Video article

Isolation of Human Umbilical Arterial Smooth Muscle Cells (HUASMC)

Maximiano P. Ribeiro[¥], Ricardo Relvas[¥], Samuel Chiquita[¥], Ilídio J. Correia[¥].

[¥] Centro de Investigação em Ciências da Saúde, Faculdade de Ciências da Saúde, Universidade da Beira Interior.

Maximiano P. Ribeiro maxprarib@gmail.com

Ricardo Relvas rrelvas@fcsaude.ubi.pt

Samuel Chiquita bananadoamor@hotmail.com

Correspondence to: Ilídio J. Correia at icorreia@ubi.pt

Short Abstract

The umbilical cords are use to isolation smooth muscle cells with different ways. In this work we used the enzymatic treatment to isolated smooth muscle cells.

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 and does not raise ethical concerns. Moreover, the UC can be the source of two different vascular vessels 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 following procedures based on the work of Jaffe *et al* [3]. After isolation the cells were kept in t-flash with DMEM-F12 supplemented with 5% of fetal bovine serum and were cultured for several passages while keeping the morphological and other phenotypic characteristics. The aim of this study was the isolation of smooth muscle cells in order to do future tests with constrictor drugs, characterization of calcium channels type L, and to study cellular and molecular aspects about the regulation of the vascular function [4].

Protocol

Procedure

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 is 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 can be repeated for several times, if needed.
5. The same procedure is repeated using RPMI 1640 supplemented with 10% of fetal bovine serum (FBS).
6. One extremity of blood vessel was closed. A 10-mL syringe is 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 was also closed.
7. The vessel was incubated with phosphate buffered saline solution (PBS), for 15 min at 37°C with agitation.
8. After incubation the ends were cuted 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 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 (2,5 g/cm^2) 25-cm^2 cell culture flask and incubated at 37°C, under a 5% CO_2 humidified atmosphere.
11. After 5–16 h, the nonadherent cells and celluar drebis were removed; 5 mL of culture medium was added; and the cells were incubated at 37°C, under a 5% CO_2 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-cm^2 cell culture flask.

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.

Acknowledgments

Hospital Amato Lusitano, Castelo Branco – Portugal

Hospital Sousa Martins, Guarda – Portugal.

Materials

Scissors (sterilized)

Needle (diameter of 0.6-1mm)

Syringe

Physiological Saline Solution (PSS)

RPMI 1640

Fetal bovine serum (FBS)

Collagenase type I

Hank's Buffered Salt Solution (HBSS)

Phosphate buffered saline solution (PBS)

DMEM-F12

Antibiotic

Collagen

References

1. Van Rijen, H. *et al.*, Gap junctions in human umbilical cord endothelial cells contain multiple connexins. *The American journal of physiology* 272 (1 Pt 1), C117 (1997).
2. Martín de Llano, J. *et al.*, Procedure to consistently obtain endothelial and smooth muscle cell cultures from umbilical cord vessels. *Translational Research* 149 (1), 1-9 (2007).

3. Jaffe, E.A., Nachman, R.L., Becker, C.G., & Minick, C.R., Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest* 52 (11), 2745-2756 (1973).
4. Cairrão, E., Santos-Silva, A., Alvarez, E., Correia, I., & Verde, I., Isolation and culture of human umbilical artery smooth muscle cells expressing functional calcium channels. *In Vitro Cellular & Developmental Biology-Animal* 45 (3), 175-184 (2009).