Proceedings of the National Science Council, ROC
Part B: Life Sciences
Vol. 19, No. 4, 1995. pp. 208-215

Isolation and Cultivation of Aortic Endothelial Cells from Spontaneously Hypertensive Rat with a Modified Tissue Explant Technique

CHUNG-FAYE CHAO*,****,+, ZU-CHIEN CHAO**, MEI-HUA LU*,****, JAU-YUAN LU*,****, AND HSING-I CHEN***,*****

**Department of Biology and Anatomy*
***Institute of Medical Sciences*
****Department of Physiology and Biophysics*
National Defense Medical Center, Taipei
*****Cell Biology Laboratory, Clinical Research Center*
Tri-Service General Hospital, Taipei
******Tzu-Chi Medical Research Center, Hua-Lien*
Taiwan, Republic of China

(Received April 18, 1995; Accepted July 17, 1995)

ABSTRACT

In the present study, we successfully established a "tissue explant technique" to obtain high yield and purity of endothelial cells from the aorta of hypertensive and normotensive rats (SHR and WKY). Small pieces of aorta were placed on fibronectin precoated petri dishes. The effects of oxygenation in the tissue preparation stage, tilting of the petri dish during the explanting period and timing of the removal of tissue blocks from petri dishes were evaluated. These procedures appeared to be critical for cell survival, tissue adhesion and minimizing of non-endothelial cell contamination. The cultured endothelial cells were characterized by morphological, immunohistochemical and biochemical examination. The cultured cells from both SHR and WKY rats showed similar endothelial cell character, positive immunofluorescence staining for the von Willebrand factor, and uptake of acetylated low-density lipoprotein (DiI-ac-LDL). The secretory function of prostacylcin I_2 (PGI_2), thromboxane A_2 and endothelin of cultured endothelial cells was measured. The results showed that the secretion of both PGI_2 and endothelin was greater in SHR than in WKY rats, but that there was no difference in thromboxane A_2 secretion. Therefore, our "tissue explant technique" can provide high yield and purity of endothelial cells with their specific biological function in vitro. It will permit us to further study the role of endothelial cells in the development of hypertension.

Key Words: rat; culture; aortic endothelial cells.

I. Introduction

In 1980, Furchgott and Zawadzki reported that acetylcholine induced relaxation of the isolated aortic strip was highly dependent on an intact endothelium. Since then, it has been well established that endothelial cells play a pivotal role in modulation of the vascular tone through the release of vasodilator and vasoconstrictor substances (Vanhoutte *et al.*, 1986; Luscher and Vanhoutte, 1986). Changes in the paracrine functions of the endothelium have also been implicated in the development of cardiovascular disorders such as hypertension, stroke, thrombosis, etc. (Moncada *et al.*, 1977; Hongo *et al.*, 1988; Vanhoutte, 1989).

To investigate the secretory function of endothelium and responses to certain perturbations, attempts have been made to isolate and cultivate endothelial cells *in vitro*. Endothelial cell culture was successfully obtained using bovine or porcine aorta (Booyse *et al.*, 1977; Feder *et al.*, 1983) or capillary (Diglio *et al.*,

+Address all correspondence and reprint requests to: Dr. Chung-Faye Chao, Department of Biology and Anatomy, National Defense Medical Center, P. O. Box 90048-502 Taipei, Taiwan, R.O.C.

1982; Gross *et al.*, 1982) and human umbilical veins (Maciag *et al.*, 1982; Mandri and William, 1983). Techniques for implantation of endothelial cells from blood vessels to the culture medium have included enzymatic digestion (Gimbrone *et al.*, 1974) and mechanical scrubbing (Lewis *et al.*, 1973). However, these methods failed to obtained endothelial cells from the rat's aorta not only because it is small, but also because the endothelial layer is resistant to enzymatic digestion and mechanical scrubbing (Diglio *et al.*, 1982). McGuire and Orkin (1987) developed the technique of "tissue explant" for the culture of endothelial cells from the rat's aorta. Small pieces of rat aortic tissue were placed on the culture medium.

The contact between the endothelial layer and the culture medium permitted outgrowth of endothelial cells. The tissue explant technique may involve the following difficulties for high yield and high purity of endothelial cells: (1) preparation of aortic tissue pieces, (2) the adhesion efficiency of the explant tissue pieces, and (3) the timing for adequate endothelial growth before out-growth of smooth muscle cells. In this study, we have considered these technical problems and consistently obtained endothelial cells culture from the aorta of hypertensive and normotensive rats. The SHR of the Kyoto strain has been the most common model for the study of genetic hypertension (Okamoto and Aorki, 1963; Yamori, 1991). To our knowledge, endothelial culture from the aortic tissue of SHR has not been reported. Disorders of the endothelial function may be involved in the pathogensis of hypertension or as a result of hypertension (Folkman *et al.*, 1979; Takeshita *et al.*, 1982). Success in obtaining endothelial culture from SHR is certainly the first step towards understanding its secretory functions in vitro.

