Vascular Regulation by endothelial Cells

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Contributor: Elisa Cairrao

Human umbilical cord (HUC) is a suitable source for isolation of endothelial cells (ECs) since it has no particular ethical impediments and is considered a non-tumorigenic and less immunogenic model. for this reason, HUC represents an advantageous experimental source for the isolation of endothelial cells. The ECs can produce/release molecules that modulate vasoconstriction and vasorelaxation by smooth muscle cells (SMC).

endothelial cells cardiovascular system umbilical vein umbilical artery smooth muscle cells

1. Introduction

Cardiovascular diseases (CVD) constitute the major cause of death worldwide, with a higher prevalence in the adult population [1][2]. Examples of CVD are coronary artery disease, peripheral arterial disease, or aortic disease.

Currently, the human umbilical cord (HUC) is a suitable source for isolation of endothelial cells (ECs) since it has no particular ethical impediments and is considered a non-tumorigenic and less immunogenic model. for this reason, HUC represents an advantageous experimental source for the isolation of endothelial cells [3]. The ECs can produce/release molecules that modulate vasoconstriction and vasorelaxation by smooth muscle cells (SMC) [4][5]. When releasing nitric oxide (NO), prostacyclin, bradykinin, and endothelium-derived hyperpolarizing factor (EDHF), the response given will be vasodilation by the SMC. If the substances released are endothelin-1 (ET-1), angiotensin II (Ang II), thromboxane A2 (TXA2), the final effect is vasoconstriction by the SMC [6].

The effect of endothelium in vivo is very difficult to study. To counteract this, several in vitro techniques were developed. Among these techniques, the use of human umbilical vein endothelial cells (HUVECs) was widely used as a source of human endothelial cells because they have some advantages. The main advantage is that it is obtained from non-pathogenic human tissues, which is not the case with other human sources, and the physiological relevance in the control of vascular pathways. Moreover, primary cultures with these cells can maintain native characteristics of endothelial cells and their intracellular signaling pathways [7].

2. Morphological Characteristics of the Human Umbilical Cord (HUC)

From a physiological point of view, the human umbilical cord (HUC) is a channel developed from the amniotic sac (which forms the epithelium), the allantois (which forms the umbilical veins and arteries), and the yolk sac [8][9].

Thus, not only the number of blood vessels but also morphological changes can contribute to the development of pathologies. We can conclude that the presence or absence of blood vessels is of extreme importance for the development of pathology.

In summary, not only the number of blood vessels but also morphological changes can contribute to the development of pathologies.

Finally, HUA does not have vasa vasorum or a nervi vasorum, nor a typical external tunica (adventitia). The function of this tunica is performed by Wharton's jelly (mucous connective tissue and fibroblasts) which is surrounded by nutritional vessels and rich in glycosaminoglycans [8][10][11].

3. Isolation of Endothelial Cells of HUC

During the last 3 decades, advances in vascular endothelial cell biology have had a great impact on the understanding of some pathophysiological conditions such as cardiovascular diseases [12][13]. Between 1922 and 1973, there was a growing interest among the scientific community in human umbilical cord vein-derived endothelial cells (HUVEC) for these investigations [1]. In 1922, studies were conducted and described the development of endothelial cells from chicken liver embryonic tissue explants [14].

In 1973, Eric A. Jaffe successfully isolated HUVEC, which grew as a homogeneous monolayer of polygonal cells with well-defined borders. Furthermore, these authors also compared HUVECs with SMC and fibroblasts, which allowed them to conclude that HUVECs contained rod-shaped cytoplasmic organelles [15]. In this procedure, veins were perfused with collagenase and incubated for 15 min at 37 °C, and endothelial cells were collected at the end [15].

Since 1974, some studies of isolation of EC have appeared intending to study the physiology of EC, most of them based on the studies and descriptions made by the following authors [16][17][18][19]. Over time, these studies have undergone some adaptations, such as the constitution of the culture medium for endothelial cells. In this sense, the constitution of the culture medium used may contain endothelial cell growth factor (ECGF), which allows long-term growth of the ECs, or an anti-PPLO agent that prevents contamination by gram-positive bacteria or mycoplasmas [17][20][21][22][23]. Culture media containing 5 ng/mL epidermal growth factor (EGF) was shown to have a higher proliferative potential compared to the anti-pleuropneumonia-like organisms' agent (anti-PPLO agent).

