Video Article

Isolation of Human Umbilical Arterial Smooth Muscle Cells (HUASMC)

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Abstract

The human umbilical cord (UC) is a biological sample that can be easily obtained just after birth. This biological sample is, most of the time, discarded and their collection does not imply any added risk to the newborn or mother's health. Moreover no ethical concerns are raised. The UC is composed by one vein and two arteries from which both endothelial cells (ECs)¹ and smooth muscle cells (SMCs)², two of the main cellular components of blood vessels, can be isolated. In this project the SMCs were obtained after enzymatic treatment of the UC arteries accordingly the experimental procedure previously described by Jaffe *et al.*³. After cell isolation they were kept in t-flask with DMEM-F12 supplemented with 5% of fetal bovine serum and were cultured for several passages. Cells maintained their morphological and other phenotypic characteristics in the different generations. The aim of this study was to isolate smooth muscle cells in order to use them as models for future assays with constrictor drugs, isolate and structurally characterize L-type calcium channels, to study cellular and molecular aspects of the vascular function⁴ and to use them in tissue engineering.

Protocol

1. Arteries are obtained from UC pieces of 3-7 cm.
2. Wharton's jelly that surrounds the arteries was carefully removed by cutting it with scissors.
3. A blunt-end needle of an outer diameter of 0.6-1mm was inserted about 7mm from one end of the dissected arteries.
4. To remove the blood remaining inside the vessels, the arteries are vertically held and perfused with, approximately 20 to 40 mL, physiological saline solution (PSS), using a 20-mL syringe connected to a needle. If necessary, this washing step was repeated for several times.
5. The same procedure was repeated using RPMI 1640 supplemented with 10% of fetal bovine serum (FBS).
6. One extremity of blood vessel was closed. A 10-mL syringe was connected to the needle and perfused with 3 mL of collagenase type I (800 unit/mL in Hank's Buffered Salt Solution (HBSS)). Then the other extremity of the vessel was also closed.
7. The vessel was incubated with phosphate buffered saline solution (PBS), for 15 min at 37°C with agitation.
8. After the ends of the vessel were cut to collect the free SMCs. The arteries were perfused with approximately 20 to 40 mL of DMEM-F12 supplemented with 5% of FBS and antibiotic to a 50-mL tube.
9. The tube was centrifuged at room temperature (250 g, 8 min), and cell pellet was resuspended in 5 mL of DMEM-F12 supplemented with 5% of FBS.
10. The cell suspension was transferred to a collagen-coated T-flask and incubated at 37°C, under a 5% CO₂ humidified atmosphere.
11. After 5-16 h, the non-adherent cells and cellular debris were removed, 5 mL of culture medium was added and the cells were incubated at 37°C, under a 5% CO₂ humidified atmosphere.
12. The cell culture media was changed every 2-3 days, until SMCs reached confluence.
13. After cells attained confluence they were seeded into two or three 25 cm² T-flasks.

Cleanup

1. Dispose of needles in sharps container.
2. Dispose of syringes in big biohazard container.
3. Put all tissue into a small biohazard bag. Close the bag and freeze at -20° Celsius until incineration.
4. Soak all instruments in virucide for at least 10 min.
5. Return unused media to fridge.
6. Dispose of gloves in biohazard waste.

Discussion

The SMC can be used as models for future assays with constrictor drugs, to isolate and structurally characterize L-type calcium channels, to study cellular and molecular aspects of the vascular function and to use them in tissue engineering. SMCs and ECs are fundamental for the development of new biomaterials that can be used in the production of semi synthetic blood vessels to be used in humans.

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