In this report, we will describe the technical procedures for a successful endothelial cells culture from SHR and normotensive control (WKY). The cultured endothelial cells were characterized by morphological, immunofluorescence and immunohistochemical examination for a specific marker, von Willebrand factor VIII (Hormia *et al.*, 1984; Wagner *et al.*, 1982; Paled, 1964), and uptake of acetylated low-density lipoprotein (DiI-ac-LDL) (Voyta *et al.*, 1984). The release of prostacyclin (PGI_2), thromboxane A_2 and endothelin from cultured endothelial cells of SHR and WKY rats was also determined.

II. Materials and Methods

1. Chemical and Reagents

Goat anti-human Factor VIII antiserum (anti-vWF) and acetylated low -density lipoprotein labeled with 1,1'-dioctadecyl-1-3,3,3',3' -tetramethylindocarbocyanine perchlorate (DiI-ac-LDL) were purchased from Biomedical Technologies (Cambridge, MA). The endothelial cell growth supplement (ECGS), FITC-conjugated rabbit anti-goat IgG, and collagenase/dispase were obtained from Boehringer Mannheim (Indianapolis, IN). Fibronectin was supplied by Sigma and cell culture medium M199 by Gibco.

2. Preparation of Fibronectin Coated Petri Dish

1% of fibronectin was dissolved in cord buffer (137 mM NaCl, 4 mM KCl, 10 mM Hepes and 11 mM glucose) and sterilized by milipore filtration. One hour prior to tissue block seeding in a petri dish, the petri dish were rinsed with 1 ml using prepared 1% fibronectin in cord buffer.

3. Endothelial Cells Culture

Male SHR and WKY rats 24 weeks old were anesthetized with intraperitoneal injection of pentobarbital (35 mg/kg). The thoracic aorta was excised under sterilized conditions. The adventitia and fat tissue of the thoracic aorta were carefully peeled off with a fine forceps. The endothelial cells were harvested using different procedures from prepared aorta as follows.

A. Enzyme Digestion and Mechanical Scrubbing

A needle carrying a 6-O silk thread was inserted carefully into the aortic lumen from one end and tied at the other end. Then, the aorta was everted by pulling the thread from one end. After the aorta was totally everted, the needle with silk thread was removed, and the open end of the aorta was also tied. The everted aorta was transferred to a petri dish containing medium with digestive enzyme (0.2% of collagenase/dispase in M199 culture medium, pH 7.4) and incubated at 37°C in an incubator for 30 min. After enzyme digestion, some of the aorta was subsequently scrubbed with a surgical knife. Then, the everted aorta was removed from the petri dish and prepared for morphological examination with light microscopy and scanning electron microscopy to observe the remaining endothelial cells on the surface of the aortic wall. The isolated endothelial cells in the medium were collected and washed with M199 culture medium. Then, the cells were seeded on petri dishes precoated with fibronectin in 20% fetal calf serum, with 30 μg/ml of ECGS in M199 medium. The plating efficiency, growth rate and endothelial cell specific markers were examined after a culture

period of two to three wk.

B. Tissue Explant Method

The prepared aorta was trimmed into small pieces of tissue (0.5 mm × 0.5 mm). During the trimming procedure, 95% oxygen and 5% air was blown into the medium at a rate of 5 ml/min through the filter. The tissue blocks were explanted on fibronectin precoated petri dishes (60 mm). The endothelial layer of the explanted tissues was placed face down onto the petri dishes. To avoid tissue dryness and detachment from the petri dish, 2 ml of M199 medium were added to just cover the tissues. Then, the dishes were placed upright in the incubator set at 45°. The dishes were allowed to lay flat after 20-24 hr incubation and were gently filled with 5 ml of culture medium (M199 medium; 20% FCS; 75 μg/ml ECGS; 1/100 dilution of anti-biotic and 100 μg/ml heparin). The cultured explant tissues were closely examined under an inverted microscope (Olympus Model CK- 600). After a culture period of five to seven days, out-growth of endothelial cells from explant tissues could be observed. The explant tissues were removed at the proper time to minimize non-endothelial cell contamination and to obtain a high yield of endothelial cells. After six wk, culture with four to five passages, the cultured endothelial cells were prepared for the following experiments.

4. Characterization of Cultured Endothelial Cells

A. Morphological Examination

The cultured endothelial cells were examined and photographed at various stages of cell growth in petri dishes under an inverted microscope (Olympus Model CK- 600).

