行政院國家科學委員會專題研究計畫　期中進度報告

子計畫三:全生物性人工血管的動物模式研究(1/3)

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計畫主持人： 歐柏榮

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中文摘要

每年有超過十萬人以上的人需要進行血管移植手術，因此對於血管的需求極為殷切，目前手術利用自體血管移植，或是利用非生物性之人造血管進行移植，但效果均不理想。本研究的主要目的是利用動物模式評估全生物性人造血管取代自然血管的可能性。利用羊膜及具有生物分解性之聚合物作為基質，並在此基質上培養平滑肌肉細胞及內皮細胞，以組成一個全生物性的人造血管，將此人造血管移植入動物的體內取代原自然血管。全生物性的人造血管必須是有生物互容性、生物可分解性，並有天然血管所具有的特性如不引起血栓，具有良好的彈性及不受寄主排斥等特性。本年度本子計畫主要的目的為準備豬羊膜作為內皮胞及平滑肌細胞的培養基質，此外，將豬心主動脈之內皮細胞及平滑肌細胞分離出，以提供體外培養全生物性的人造血管。豬羊膜由分娩之母豬取得，經以 PBS 緩衝溶液清洗後，切成五公分見方，置於 50%的甘油儲存。豬內皮細胞及平滑肌細胞由豬心主動脈分離並培養於 M199 培養基中，以第八因子及 M22 基因表現偵測所得之內皮細胞及平滑肌細胞，結果顯示分離此二種細胞可得極純之細胞。

關鍵詞：生物性、人造血管、羊膜

（一） 英文摘要。（五百字以內）

Each year, more than 100,000 vascular bypass graft procedures are performed, creating an important demand for vascular grafts. Currently, autogenous saphenous veins, synthetic grafts or acellular human umbilical veins are used as grafting conduit. However, the results were far from satisfactory. The objective of this study is to evaluate the potency of biological blood vessel equivalent in animal model. Porcine smooth muscle cells and endothelial cells will be seeded on biodegradable polymer and AM base matrix. The objectives of this year were to prepare the amniotic membranes (AM) from porcine and isolate the endothelium cell and smooth muscle cells from aorta. The AM were obtained from full-term pregnant female porcine. AM were collected and washed with PBS. And then stored in DMEM containing 50% glycerol at -80℃. Endothelium cell and smooth muscle cells were isolated from porcine aorta. These cells were cultured in M199 medium. Factor VIII and SM2$2\alpha$ genes were used to identify endothelium and smooth muscle cells respectively.

Keywords: Biological, artificial blood vessel, amniotic membranes

前言

Each year, more than 100,000 vascular bypass graft procedures are performed, creating an important demand for vascular grafts (Williams, 2000). Currently, autogenous saphenous veins, synthetic grafts or acellular human umbilical veins are used as grafting conduit. However, the results were far from satisfactory. In the future, tissue engineered arteries may serve as an alternate source of vascular grafts for the patients. In last two decades, there are several artificial blood vessel under investigate such as (1) synthetic grafts (2) Matrix template or acceluar tubes of ectracellular matrix (3) tissue-engineered blood vessels (4) artificial arteries derived from peritoneal granulation tissue in body "bioreactor" (Thomas et al., 2003). The successful artificial blood vessel must meet following requirements. (1) It has to be biocompatible and biodegradable. (2) It has to be leak-proof and thromboresistant, but with adequate porosity for healing and angiogenesis. (3) It has to have appropriate mechanical properties such as strength, kink resistance, and compliance. (4) It has to possess appropriate vasoactive physiological properties such as contrict or relax (Anita et al., 2003).

Porcine are popular for experimental study, as they have similar anatomy and physiology to that of humans (Rashid et al., 2004). Porcine models have been used to assess the durability and functionality of tissue engineering conduits. Niklason and co-worker developed vascular grafts using biodegradable polyglycolic acid (PGA) scaffolds chemically modified by sodium hydroxide. Onto this were seeded bovine SMCs and ECs before these constructs were pulsed in a flow circuit within bioreactors for 8 weeks. On implantation in a porcine model these grafts showed patency after 24 days by angiography (Niklason et al., 1999).

研究目的

The objective of this study was to evaluate the patency of biological blood vessel equivalent in animal model. Porcine smooth muscle cells and endothelial cells were seeded on biodegradable polymer and amniotic membrane base matrix. The conduits will be implanted into animal to assess the practical usage in vivo.

Spicific Aim

The specific aim of first year is to prepare porcine AM (amniotic membrane), endothelium and smooth muscle cells for the construction of blood vessel equivalents.

研究方法

Material and methods

1. Animals

Porcine was used in this study to provide AM, EC and SMC. Animal care and use were performed in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

2. Preparation of Amniotic membranes

The amniotic membranes were obtained from full-term pregnant female porcine. After cesarean section, AM were collected and washed with PBS containing antibiotics (100U/ml penicillin and 100ug /ml streptomycin). The AM, a thin sheet consisting of the epithelium, basement membrane and some underlying compact stroma, were separated from the bulk of the

tissue and stored in DMEM containing 50% glycerol at -80℃.