Meanwhile, some authors performed with the same umbilical cord the culture of HUVECs and the culture of HUAECs, and observed that cell isolation by enzymatic digestion is easy and fast when the artery and vein are used from a single umbilical cord [18]. Furthermore, other authors performed the isolation of the HUVECs and the HUCMSC (umbilical cord mesenchymal stem cells) from the same umbilical cord [24], as shown in **Table 1** . The

culture of HUCMSC may also allow us to direct the fate towards an endothelial cell lineage, which can represent economical and commercially viable option manly for cell replacement therapy due to its noninvasive collection procedure [24].

Table 1. Conditions used for isolation of endothelial cells.

Studies Performed	Digestive Enzyme	Medium Used	Centrifugation Conditions	Coating	Cells	Protocol Based on	Observations
Maruyama et al. (1963) [25]	0.2% Trypsin	YLH	-	Rat-tail Collagen (coverslip)	HUVECs	-	The cells were cultured in sheets
Jaffe et al. (1973) [15]	0.2% Collagenase	TC 199	250 g 10 min	-	HUVECs	Based on Maruyama et al.	ECs differentiation based on morphologic and immunologic criteria
Mann et al. (1989) [26]	Collagenase type II	M199	-	1% Gelatin	HUVECs	Based on Jaffe et al.	ECs were cultured on microcarrier beads
Sobrevia et al. (1995) [27]	Collagenase	M199	-	-	HUVECs	-	HUC from gestational diabetic pregnancies
Marin et al. (2001) [17]	Collagenase A	M199	1500 r/min 5 min	1% Gelatin	HUVECs	-	Three different cords were used to limit the variability of ECs
Ulrich-mersenich et al. (2002) [18]	Dispase II and Collagenase tipo IV	M199	1500 rpm 15 min	Fibronectin	HUVECS and HUAECS	Based on Ko et al. (1995)	Isolation of ECs and SMC from the same vessel of HUC obtain the best results
Larrivee et al. (2005) [28]	Collagenase A	MCDB 131	300 g 5 min	0.2% Gelatin	HUVECs	-	ECs successful cryopreservation
Mahabeleshwar et al. (2006) [29]	0.1% Collagenase	Endothelial cell growth medium	320 g 10 min	Gelatin	HUVECs	-	Obtention pure cultures of ECs
Baudin et al. (2007) [21]	0.2% Collagenase	M199	750 g 10 min	Fibronectin	HUVECs	Based on Jaffe et al.	HUVECs can keep phenotypic

Studies Performed	Digestive Enzyme	Medium Used	Centrifugation Conditions	Coating	Cells	Protocol Based on	Observations
							features in free serum medium for up 12 h
Crampton et al. (2007) [30]	Collagenase	M199	1200 rpm 5 min	Gelatin	HUVECs	-	Used in angiogenesis assay
Cheung et al. (2007) [22]	0.1% Collagenase	M199	250 g 10 min Room temperature	Gelatin	HUVECs	-	Confluent T25 flask was obtained in 4–5 days. Cells beyond the 6th passage should be discarded.
Martin de llano et al. (2007) [16]	Collagenase type I and Dispase	M200 or M231	10 g 10 min	Fibronectin	HUVECs and HUAECs	-	Birth weight does not have influence on the time request to obtain grown cultures
Casanello et al. (2009) [31]	Collagenase	M199	-	-	HUVECs	Based on Sobrevia et al.	ECs maintain the protein expression until 5th passage
Kadam et al. (2009) [24]	Dispase II and Collagenase type IV	M199	1500 rpm 15 min	Fibronectin	HUVECS and hUCMSCs	Based on Ulrich-mersenich et al.	Two-steps protocol for the simultaneous isolation of ECs and hUCMSCs
Krause et al. (2012) [32]	Collagenase	M199	-	-	HUVECs and HUAECs	Based on Casanello et al.	ECs are responsive to hypoxia
Pang et al. (2012) [33]	Collagenase	M199	1000 g 5 min	-	HUVECs	Based on Jaffe et al.	ECs are responsive to cytokine stimulation
Siow et al. (2012) [13]	Collagenase	M199	1000 rpm 5 min	Gelatin	HUVECs	Based on Jaffe et al.	Confluent culture of ECs after 2–3 days will start to detach and