B. Immunofluorescence Stain for von-Willebrand Factor VIII

The cultured endothelial cells at passage 3 were allowed to grow on fibronectin precoated glass slides and were fixed with acetone at room temperature for 10 min. After air drying, the cells were washed with phosphate buffer saline (PBS). The slides were blocked with normal goat serum (1:10 in PBS buffer) and then incubated with rabbit anti-human Factor VIII (1:100) antiserum at 37°C for 1 hr. They were thoroughly washed with PBS buffer. Goat anti-rabbit IgG conjugated with FITC (1:32) was reacted with the cells at 37°C for 1 hour. The slides were washed and mounted with coverslips in 10% glycerol. The cells were observed and photographed under a fluorescence microscope (Olympus).

C. DiI-ac-LDL Uptake Examination

The cultured endothelial cells growing on the coverslips were treated with 20 μg/ml of the DiI-ac-LDL solution in culture medium and incubated at 37°C in a 5% CO_2 incubator for 4 hr. After removal of excessive DiI-ac-LDL with PBS washing, the cells were fixed in 10% neutral formalin for 5 min and mounted on slides with glycerol mounting medium. The cells on the slides were examined under a fluorescence microscope (excitation 580 nm) and photographed.

D. Measurement of PGI_2, Thromboxane A_2 and Endothelin Secretion Using the RIA Method

Cultured endothelial cells from SHR and WKY aorta was detached with 0.05% trypsin (Gibco) and 1×10^5 cells were seeded on 24-well petri dishes precoated with fibronectin. When the cells reached a confluence state, the culture medium was washed out, and the cells were incubated with PBS (1 ml) containing 1 mM of $CaCl_2$ and $MgCl_2$ incubation medium at 37°C for 10 min. The incubation medium was collected and stored at −20°C in preparation for measurement of PGI_2, thromboxane A_2 and endothelin. The cell number in each well was counted using a hemocytometer. Determination of PGI_2 and thromboxane A_2 was carried out by measuring the formation of their stable metabolites 6-keto-$PGF_{1\alpha}$ (Yamori, 1991) and thromboxane B_2 (De Nucci *et al.*, 1988; Konieckowski *et al.*, 1983; Moncada and Vane, 1979), respectively. The concentration of 6-keto-$PGF_{1\alpha}$, thromboxane B_2 and endothelin was determined by radio-immunoassay and normalized with the number of endothelial cells (pg/1×10^5 cells).

III. Results

1. Primary Culture of Rat Aortic Endothelial Cells

Seeding of endothelial cells from rat aorta using the "enzyme digestive method" and "mechanical scrubbing" was evaluated in this experiment. The everted aorta from 6 rats was incubated with 0.2% collagenase and dispase for 30 min. Only a few cells could be collected from the medium using this method and the cells seeded on the petri dish also could not proliferate. Light and scanning electron microscope revealed that the endothelial layer of the aorta remained virtually intact (Fig. 1, A and B). In 4 other aortic tissues, the concentration of collagenase was increased to 0.3%, and 0.05% trypsin was added for enzyme digestion. There was little improvement of the endothelial cell seeding. In an additional 6 rat

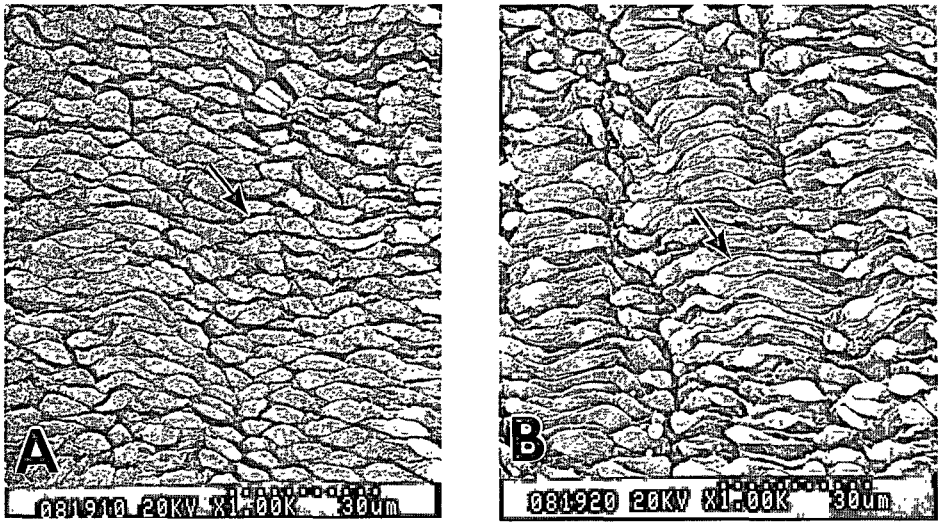

Fig. 1. Scanning electron microscopic photography of the aortic intima layer from 24 wk SHR. A. Before enzyme digestion. B. After enzyme digestion. The endothelial cells (arrow) still exist on the surface of the thoracic aortic intima layer. (bar = 30μm)

Table 1. Endothelial Cell Outgrowth from Explant Aortic Tissue Blocks with Oxygenation or Without Oxygenation During Tissue Block Preparation

	Oxygenation	No oxygenation
Total tissue blocks	78	74
Tissue blocks with endothelial cell outgrowth	64	7
Tissue blocks without endothelial cell outgrowth	14	67
Outgrowth rate %	82	9

Total number of aortic tissue blocks obtained from 6 animals; all dishes were at the 45 degree tilting position.

