ikhlas jenie turnitin 6

by Ikhlas Jenie

Submission date: 24-Sep-2019 12:35PM (UTC+0700)
Submission ID: 1178900783
File name: DEVELOPMENT_OF_HUMAN_ENDOTHELIAL_CELL_CULTURE.pdf (178.74K)
Word count: 1665
Character count: 9373

DEVELOPMENT OF HUMAN ENDOTHELIAL CELL CULTURE METHOD (HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS) FOR RESEARCH ANTI-AGING CARDIOVASCULAR

Titiek Hidayati[1], Ardi Pramono[2], Muhammad Ikhlas Jenie[3]
[1]*Departemen Epidemiology, Family Medicine and Public Health, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta*
[2]*Departemen Biochemistry and Anesthesiology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta*
[3]*Departemen Physiology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta*
The study was financed by the "competitive grants scheme", Indonesia Higher Education

ABSTRACT

Introduction: The morbidity and mortality of cardiovascular disease in Indonesia is high. Endothelial cells play a key role in the pathogenesis of cardiovascular disease. Endothelial role as regulator in hemostasis and coagulation, vasomotor regulation, angiogenesis and permeability. Until now, the development of culture methods of human umbilical vein endothelial cells (HUVEC) are based on foreign protocols that are often not in accordance with the laboratory conditions in Indonesia. Need to develop a model of HUVEC cell cultures for testing the mechanism of pathogenesis of cardiovascular disease in cultured endothelial cells. Culture of human endothelial cells from umbilical vein would be presented as a model of anti-aging research cardiovascular endothelial cells in vitro.

Method: The study was conducted with the design of experiments in vitro on HUVEC cells. Vein endothelial cells isolated from the placenta. Activity in the research is to pilot plant umbilical vein endothelial cells (HUVEC) and standardize the incubation time and the concentration of collagenase as a determinant factor of growth and cell morphology. Incubation time and the concentration of collagenase is able to foster the culture of HUVEC best set as a standard method in HUVEC culture protocol.

Results: The study has gained HUVEC culture method in accordance with the conditions Gadjah Mada University LPPT laboratory. Growth and development of collagenase HUVEC optimal incubation takes 1hour. Collagenase concentration which produces optimal HUVEC culture is 12 micrograms / ml.

Conclusion: The study has been able to determine the incubation time and the concentration of collagenase that produces optimal HUVEC culture
Keywords: HUVEC; incubation time; concentration of collagenase

INTRODUCTION

Morbidity and mortality of

cardiovascular disease is high. Cardiovascular disease is the leading cause of death in the group of degenerative diseases. The disease is expected to increase. Endothelial cells play an important role in the process of cardiovascular disease due to the degenerative process. Prevention or control of cardiovascular disease and its complications requires understanding the mechanisms of the pathogenesis of cardiovascular disease and its complications in bio molecular, both in vivo and in vitro. Endothelium is a component of the natural defense system that interacts directly with the reactive radicals in the body. As the wrapping layer organ in the body, the endothelium has a system of antioxidants as a natural defense system against reactive radicals. Endogenous antioxidant system involves a transcription factor Nrf2, iNOS as enzymes and TLR-4 receptors (Cines et al., 1998). Exposure on endothelial reactive radicals leads to atherogenesis by involving platelets and various inflammatory and proinflammatory cytokine modulators.

Human umbilical vein endothelial cells (HUVEC) proposed as a model of endothelial cells in vitro. Atherogenesis mechanism is still unclear needs studied more seriously to get a new drug discovery as a deterrent and to tackle atherosclerosis. Model maternal placental cells

are as more profitable, compared with endothelial cells from human aorta, aortic cows and mice. The reason is placental of human origin, and easily obtained, and a larger lumen size. Since culture HUVEC successfully carried out in the early 70s, up to the 21st century had been published about 50,000 publications associated with endothelial cells (Nachman & Jaffe, 2004).
Exposure reactive radicals in the blood vessels activate endothelial cells in the process of atherogenesis through the mechanism of chronic inflammation induced by oxidative stress. Antioxidant and immunomodulatory agent may inhibit the pathogenesis of cardiovascular disease. Consumption of herbal antioxidants including nigella sativa seed oil are expected to inhibit the degenerative processes in the body. As immunomodulators, bioactive nigella sativa has broad activity. Bioactive nigella sativa can reduce inflammatory reactions in response to asthma and toxic materials (Plumbum) (Massadeh et al., 2007), decrease the secretion of histamine by mast cells (El-Dakhakhny et al., 2000), increasing the phagocytic activity of macrophages in vivo in hamsters induced by streptozotocin (Fararh et al., 2004). Timokuinon proven to reduce reaction inflammatory on bronchus of mice, lowered Ig E and specific Ig G-OVA, lowering IL-5, IL-4, IL-13 and increase IFN

γ in mice induced by ovalbumin (El-Gazzar et al., 2006), whereas the same researchers have studied about the effect of yawning nigella sativa oil can decrease leukotriene (El-Gazzar et al., 2006). The main active substance does not evaporate MBJH oil is unsaturated fatty oil being linoleic and linolenic. The main active substance is oil evaporate timokuinon, nigelon and nigelin (Farrah et al, 2004; Nickavar et.al., 2003). Unsaturated fatty acids and is a powerful antioxidant timokuinon (El sayed & Fukuhima, 2003; Mousa et al, 2004; Randhawa et al., 2002).

