to terminate the digestion, and then centrifuge (rotate at 1100r/min for 3min). Finally, resuspend the cells in 2ml of 25% special culture medium for the above-mentioned processed samples, pass in 5% CO, and place them in a 37-degree incubator. After 48 hours, the medium is changed, and then regular observations under the microscope are carried out.

3.3 Subculture

Take the cells cultured for 48 hours out of the incubator and observe their growth under a microscope. Subculture can be carried out when the culture has grown to 80%~90% of the field of view. Discard the remaining culture medium in the cell culture dish, clean the culture dish with DPBS to remove the residual culture solution, and then add Try digestion solution to digest 30S in a 37℃, 5% CO2 incubator to help separate the adherent growth cells, under the microscope Stop the digestion after observing the cells to become round, then add twice Try volume of 10% DMEM medium and mix well. Use a pipette to transfer the mixed liquid to a 15mL centrifuge tube for centrifugation, and discard the supernatant after centrifugation. Then add 2 mL of 10% DMEM medium to the remaining cell pellet to resuspend, pipette and mix well, and divide it into two culture flasks, each with 1 mL in each flask, and continue to add 3 mL of medium in each flask. Shake in the cross method, loosen the bottle cap and place it in a 37°C, 5% CO2 incubator to continue culturing, and regularly check its growth status under a microscope. When the cell growth status is poor, antibiotics can be added to prevent bacterial growth.

4. Conclusion

After subculture for a period of time, the growth status was observed under the microscope, and it was found that the lymphatic smooth muscle cells cultured by trypsin digestion method grew well, and the number of dead cells was much smaller than that of traditional digestive enzyme culture method and patch method. Compared with other traditional lymphatic smooth muscle cell culture methods, the enzymatic digestion method is easy to operate, and the cell subculture has a high survival rate, which is more suitable for the culture of laboratory lymphatic smooth muscle cells.

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