Table 2. Comparison of Endothelial Cell Outgrowth from Tissue Explant on Petri Dishes in Horizontal or Tilting Positions

	Horizontal position	45° tilting position
Total tissue blocks	80	82
Tissue blocks with endothelial cell outgrowth	12	69
Tissue blocks without endothelial cell outgrowth	68	13
Outgrowth rate %	15	84

Total number of aortic tissue blocks obtained from 6 animals; tissue was prepared under oxygenated conditions.

aortic tissues, enzyme digestion was followed by mechanical scrubbing. A total of 103 cells were collected for cell culture in the petri dish. After a culture period of 2 wk, cell survival and growth were limited.

In the "tissue explant technique," two factors appeared to be crucial for cell survival and outgrowth from tissue explant in the medium. The first was the oxygen supply during the trimming procedure for tissue blocks. In each rat, the aorta was carefully cut into approximately 20 tissue blocks, and the tissue blocks were picked up and placed on one petri dish with the intima layer face down in the culture medium. During preparation, 6 aortic tissues were provided with oxygenation while 6 other tissues did not receive oxygenation. Table 1 shows the outgrowth rate in the tissue explant of two groups with and without oxygenation. The outgrowth was observed 7 days after tissue explantation. It is obvious that trimming of the tissues without oxygenation seriously affected the rate of endothelial culture. The outgrowth rate of tissue blocks without oxygenation was about 11% of the value of that with oxygenation. Close adhesion of the tissue explant with the surface of the petri dish precoated with fibronectin was apparently another important factor for cell outgrowth. Initially, large variation in the successful outgrowth occurred in explantation when the petri dish was placed inside the incubator in the usual (horizontal) position. We though that tiling the petri dish might keep the amount of culture medium between the tissue block and dish surface to a minimum, thereby enhancing surface contact. Table 2 shows the outgrowth of endothelial cells on the petri dish in a horizontal and 45° tilting position. Tilting the petri dish greatly

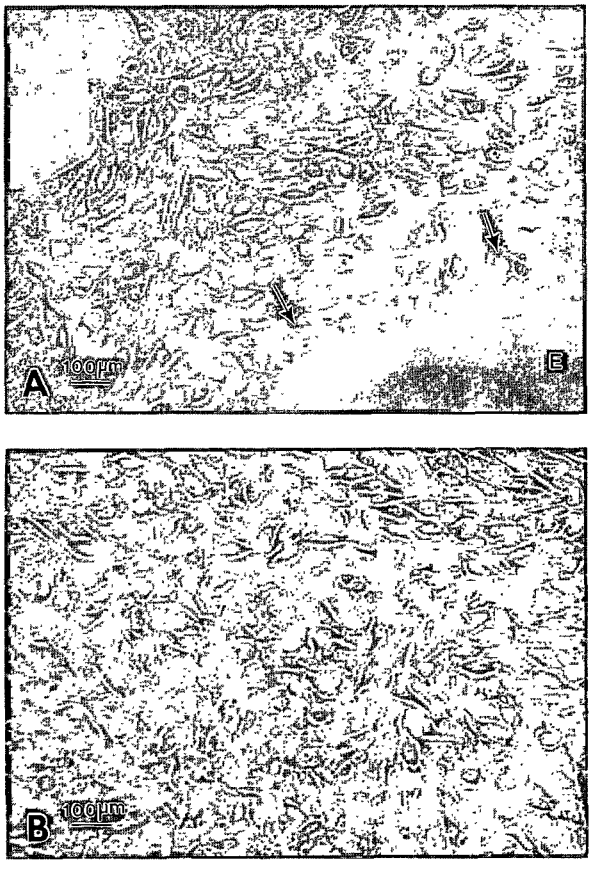

Fig. 2. Endothelial cell (arrow) outgrowth from rat aortic tissue explant (E). A. Tissue explant at day 7. B. Cultured endothelial cell growth to a confluence status at day 21. (bar = 100μm).

