# Endothelial Cell Culture From Human Umbilical Cord Vein

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## Abstrak

*Sel endotel memegang peranan penting dalam berbagai proses fisiologis maupun patologis. Fungsi sel endotel pada peristiwa biologik dapat diketahui lebih baik bila sel endotel dapat diisolasi dan dikultur secara invitro. Keberhasilan mengultur sel endotel di daerah tropis bergantung pada cara mengatasi kontaminasi bakteri dan jamur. Telah dilakukan penelitian mengisolasi dan mengultur sel endotel dari v. umbilikalis tali pusat mempergunakan metode Jaffe dengan modifikasi. Dari 50 tali pusat yang dipergunakan, 35 memberikan kultur sel yang konfluen. Dari hasil penelitian ini mungkin dapat dikembangkan penelitian lebih lanjut mengenai fungsi sel endotel yang sangat penting pada beberapa proses fisiologis maupun patologis.*

## Abstract

*Endothelial cells play an important role in various physiologic and pathologic processes. Their function in biologic events can be better understood if these endothelial cells can be isolated and cultured in vitro. The success of endothelial cell culture in the tropics depends upon controlling bacterial or fungal contamination. An attempt was made to isolate and culture endothelial cells from human umbilical cord vein using an adaptation of the method employed by Jaffe.[4] Out of 50 umbilical cord vein samples cultured, 35 produced confluent cultures. It is hoped that this present trial will be the bases of further studies on the functions of endothelial cells in physiologic and pathologic processes.*


***Keywords*** : *Human umbilical vein endothelial cell culture, Endothelial cell isolation, Bacterial and fungal contamination, Confluent growth.*

## INTRODUCTION

Endothelial cells play a major role in various physiologic processes, such as hemostasis, vascular permeability, and other vascular responses to physiologic or pathologic stimuli. Abnormalities in endotelial cell structure and function can cause vascular diseases, such as thrombosis, atherosclerosis, and vasculitis.[1,2] These various functions can be studied if the cells can be isolated and cultured *in vitro*.

Endothelial cells can be isolated and cultured from bovine aorta, human umbilical cord vein, pulmonary artery, etc.[3] Many investigators from other countries have reported success in isolating and culturing endothelial cells. Long-term *in vitro* cell culture in tropical countries is challenging because of the problem of fungal and bacterial contamination.[3]

This investigation hopes to pave the way for other investigators interested in studying endothelial cell functions through cell culture.

## METHODS

Endothelial cells can be easily isolated from human umbilical cord veins of neonates. Collagenase can selectively digest the subendothelial basement membrane leaving the internal elastic layer intact. This loosens the endothelial cells without fibroblast contamination.

In this study, the umbilical cords were obtained from neonates born in the Budi Kemuliaan Maternity Hospital, Jakarta. They were *at term*, of normal births, with no intrapartum infection, and with clear amniotic

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fluid. The mothers consented to donate 20 - 25 cm of their newborns' umbilical cord. The cord was severed from the placenta immediately after birth.

The cord was inspected and all areas with clamp marks were cut off. It was then placed in a sterile container filled with cord buffer (0.14 M NaCl, 0.004 M KCL, 0.001 M phosphate buffer, pH 7.4, 0.011 M glucose) and held at $4^0$C until processing.[4] To control bacterial contamination during the process of obtaining the cord, 15 mg of gentamycin and 100 mg of ampicillin were added to every 100 ml of cord buffer.

After the clamp marks have been discarded, the umbilical cord vein was cannulated with a blunt needle. The vein was then perfused with 50 ml of cord buffer at $37^0$C to wash out any blood left in the vein. The other end of the vein was also cannulated with a polyethylene tubing. The cord was perfused again and allowed to drain, to insure the solution runs out of the tubing. The polyethylene tube was clamped and the umbilical vein infused with 5 ml of 0,4 % collagenase (*Clostridium histolyticum, type I, Sigma*). The umbilical cord with polyethylene tube and clamps are then wrapped in aluminium foil and incubated for 15 minutes at $37^0$C.

Only carefully selected umbilical cords should be used. Cords damaged by clamps should be discarded, since it can cause contamination to the fibroblast culture. The collagenase incubation time must not exceed 15 minutes, or the basement membrane can be digested and the underlying structures disrupted.