Thus the need to develop a test model in vitro endothelial cells for testing the mechanism of atherogenesis and nigella sativa, which is expected to provide enough specific information about the function and endothelial response. Culture of human endothelial cells from human umbilical vein or umbilical vein endothelial cells (HUVEC) will be proposed as a model of endothelial cells in vitro (Randhawa et al., 2002).

METHODS

1. Collection of cord

Collection of umbilical cord is to obtain pieces of cord that can be used for the isolation of endothelial cells. Terms cord for culturing endothelial cells (HUVEC) are in good condition (not broken) and long enough (at least 15 cm).

2. Isolation of endothelial cells

Isolation of endothelial cells aims to remove the endothelial cells from umbilical vein wall enzymatically in sufficient quantity and condition in life. With the isolation so that it can be done planting a primary culture of human umbilical vein endothelial cell culture (HUVEC). Implementation is done aseptically in a biosafety cabinet (BSC) level 2 with enzymatic method.

Materials needed are the umbilical cord in the buffer solution and antibiotics, medium complete DMEM glucose, FBS aliquot (FBS A) qualified, Pen Strep aliquot, Fungizone aliquot, PBS Pen Strep 1%, Collagenase sterile (7 mg / 7ml sterile PBS (without Ca Cl and Mg Cl), Glutamine 100x, Alcohol 70%.

Tools needed are incubators, BSC level2, centrifugation, hot plate, clamps, tweezers network, needle cannula, syringes 10cc, glass bottle, funnel glass, tube conical 15cc, sterile gauze, a petri dish of glass, flask, blue yellow white tips, micropipette,

The procedure of research carried on in the research laboratory LPPT UGM.

a. Umbilicus cleared from the network and the rest of the blood with sterile gauze moistened with 70% alcohol. Each end of the umbilicus is cut transversely

so that the visible presence of two arteries and veins, characteristic veins has thicker walls, large and elastic.

b. A cannula is inserted into the end of the vein approximately 1 cm, then tied tightly with string. Veins washed with FBS A through a cannula that is preinstalled with using a 20cc syringe. The foregoing is done 2-3 times. Once clean, the end of the umbilicus using either tied with strong ties and clamped.

c. Collagenase solution is inserted, while the syringe should be left attached to the cannula. Umbilicus subsequently warmed to room temperature for 60 minutes or incubated.

d. Collagenase solution containing endothelial then removed from the umbilicus a way to suck through a syringe mounted on the tip of the cannula. Collagenase is then inserted into a 18cc sterile centrifuge tube. Umbilicus 8cc rinsed with a solution of FBS A to rinse the remaining endothelial cells, for subsequent inclusion in a centrifuge tube, which already contains collagenase solution. The solution containing the endothelial centrifuged at a speed of

1000rpm for 8 minutes, in order to obtain pellets that contain endothelial cells. The supernatant was discarded, then added 4 ml culture medium in pellet and suspended with pipetting methods are used so that the endothelial cells can be separated. The solution was transferred into a flash 25cm^2 that has been coated with 0.2% gelatin solution, then flash is inserted into the 5% CO2 incubator at a temperature of 37°C for 30 minutes.

3. Culture of endothelial cells, stimulation and activation of endothelial cells.

The purpose of this phase is to grow endothelial cells that have been isolated in the growing medium. Preparations containing flash endothelial cells then added to the culture medium (RPMI + 20% FBS), and incubated for 3-4 days until confluent endothelial cell cultures and monolayer.

4. Standardization of incubation time and concentrations of collagenase.

The goal of getting collagenase incubation time that is able to produce best cell culture result.

RESULTS AND DISCUSSION

1. The result of the isolation and culture of HUVEC early

HUVEC culture results are

shown in Figure 1.
The survey results revealed that HUVEC can grow well. The results showed the cell culture HUVEC grow quickly shown on the fourth day changes in cell morphology, migration and elongation in cultured cells. Cell culture results also showed only slightly contaminants. On day six of culture seemed to confluent and ready to be harvested for use in vitro testing.

2. The results of HUVEC culture with the incubation time variations collagenase

The results of cell culture based on the time of induction of collagenase are presented in Figure 2.
Based on the results of standardization known that the incubation time long incubation collagenase are on the best culture results are for 60 minutes. The concentration of collagenase, which provides optimal culture results, was 0.5%. The results provide a new protocol modify protocol of HUVEC.com. Which according huvec.com collagenase incubation only performed for 10 minutes. The results showed that incubation of collagenase for 10 minutes yet provide optimal culture results as presented in Figure 2 c and d. This research result is slightly different from previous studies (Galley & Webster, 2004).

CONCLUSION

The results showed that it had been successfully cultured HUVEC

with the addition of collagenase
incubation time and concentration.
HUVEC culture can be used to test
antiaging.

ikhlas jenie turnitin 6

ORIGINALITY REPORT

5%	3%	2%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

2%

★ Winny Setyonugroho, Thomas Kropmans, Kieran M Kennedy, Brian Stewart, Jan van Dalen. "Calibration of communication skills items in OSCE checklists according to the MAAS-Global", Patient Education and Counseling, 2016

Publication

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		