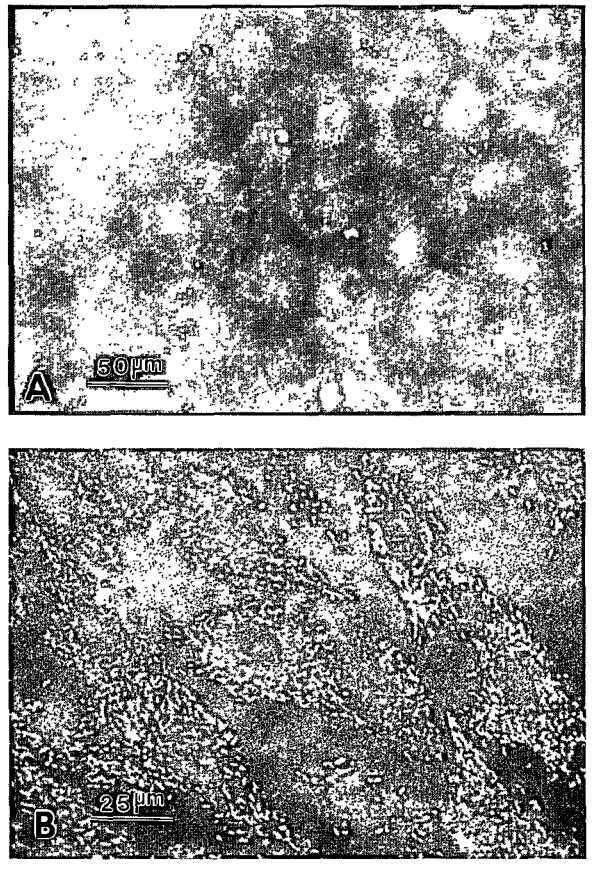

Fig. 3. A. Immunofluorescence examination of DiI-ac-LDL uptake activity of culture rat aortic endothelial cells. Abundant perinuclear punctate fluorescence is visible in most of the cells. (bar = 50μm). B. Immunofluorescence staining of vWF in culture aortic endothelial cells. Fluorescence positive granules are visible in most of the cells. (bar = 25μm).

enhanced the adhesion efficiency of the tissue block on the fibronectin precoated petri dish and eventually increased endothelial cell outgrowth from 15% to 84%. In general, endothelial cell outgrowth from explant tissue could be observed within three to five days (Fig. 2A). When the cells grew to an appropriate density, the explant tissues were removed from petri dishes to minimize non-endothelial cell contamination. The endothelial cells without explant tissue were allowed to grow to a confluent state, which usually took 14-20 days (Fig. 2B). Then, the cells were divided to obtain a one to two dilution and transferred to other dishes. After the third passage, we obtained about 6×10^6 cells per aorta. Thereafter, the cells were capable of growing in petri dishes without fibronectin precoating, and ECGS could also be omitted in culture medium. Over five passages, the phenotype of the culture endothelial cells was altered. Therefore, cells used for characterization and for further experiments were confined to the third passage.

2. Characterization of Cultured Endothelial Cells

The cultured endothelial cells from rat aorta were observed under a phase contrast microscope from outgrowth of explant tissue to the third passage. The cells from both SHR and WKY rats presented a cobblestone pattern (Fig. 2B). The growth rate of the third passage of the cells was about 26 hr for the doubling time. In SHR and WKY, a fluorescence positive granule could be observed in the perinuclear region of cultured endothelial cells after treatment with anti-vWF antiserum conjugated with FITC (Fig. 3A). According to Voyta *et al.* (1984), DiI-ac-LDL uptake activity mediated by their specific LDL receptor was very high in the production of endothelial cells. Thus, cultured endothelial cells were treated with the metabolic probe DiI-ac-LDL. Uptake of DiI-ac-LDL by endothelial cells was observed under a fluorescence microscope (Fig. 3B). The purity of

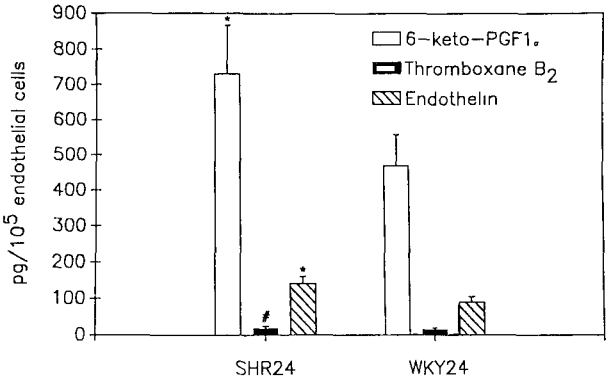

Fig. 4. 6-keto-$PGF_{1\alpha}$ (PGI_2 metabolite), thromboxane B_2 (thromboxane A_2 metabolite) and endothelin in supernatant after 30 min. incubation of cultured endothelial cells from SHR and WKY (24 wk) rats as measured by RIA assay. Both 6-keto-$PGF_{1\alpha}$ (729.6 ± 136.9 vs 471.2 ± 88.8) and endothelin (140.7 ± 20.2 vs 90.8 ± 14.6) secretion from endothelial cells was higher in SHR than in age matched WKY rats, but there was no significant difference in thromboxane B_2 secretion (18.6 ± 4.8 vs 16.1 ± 3.9). The statistic data were analyzed by student *t*-test.
* $p < 0.05$ SHR compared with WKY rats, # $p > 0.05$ SHR compared with WKY. Values are mean ± SEM (n = 6).

the cultured endothelial cells was calculated from both anti-vWF and DiI-ac-LDL at the third passage. The purity of the endothelial cells ranged from 92-96% and averaged 93% in the total cell population from aortic tissues of 6 SHRs and 6 WKYs.