After incubation, the collagenase solution containing the endothelial cells was flushed top to bottom and perfusing the vein with 10 - 20 ml of cord buffer. The effluent was collected in a sterile concical centrifuge tube containing 0.5 - 1.0 ml fetal calf serum (FCS, Sigma). The FCS will neutralize and halt further endothelial digestion by collagenase. The cells were sedimented at 250 g for 5 minutes. The precipitate was resuspended by trituration in 2 ml of culture medium (TC 199), to which antibiotics, an antifungal drug, and FCS have been added. Each 80 ml of TC 199 was given 20 ml FCS, 15 mg gentamycin, 100 mg ampicillin, and 0.4 ml fungizone. The antibiotics and antifungal were twice the usual dose. All culture medium was passed through a 2 mu sterile filter.

The cell suspension was cultured in a T-25 flask (Falcon Plastics) lined with 0.2% gelatin solution. The cells were fed with a complete change of fresh culture medium after 24 hours and twice in the following week. Endothelial cell growth was monitored and photographed every day through a phase contrast microscope. Although growth factors can simulate cell replication in culture, they will not be needed if cell proliferation is sufficiently profuse. Generally, the endothelial cells will achieve confluent growth ( a monolayer growth covering the entire surface of the flask base ) on day 3 or 4.[5]

## RESULTS

There were 50 umbilical cord vein samples received. Endothelial cells were successfully isolated from 42 samples, from which 35 confluent growths were produced. Isolation was less successful in 8 samples and the cultures failed to yield confluent growths. Confluent monolayers were achieved on day 4 (Figure 1).

Bacterial contamination occurred to 7 cultures on day 2 and to 4 cultures after day 3. Fungal contamination appeared later, after day 6. Only 10 cultures survived more than 6 days with only 1 fungal contamination.

## DISCUSSION

The failure of endothelial cells to grow in tissue culture is mainly due to contamination of fibroblasts or blood cells. At the risk of flushing away some endothelial cells not adhering to the T-flask surface along with the fibroblasts and blood cells, a complete change of fresh culture medium after 60 minutes of culture can reduce the possibility of contamination by those cells.

In this trial, 0.4 % collagenase with an incubation time of 15 minutes is applied. Jaffe used 0.1 % collagenase and 10 minutes of incubation. The range may vary from 0.1 - 0.4% for collangenase and 10 - 30 minutes for the incubation time.[6]

Endothelial cell morphology is identified through a phase contrast microscope. The cells were homogenous, closely opposed, large, and polygonal with an oval, centrally located nucleus. The findings (figure 2) were consistent with endothelial cells cultured by Jaffe. Ideally, a transmission electron microscope should be used to identify these endothelial cells.

When confluent cell growth was achieved, the cells were harvested with 0.2% tryspsin-0.05% EDTA and subcultured. On day 1 of the subculture, cell growth was identical to the primary endothelial cell culture.

These endothelial cells were found to be capable of migrating towards an appropriate stimulus. They can therefore be used in an angiogenesis assay.

Cultured endothelial cells are micoscopically distinct from cultured fibroblasts. Cultured fibroblasts grow close to one another in parallel arrays with whorling and multiple overlapping layers. With a phase contrast microscope, they can be seen as long, slender,