Studies Performed	Digestive Enzyme	Medium Used	Centrifugation Conditions	Coating	Cells	Protocol Based on	Observations
							decrease viability
Lattuada et al. (2013) [34]	0.1% Collagenase A	M199	463 g 15 min	-	HUVECs	Based on Jaffe et al.	Gravitational field-flow fractionation is the new method to easy isolate ECs from HUC
Lei et al. (2016) [35]	0.2% Collagenasee	M199	800 g 5 min	Gelatin	HUVECs	-	Used a new instrument for insertion of HUC
Krause et al. (2016) [36]	Collagenase	M199	-	-	HUAECs	Based on Krause et al. (2012)	ECs pure culture can be used for protein expression
Amrithraj et al. (2017) [37]	Collagenase	EGM-2	1200 rpm 5 min	Gelatin	HUVECs	Based on Crampton et al.	ECs culture from gestational diabetic pregnancies maintain metabolic and molecular imprints of maternal hyperglycemia
Brodowaski et al. (2017) [38]	0.2% Collagenase	EGM	-	-	HUVEC	Based on Jaffe et al.	EC culture from preeclamptic women
Di tomo et al. (2017) [39]	Collagenase 1	M199	-	1.5% Gelatin	HUVECs	-	EC culture was obtained with explants
Suhaila et al. (2017) [40]	Collagenase type I	M199	1500 rpm 5 min	They tested various concentrations of gelatin	HUVECs	-	The 0.2% gelatin obtain the best results
Thormodsson et al. (2018) [41]	Collagenase	M199	140 g 5 min	-	HUVECS and HUAECs	-	Using Nunclon^{TM} Δ T-25 flasks: the cells attach without any problem

Studies Performed	Digestive Enzyme	Medium Used	Centrifugation Conditions	Coating	Cells	Protocol Based on	Observations
Yang et al. (2018) [19]	0.05% Collagenase I	EGM-2	250 g 5 min	Fibronectin	HUVECs	Based on Jaffe et al.	The cells were negative for CD34, CD45, and human leukocyte antigen–DR isotype (HLA-DR)
Provitera et al. (2019) [42]	0.1% Collagenase A	M199	463 g 15 min	-	HUAECs and HUVECs	Based on Lattuada et al.	Cells were used at P0
Pipino et al. (2020) [43]	Collagenase type1A	DMEM	-	1.5% Gelatin	HUVECs	Based on Di tomo et al.	Cells between the 3rd and 7th passages were used
Psefteli et al. (2021) [44]	0.2% Collagenase	M199	-	Gelatin	HUVECs	Based on Jaffe et al.	Cells between P0 and P3 were used for protein expression

References

1. Medina-Leyte, D.J.; Domínguez-Pérez, M.; Mercado, I.; Villarreal-Molina, M.T.; Jacobo-Albavera, L. Use of Human Umbilical Vein Endothelial Cells (HUVEC) as a Model to Study Cardiovascular Disease: A Review. Appl. Sci. 2020, 10, 938.
2. Lau, S.; Gossen, M.; Lendlein, A.; Jung, F. Venous and Arterial Endothelial Cells from Human Umbilical Cords: Potential Cell Sources for Cardiovascular Research. Int. J. Mol. Sci. 2021, 22, 978.
3. Campesi, I.; Franconi, F.; Montella, A.; Dessole, S.; Capobianco, G. Human Umbilical Cord: Information Mine in Sex-Specific Medicine. Life 2021, 11, 52.
4. Galley, H.F.; Webster, N.R. Physiology of the endothelium. Br. J. Anaesth. 2004, 93, 105–113.
5. Kumar, G.; Dey, S.K.; Kundu, S. Functional implications of vascular endothelium in regulation of endothelial nitric oxide synthesis to control blood pressure and cardiac functions. Life Sci. 2020, 259, 118377.