3. Endothelial Cell Secretion of PGI_2, Thromboxane A_2 and Endhtoehlin

The secretions of PGI_2, thromboxane A_2 and endothelin were measured from both SHR and WKY rat cultured endothelial cells by RIA. The results showed that the PGI_2 (729.6 ± 136.9 vs 471.2 ± 88.8 pg/10^5 cells) and endothelin (140.7 ± 20.2 vs 90.8 ± 14.6 pg/10^5 cells) secretion were higher in adult SHR than in WKY, but that there was no significant difference in thromboxane A_2 (18.6 ± 4.8 vs 16.1 ± 3.9 pg/10^5 cells) secretion between SHR and WKY (Fig. 4).

IV. Discussion

Endothelial cells have been successfully isolated from human pulmonary artery and vein (Johnson, 1980), varicose vein (Ryan and White, 1980), saphenous vein, capillaries, umbilical vein and from bovine and canine aorta (Buonassisi and Root, 1975; Cole *et al.*, 1986; Folkman *et al.*, 1979; Macerak *et al.*, 1979). The methods commonly used were enzyme digestion or mechanical scrubbing (Jaffe *et al.*, 1973; Macerak *et al.*, 1979; Pearson *et al.*, 1983). An attempt to isolate endothelial cells from the aorta of small animals such as rats with enzyme digestion or combined with mechanical scrubbing failed to achieve satisfactory results for *in vitro* endothelial culture. In the present study, light and scanning electron microscopic examination revealed that endothelial cells on the inner surface of rat aorta after enzyme digestion or with mechanical scrubbing still attached very well to the intima layer of the aorta. This finding indicates that rat aortic endothelial cells are resistant to enzyme digestion or mechanical scrubbing. Pease and Paule (1960), and Marrillees and Scot (1981) demonstrated that the subendothelial space of rat aortic intima was reduced, and that the endothelial cells attached directly to the irregular inner surface of the internal elastic lamina.

In our "modified Tissue Explant Technique," oxygen supply is essential for endothelial cell survival in processing the tissue block preparation. Without oxygenation into the medium, the final outgrowth rate of explant tissue blocks was very low. During the explantation period, there were two other factors which affected the efficiency of tissue block adhesion to the petri dish. When the specimens were covered with a minimal volume of medium and the petri dish was tilted to about 45°, there was about a six-fold increase in the efficiency of tissue block adhesion to the petri dish.

The timing for removal of tissue blocks from petri dishes was very important to minimize non-endothelial cell contamination. In our observation, smooth muscle contamination was largely prevented when the tissue blocks were removed from petri dishes at the time of cell outgrowth about 2 mm away from the tissue block.

The endothelial cells in culture showed a cobblestone pattern and could remain unaltered for five to seven passages in petri dishes without fibronectin precoating. Thereafter, the cells started to undergo disorganization and morphological alteration. These cells could be recovered by reculturing the cells in petri dishes precoated with fibronectin (data not shown). This indicates that the fibronectin matrix is essential for long-term maintenance of rat aortic endothelial cells in culture.

The von Willebrand factor has been widely accepted as a marker for identification of endothelial cells in culture although this factor can also be found in platelet, megakaryocyte and mast cells (Feder *et al.*, 1983; Zetter, 1981). DiI- Acetylated LDL uptake activity is another characteristic for identification of

endothelial cells (Pitas *et al.*, 1981; Voyta *et al.*, 1984). Therefore, the purity of cultured endothelial cells was examined using immunocytochemical assays for these two characteristics. According to our results, about 92-96% of the cultured cells showed positive response.

Local vascular tone is regulated by vasoconstrictors, endothelin and thromboxane A_2, and vasodilator PGI_2 secreted from endothelial cells (Yanagisawa *et al.*, 1988). In our cultured rat aortic endothelial cells, both endothelin and PGI_2 secretion were higher in 24 wk old SHR than in age matched WKY, but there was no significant difference in thromboxane A_2 secretion.

To the best of our knowledge, this study is the first to report on a successful endothelial cell culture with high yield and purity from spontaneously hypertensive rats. The morphology, marker identification and secretory functions appear to be similar to those observed *in vivo*. We believed that this technical improvement can potentially provide a tool for study of the role of endothelial cells in the development of hypertension.