3. Preparation of endothelium and smooth muscle cells from Aorta

Endothelial cells were isolated according to the procedureof Sharma and Davis with modification. Freshsegments of thoracic aorta from 7-month-old pig was collected. A thin layer was gently scraped from each arteriallumen by sterile surgical blades and transferred into a sterile flask to incubate with 1 mg/ml collagenase (typeII) at 37 °C for 10 min to release the endothelial cells. The mixture was centrifuged and the supernatant discarded. The pellets were washed twice in sterile basal medium with additional antibiotics and plated in sterile tissue culture dishes with culture medium (basal medium supplemented with 10% FBS) in a humidified atmosphere of5% CO2 and 95% air at 37°C. The primary culture of PAECs was passed and maintained routinely in culture medium at 37°C and the culture medium was changed every 2–3 days until the cells reached confluence. PAECs were identified by a Dil-AcLDL uptake assay and subsequently examined by fluorescent microscopy. Microscopic examination was used to observe cell morphologyand monitor cell growth. The 10th through 12th passages of PAEC primary cultures were used for construction of artificial blood vessel..

After removing endothelium cells, trypsin was added into the thoracic aorta matrix and incubated for 5 5- 10 mins. A thin layer of matrix membrane was gently scraped from arterial lumen by sterile surgical blades. The artery was dissected into segments of approximately 0.5cm^2. The segments were placed intimal side down in Petri dishes and maintained in CO_2 incubator for 5 to 10 days. The smooth muscle cells migrated off the segments and become established in two-dimentional culture.

結果與討論

1. **Preparation of AM**

AM was prepared from full-term pregnant female porcine. Before using, the epithelium cells have to be removed. To prevent the contamination, AM was sterilized by UV illumination. Endothelium cells and smooth muscle cells were grown on AM firmly. This result indicates that AM is a good matrix for endothelium and smooth muscle cells.

2. **Isolation of endothelium and smooth muscle**

Endothelial cells were isolated from porcine thoracic aorta and the primary culture of PAECs was used as experimental material in this study (Figure 1). Results from the uptakeof Dil-AcLDL showed that PAECs were brilliantly fluorescent, while the fluorescent intensities of other celltypes (rat L8 myoblasts and porcine smooth muscle cells) were near background level (data not shown). In addition, expression of factor VIII was examined by RT-PCR. The result showed that factor VIII expressed in PAECs (Figure 2).

Smooth muscle cells were isolated from aorta and smooth muscle specific marker, SM22α, was used to identify the cells. The morphology of smooth muscle cells is different from PAECs (Figure 3). Expression of SM22α was detected in these cells (Figure 4).

結論與建議

It is concluded that AM is a good matrix base membrane for the culture of endothelium and smooth muscle cells.

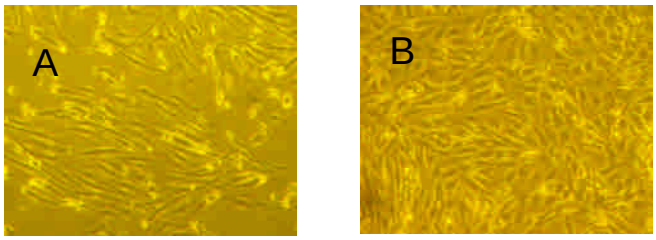

Figure 1. PAEC isolated from porcine.
(A): 8th passage; (B): 19th passage

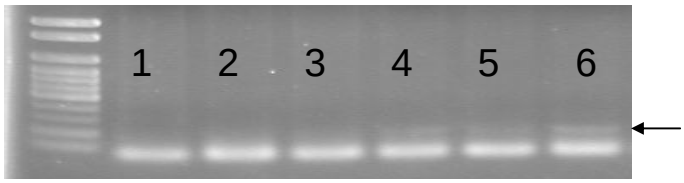

Figure 2. Expression of Factor VIII in PAECs.

Lane 1, 2, 3: smooth muscle; Lane 4, 5, 6:PAEC

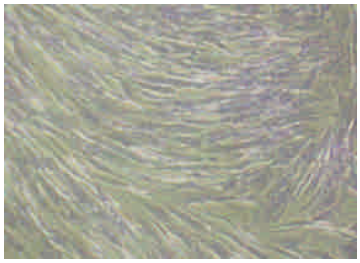

Figure 3. Smooth muscle cells isolated from porcine.

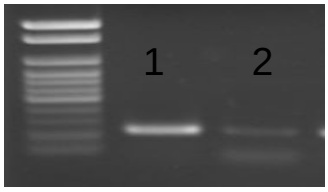

Figure 4. Expression of SM22 α in smooth muscle cells. Lane 1:Smooth muscle cells; Lane 2: PAEC

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計畫成果自評

The results from first year of this projective were promising. In the next year, we should be able to construct the biological artificial blood vessel.