6. Alonso, D.; Radomski, M.W. The nitric oxide-endothelin-1 connection. Heart Fail. Rev. 2003, 8, 107–115.
7. Storch, A.S.; de Mattos, J.D.; Alves, R.; Galdino, I.d.S.; Rocha, H.N.M. Methods of Endothelial Function Assessment: Description and Applications. Int. J. Cardiovasc. Sci. 2017, 30, 262–273.
8. Lorigo, M.; Mariana, M.; Feiteiro, J.; Cairrao, E. Human Umbilical Artery Smooth Muscle Cells: Vascular Function and Clinical Importance. In Horizons in World Cardiovascular Research; Nova Science Publishers: New York, NY, USA, 2019; Volume 16, pp. 81–137.
9. Yampolsky, M.; Salafia, C.M.; Shlakhter, O.; Haas, D.; Eucker, B.; Thorp, J. Modeling the variability of shapes of a human placenta. Placenta 2008, 29, 790–797.
10. Bosselmann, S.; Mielke, G. Sonographic Assessment of the Umbilical Cord. Geburtshilfe Frauenheilkd. 2015, 75, 808–818.
11. Kellow, Z.S.; Feldstein, V.A. Ultrasound of the placenta and umbilical cord: A review. Ultrasound Q. 2011, 27, 187–197.
12. Vaccaro, P.S.; Joseph, L.B.; Titterington, L.; Stephens, R.E. Methods for the Initiation and Maintenance of Human Endothelial Cell Culture. Vasc. Surg. 1987, 21, 391–400.
13. Siow, R.C. Culture of human endothelial cells from umbilical veins. Methods Mol. Biol. 2012, 806, 265–274.
14. Lewis, W.H. Endothelium in tissue cultures. Am. J. Anat. 1922, 30, 39–59.
15. 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. Investig. 1973, 52, 2745–2756.
16. Martin de Llano, J.J.; Fuertes, G.; Garcia-Vicent, C.; Torro, I.; Fayos, J.L.; Lurbe, E. Procedure to consistently obtain endothelial and smooth muscle cell cultures from umbilical cord vessels. Transl. Res. 2007, 149, 1–9.
17. Marin, V.; Kaplanski, G.; Grès, S.; Farnarier, C.; Bongrand, P. Endothelial cell culture: Protocol to obtain and cultivate human umbilical endothelial cells. J. Immunol. Methods 2001, 254, 183–190.
18. Ulrich-Merzenich, G.; Metzner, C.; Bhonde, R.R.; Malsch, G.; Schiermeyer, B.; Vetter, H. Simultaneous Isolation of Endothelial and Smooth Muscle Cells from Human Umbilical Artery or Vein and Their Growth Response to Low-Density Lipoproteins. In Vitr. Cell. Dev. Biol.-Anim. 2002, 38, 265–272.
19. Yang, S.J.; Son, J.K.; Hong, S.J.; Lee, N.E.; Shin, D.Y.; Park, S.H.; An, S.B.; Sung, Y.C.; Park, J.B.; Yang, H.M.; et al. Ectopic vascularized bone formation by human umbilical cord-derived mesenchymal stromal cells expressing bone morphogenetic factor-2 and endothelial cells. Biochem. Biophys. Res. Commun. 2018, 504, 302–308.

20. Henriksen, T.; Evensen, S.A.; Elgjo, R.F.; Vefling, A. Human fetal endothelial cells in cluture. Scand. J. Haematol. 1975, 14, 233–241.
21. Baudin, B.; Bruneel, A.; Bosselut, N.; Vaubourdolle, M. A protocol for isolation and culture of human umbilical vein endothelial cells. Nat. Protoc. 2007, 2, 481–485.
22. Cheung, A.L. Isolation and culture of human umbilical vein endothelial cells (HUVEC). Curr. Protoc. Microbiol. 2007, 4.
23. Kunkanjanawan, H.; Kunkanjanawan, T.; Khemarangsan, V.; Yodsheewan, R.; Theerakittayakorn, K.; Parnpai, R. A Xeno-Free Strategy for Derivation of Human Umbilical Vein Endothelial Cells and Wharton's Jelly Derived Mesenchymal Stromal Cells: A Feasibility Study toward Personal Cell and Vascular Based Therapy. Stem Cells Int. 2020, 2020, 8832052.
24. Kadam, S.S.; Tiwari, S.; Bhonde, R.R. Simultaneous isolation of vascular endothelial cells and mesenchymal stem cells from the human umbilical cord. In Vitr. Cell. Dev. Biol.-Anim. 2009, 45, 23–27.
25. Maruyama, Y. The human endothelial cell in tissue culture. Z. Zellforsch. Mikrosk. Anat. 1963, 60, 69–79.
26. Mann, G.E.; Pearson, J.D.; Sheriff, C.J.; Toothill, V.J. Expression of amino acid transport systems in cultured human umbilical vein endothelial cells. J. Physiol. 1989, 410, 325–339.
27. Sobrevia, L.; Cesare, P.; Yudilevich, D.L.; Mann, G.E. Diabetes-induced activation of system y+ and nitric oxide synthase in human endothelial cells: Association with membrane hyperpolarization. J. Physiol. 1995, 489, 183–192.
28. Larrivee, B.; Karsan, A. Isolation and culture of primary endothelial cells. Methods Mol. Biol. 2005, 290, 315–329.
29. Mahabeleshwar, G.H.; Somanath, P.R.; Byzova, T.V. Methods for isolation of endothelial and smooth muscle cells and in vitro proliferation assays. Methods Mol. Med. 2006, 129, 197–208.
30. Crampton, S.P.; Davis, J.; Hughes, C.C. Isolation of human umbilical vein endothelial cells (HUVEC). J. Vis. Exp. 2007, 3, e183.
31. Casanello, P.; Krause, B.; Torres, E.; Gallardo, V.; González, M.; Prieto, C.; Escudero, C.; Farías, M.; Sobrevia, L. Reduced l-arginine transport and nitric oxide synthesis in human umbilical vein endothelial cells from intrauterine growth restriction pregnancies is not further altered by hypoxia. Placenta 2009, 30, 625–633.
32. Krause, B.J.; Prieto, C.P.; Munoz-Urrutia, E.; San Martin, S.; Sobrevia, L.; Casanello, P. Role of arginase-2 and eNOS in the differential vascular reactivity and hypoxia-induced endothelial response in umbilical arteries and veins. Placenta 2012, 33, 360–366.
33. Pang, H. Make Human Umbilical Vein Endothelial Cells from Cords. Bio-Protocol 2012, 2, e204.