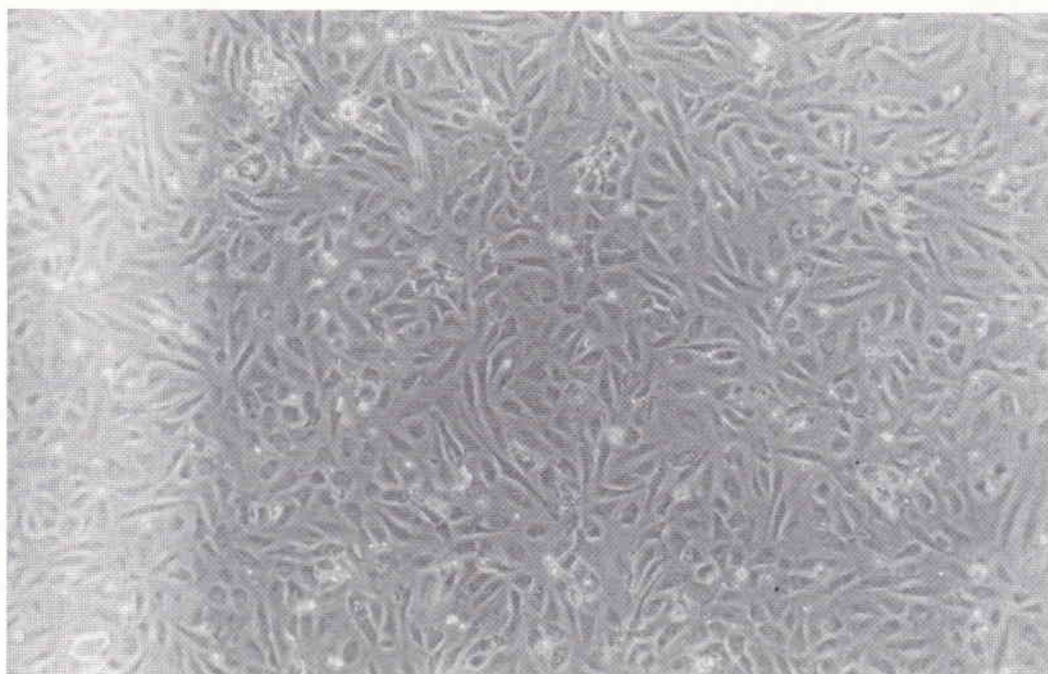

*Figure 1. The morphology of human endothelial cells at confluence stage (100 x)*

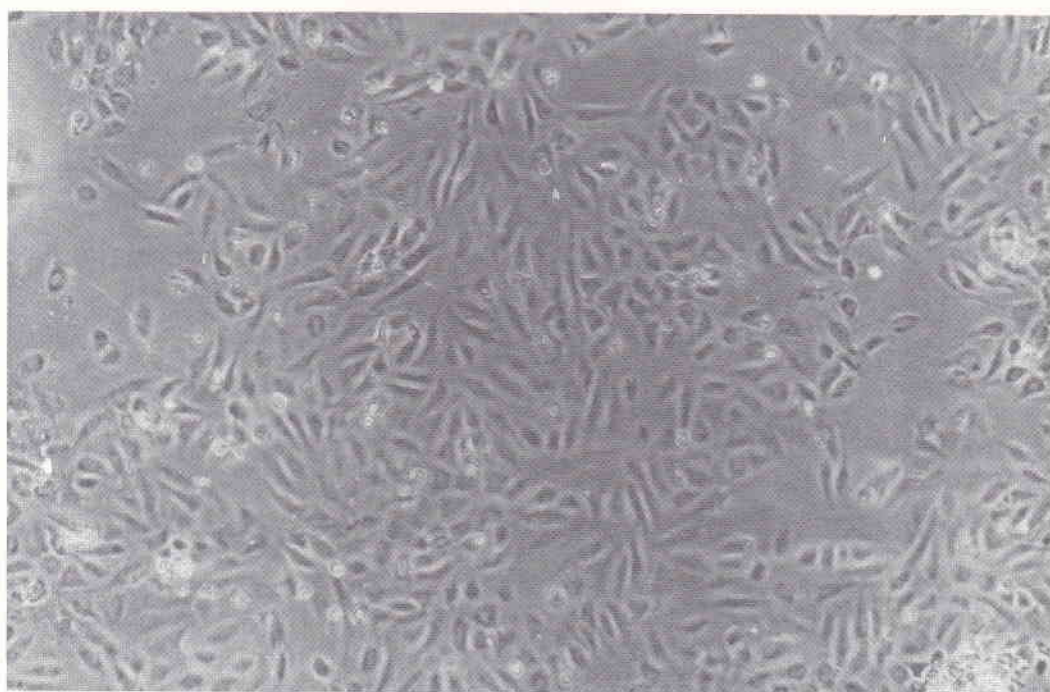

*Figure 2. The endothelial cells, 24 hours after seeding*

and spindle-shaped cells. Unlike endothelial cells, fibroblasts do not have the ability of migrating during an angiogenic reaction.

No additional growth factor was required, since the cultures yielded dense, confluent cell growth able to survive up to 7 - 8 days. The endothelial cells probably produce sufficient growth factor by autocrine or paracrine secretion.

It seems that double doses of antibiotics and antifungal drugs are needed to control bacterial and fungal contamination in the tropics. These high doses were not found to be detrimental to cell culture growth.

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