Acknowledgments

Research grants were provided by the National Science Council, Republic of China (NSC 80-0412-B016-137 and NSC 81-0412-B016-003).

References

Booyse, F.M., Quarfoot, A.J., Bell, S., Fass, D.N., Lewis, J.C., Mann, K.G., *et al.* (1977) Cultured aortic endothelial cells from with von Willebrand disease: in vitro model for studying the molecular defect(s) of the disease. *Proc. Natl. Acad. Sci. USA*, **74**:5701-5706.

Buonassisi, V. and Root, M. (1975) Enzymatic degradation of heparin-related mucopolysaccharide from the surface of endothelial cell cultures. *Biochemica Biophysica Acta*, **385**:1-10.

Cole, O.F., Fan, T.-P.D., and Lewis, G.P. (1986) Isolation, characterization, growth and culture of endothelial cells from the rat aorta. *Cell Biol Int. Rep.*, **10**:399-405.

De Nucci, G., Thomas, R., D'Orleans-Juste, P., Antunes, E., Walder, C., Warner, T.D. (1988) Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. USA*, 9797-9800.

Diglio, C.A., Grammas, P., Giacomelli, F., and Wiener, J. (1982) Primary culture of rat cerebral microvascular endothelial cells. Isolation, growth and characterization. *Lab. Invest.*, **46**:554-563.

Feder, J., Marasa, J.C., and Olander, J.V. (1983) The formation of capillary-like tubes by calf aortic endothelial cells grown *in vitro*. *J. Cell Physiol.*, **116**:1-6.

Folkman, J., Marasa, J.C., and Olander, J.V. (1979) The formation of capillary-like tubes by calf aortic endothelial cells grown *in vitro*. *Proc. Natl. Acad. Sci. USA*, **76**:5217-5221.

Furchgott, R.F. and Zawazki, J.V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**:373-376.

Gimbrone, M.A. Jr., Cotran, R.S., and Folkman, J. (1974) Human vascular endothelial cells in culture: growth and DNA Synthesis. *J. Cell Biol.*, **60**:673-684.

Gross, J.L., Moscatelli, D., Jeffa, E.A., and Rifkin, D.B. (1982) Plasminogen activator and collagenase production by cultured capillary endothelial cells. *J. Cell Biol.*, **95**:974-981.

Hongo, K., Kassell, N.F., Nakagomi, T., Sasaki, T., Tsukahara, T., Orawa, H. (1988) Subarachnoid hemorrhage inhibition of endothelium-derived relaxing factor in rabbit basilar artery. *J. Neurosury.*, **69**:247-253.

Hormia, M., Lehto, V.P., and Virranem, I. (1984) Intracellular localization of factor VIII-related antigen and fibronectin in cultured human endothelial cells: evidence for divergent routes of intracellular translocation. *Eur. J. Cell Biol.*, **33**:217-220.

Jaffe, E.A., Nachman, R.L., Becker, C.G., and Minick, C.R. (1973) Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J. Clin. Invest.*, **52**:2745-2756.

Johnson, A.R. (1980) Human pulmonary endothelial cells in culture activities of cells from arteries and cells from veins. *J. Clin. Invest.*, **65**:841-850.

Konieckowski, M., Dunn, J.M., Stock, J.E., and Hassid, A. (1983) Glomerular synthesis of prostaglandins and thromboxane in spontaneously hypertensive rats. *Hypertension*, **5**:446-452.

Lever, A.F. (1986) Slow pressor mechanisms in hypertension. A role for hypertrophy of resistance vessels? *J. Hypertension*, **4**:515-524.

Lewis, L.J., Hoak, J.C., Maca, R.D., and Fry, G.L. (1973) Replication of human endothelial cells in culture. *Science*, **181**:1453-1454.

Luscher, T.F. and Vanhoutte, P.M. (1986) Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension*, **8**:344-348.

Macerak, E.J., Howard, B.V., and Kefalides, N.A. (1977) Properties of calf endothelial cells in culture. *Lab. Invest.*, **36**:62-71.

Maciag, T., Kadish, J., Williams, L., and Weinstein, R. (1982) The organizational behavior of human umbilical vein endothelial cells. *J. Cell Biol.*, **94**:511-520.

Mandri, J.A. and William, S.K. (1983) Capillary endothelial cell cultures: phenotypic modulation by matrix components. *J. Cell Biol.*, **97**:153-165.

McGuire, P.G. and Orkin, R.W. (1987) Methods in laboratory investigation. Isolation of rat aortic endothelial cells by primary explant techniques and their phenotypic modulation by define substrata. *Lab. Invest.*, **57**:94-105.

Menconi, M.J., Hahn, G., and Polgar, P. (1984) Prostaglandin synthesis by cells comprising the calf pulmonary artery. *J. Cell Physiol.*, **120**:163-168.