34. Lattuada, D.; Roda, B.; Pignatari, C.; Magni, R.; Colombo, F.; Cattaneo, A.; Zattoni, A.; Cetin, I.; Reschiglian, P.; Bolis, G. A tag-less method for direct isolation of human umbilical vein endothelial cells by gravitational field-flow fractionation. Anal. Bioanal. Chem. 2013, 405, 977–984.
35. Lei, J.; Peng, S.; Samuel, S.B.; Zhang, S.; Wu, Y.; Wang, P.; Li, Y.F.; Liu, H. A simple and biosafe method for isolation of human umbilical vein endothelial cells. Anal. Biochem. 2016, 508, 15–18.
36. Krause, B.J.; Hernandez, C.; Caniuguir, A.; Vasquez-Devaud, P.; Carrasco-Wong, I.; Uauy, R.; Casanello, P. Arginase-2 is cooperatively up-regulated by nitric oxide and histone deacetylase inhibition in human umbilical artery endothelial cells. Biochem. Pharmacol. 2016, 99, 53–59.
37. Amrithraj, A.I.; Kodali, A.; Nguyen, L.; Teo AK, K.; Chang, C.W.; Karnani, N.; Ng, K.L.; Gluckman, P.D.; Chong, Y.S.; Stunkel, W. Gestational Diabetes Alters Functions in Offspring's Umbilical Cord Cells with Implications for Cardiovascular Health. Endocrinology 2017, 158, 2102–2112.
38. Brodowski, L.; Burlakov, J.; Hass, S.; von Kaisenberg, C.; von Versen-Hoynck, F. Impaired functional capacity of fetal endothelial cells in preeclampsia. PLoS ONE 2017, 12, e0178340.
39. Di Tomo, P.; Lanuti, P.; Di Pietro, N.; Baldassarre MP, A.; Marchisio, M.; Pandolfi, A.; Consoli, A.; Formoso, G. Liraglutide mitigates TNF-alpha induced pro-atherogenic changes and microvesicle release in HUVEC from diabetic women. Diabetes Metab. Res. Rev. 2017, 33, e2925.
40. Suhaila, R.N.; Safuan, S. Isolation Methods and Culture Conditions of Human Umbilical Vein Endothelial Cells from Malaysian Women. Sains Malays. 2017, 46, 463–468.
41. Thormodsson, F.R.; Olafsson, I.H.; Vilhjalmsson, D.T. Preparation and Culturing of Human Primary Vascular Cells. Methods Mol. Biol. 2018, 1779, 355–369.
42. Provitera, L.; Cavallaro, G.; Griggio, A.; Raffaeli, G.; Amodeo, I.; Gulden, S.; Lattuada, D.; Ercoli, G.; Lonati, C.; Tomaselli, A.; et al. Cyclic nucleotide-dependent relaxation in human umbilical vessels. J. Physiol. Pharmacol. 2019, 70, 619–630.
43. Pipino, C.; Shah, H.; Prudente, S.; Di Pietro, N.; Zeng, L.; Park, K.; Trischitta, V.; Pennathur, S.; Pandolfi, A.; Doria, A. Association of the 1q25 Diabetes-Specific Coronary Heart Disease Locus With Alterations of the gamma-Glutamyl Cycle and Increased Methylglyoxal Levels in Endothelial Cells. Diabetes 2020, 69, 2206–2216.
44. Psefteli, P.M.; Kitscha, P.; Vizcay, G.; Fleck, R.; Chapple, S.J.; Mann, G.E.; Fowler, M.; Siow, R.C. Glycocalyx sialic acids regulate Nrf2-mediated signaling by fluid shear stress in human endothelial cells. Redox Biol. 2021, 38, 101816.