Merrilees, M.J. and Scott, L. (1981) Culture of rat and pig aortic endothelial cells: differences in their isolation, growth rate and glycosaminoglycan synthesis. *Atherosclerosis*, **38**:19-26.

Moncada, S. and Vane, J.R. (1979) Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2 and prostacyclin. *Pharmacol. Rev.*, **30**:293-331.

Moncada, S., Hermann, A.G., Higgs, E.A., and Vane, J.R. (1977) Differential formation of prostaglandin (PGX or PGI_2) by layers of arterial wall. An explantation for the antithrombotic properties of vascular endothelium. *Throm. Res.*, **11**:323-344.

Okamoto, K. and Aorki, K. (1963) Development of a strain of spontaneously hypertension rats. *Jpn. Circ. J.*, **27**:282-293.

Pearson, J.D., Slakey, L.L., and Gordon, J.L. (1983) Stimulation

of prostaglandin production through purinoreceptors on cultured procine endothelial cells. *Biochemical Journal*, **214**:273-276.

Pease, D.C. and Paule, W.J. (1960) Electron microscopy of elastic arteries-The thoracic aorta of the rat. *J. Ultrastr Res.*, **3**:469-483.

Pitas, R.E., Innerarity, T.L., Weinstein, J.N., and Mahley, R.W. (1981) Acetoacetylated lipoprotein used to distinguish fibroblasts from macrophage *in vitro* by fluorescence microscopy. *Arteriosclerosis*, **1**:177-185.

Ryan, U.S. and White, L.A. (1985) Varicose veins as a source of adult human endothelial cells. *Tissue Cell*, **17**:171-176.

Takeshita, A., Imaizumi, T., Ashihara, T., Yamamoto, K., Hoka, S., and Nakamura, M. (1982) Limited maximal vasodilator capacity of forearm resistance vessels in normotensive young men with a familial prediposition to hypertension. *Circ. Res.*, **50**:671-677.

Vanhoutte, P.M., Rubanyi, G.M., Miller, V.M., and Houston, D.S. (1986) Modulation of vascular smooth muscle contraction by the endothelium. *Ann. Rev. Physiol.*, **48**:307-320.

Vanhoutte, P.M. (1989) Endothelium and control of vascular function. State of the art lecture. *Hypertension*, **13**:658-677.

Voyta, J.C., Via, D.P., Butterfield, C.E., and Zetter, B.R. (1984) Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. *J. Cell Biol.*, **99**:2034-2040.

Wagner, D.D., Olmsted, J.B., and Marder, V.J. (1982) Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. *J. Cell Biol.*, **95**:355-361.

Weible, E.R., Palde, G.E. (1964) New cytoplasmic components in arterial endothelia. *J. Cell Biol.*, **23**:101-112.

Yamori, Y. (1991) Studies on spontaneous hypertension development from animal models toward man. *Clin. Exper. Hyper-Theroy and Practice*, **5**:631-644.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M.S., Mitsui, Y. *et al.* (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (London)*, **332**:441-415.

Zetter, B.R. (1981) The endothelial cells of large and small vessels. *Diabetes*, **30(suppl. 2)**:24-28.

以“組織塊種植法”體外培養自發性高血壓大白鼠主動脈內皮細胞之研究

趙壯飛 • 趙子健* • 呂美華 • 盧昭元 • 陳幸一**

國防醫學院生物及解剖學科暨三軍總醫院醫學研究部
*國防醫學院醫學科學研究所
**國防醫學院生理及生物物理學科暨慈濟醫學研究中心

摘　要

本實驗以“組織塊種植法”成功地由原發性高血壓大白鼠及正常血壓大白鼠之主動脈培養出高純度的內皮細胞。首先，將血管塊置於以纖維網蛋白處理過之培養皿上，然後再分別測試不同條件下對內皮細胞生長的影響，包括：處理血管時氧的供給、植種血管塊時培養皿的傾斜及血管塊移除的時間等，結果顯示這些條件顯然對細胞的生存、組織之貼附及減少非內皮細胞之污染等非常重要。培養之細胞再以形態上、免疫組織化學上及生化上之方法來定性時顯示這些細胞對von Willerbrand因子之抗體呈正反應，且可吞噬DiI-ac-LDL，可見其具內皮細胞細胞之特徵。同時，這些內皮細胞分泌前列腺素I_2、血栓素及內皮素之能力也被測試，結果顯示培養之自發性高血壓大白鼠內皮細胞分泌前列腺素I_2及內皮素之能力比正常血壓大白鼠之內皮細胞高，但其分泌血栓素之能力則相同。因此，以“組織塊種植法”可在體外成功地培養出具生物功能之高純度內皮細胞，利用這個方法可使我們更進一步的研究內皮細胞在高血壓形成過程中所扮演的角色。