

A prototype tissue engineered blood vessel using amniotic membrane as scaffold

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ABSTRACT

In this study, we used amniotic membrane (AM), a natural extracellular matrix, as a scaffold for the fabrication of tissue engineered blood vessels (TEBVs). The inner surface of the denuded glutaraldehyde cross-linked AM tube was endothelialized with porcine vascular endothelial cells (ECs) and subjected to a physiological (12 dyne cm^{-2}) shear stress (SS) for 2 and 4 days. The results showed that after applying SS, an intact EC monolayer was maintained in the lumen surface of the TEBV. The ECs were aligned with their long axis parallel to the blood flow. The immunofluorescent microscopy showed that the intercellular junctional proteins, PECAM-1 and VE-cadherin, were surrounding the EC periphery and were better developed and more abundant in SS-treated TEBVs than the static controls. The Western blot indicated that the expressions of PECAM-1 and VE-cadherin were increased by $72 \pm 9\%$ and $67 \pm 7\%$, respectively, after shear stress treatment. The distribution pattern of integrin $\beta 1$ was mainly at the interface of ECs and AM in static TEBVs but it was extended to the cell–cell junctions after SS treatment. The SS promoted the expression of integrin $\alpha_v\beta_3$ without altering its distribution in TEBV. The results suggest that glutaraldehyde cross-linked AM tube can potentially be used as a scaffold biomaterial for TEBV fabrication. Most importantly, the use of an AM tube shortened the TEBV fabrication.

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1. Introduction

The landmark report by Weinberg and Bell in 1986 [1] opened the door for the development of cardiovascular tissue engineering. Over the last three decades, numerous prototypes of tissue engineered blood vessels (TEBVs) using living vascular cells and natural matrix molecules have been reported [2–7]; however, the production of a TEBV without synthetic scaffolds yet with sufficient mechanical strength remains a challenge. Recently, a TEBV using a new method, termed sheet-based tissue engineering, has been developed [8,9]. This method utilized matrix sheet laid down by fibroblasts that are cultured in conditions promoting matrix synthesis. The matrix sheets comprised living fibroblasts and well organized natural matrix proteins. They showed that the cohesive matrix could be detached from the culture dish and layered into three-dimensional tissues or organs with a mechanical strength significantly higher than normal physiological loads [9]. Although the sheet-based TEBV marks a significant advancement in TEBV development, displaying adequate mechanical strength without

relying upon synthetic scaffolds, harvesting cells and growing new vessels are time-consuming processes that limit a wide clinical application of sheet-based TEBVs [10].

Amniotic membrane (AM), a natural extracellular matrix sheet, is the innermost layer of the placental membrane. It consists of a single layer of epithelium, a basement membrane and an avascular stroma. The AM basement membrane has been shown to contain type IV and type VII collagen, fibronectin, and laminins 1 and 5 [11], and to be an ideal substrate for supporting the growth of epithelial cells. Moreover, the laminins have been shown to be one of the basement membrane components that are important in facilitating epithelial adhesion [12]. De-epithelialized AM showed no immunoreactivity and its long-term patency in ocular surface transplantation has been demonstrated in many clinical reports [13–15].

We recently showed that porcine vascular endothelial cells (ECs) cultured on the AM exhibit a reduced expression of E-selectin and P-selectin compared to those cultured on the plastic surface, and the leukocyte adhesion to the AM-based EC surface is also correspondingly reduced [16]. In addition, the AM-based ECs express higher VE-cadherin and integrin, suggesting a better substrate attachment and stronger EC intercellular adhesion than plastic cultures [16]. These results suggest that AM can be an ideal natural matrix for the attachment of vascular ECs. In the present study, we explored the possible use of glutaraldehyde cross-linked AM

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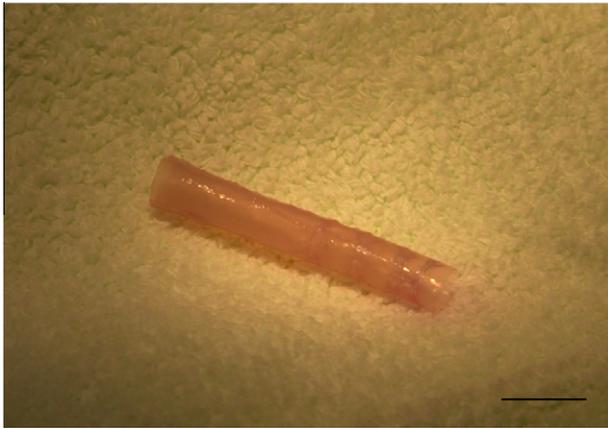


Fig. 1. A glutaraldehyde cross-linked AM tube, 15 mm long with inner-diameter 3 mm. Scale bar = 5 mm.

as a scaffold for the fabrication of TEBV. The inner surface of the glutaraldehyde cross-linked AM tubes was endothelialized with porcine vascular ECs. The EC layer was subjected to a physiological (12 dyne cm^{-2}) or sub-physiological shear stress for various lengths of time and the biological properties for cell adhesion/junction were characterized. The results suggested that the glutaraldehyde cross-linked AM tube can be used as a scaffold for TEBV and shortened the TEBV fabrication time.

2. Materials and methods

2.1. Preparation of AM and scaffold fabrication

Human AM was obtained from the Department of Obstetrics and Gynecology, Veterans General Hospital (Taichung, Taiwan) with proper informed consent, according to the Declaration of Helsinki. The AM was aseptically washed three times in phosphate buffered saline (PBS) containing $50 \mu\text{g ml}^{-1}$ penicillin, $50 \mu\text{g ml}^{-1}$ streptomycin, $2.5 \mu\text{g ml}^{-1}$ amphotericin B, and 25 ng ml^{-1} gentamycin (all from Invitrogen) and then incubated in 0.25% trypsin (Invitrogen) for 20 min at 37°C to loosen up the epithelial layer. The epithelial cells were then removed by gentle scraping with a cell scraper. The denuded AM was rinsed with sterile milli-Q H_2O twice

and trimmed into a proper size. The AM was rolled around a Teflon mandrel (diameter 0.3 cm) for 10–15 rounds and then air dried at room temperature under sterile conditions. The dried AM tube was treated with 0.1% glutaraldehyde (in 0.01 N acetic acid, 4°C) for 24 h to induce matrix cross-linking. The cross-linked AM tube was submerged in the glycine solution (0.1 M) for 30 min to quench the excessive aldehyde. The tube was then rinsed several times with sterile double distilled water and stored at 4°C for further use (Fig. 1). To test whether these TEBVs are capable of holding pressure within the physiological and pathophysiological range, the TEBVs were cannulated and pressurized with PBS in a series of pilot studies. The luminal pressure was increased in a step-wise manner to the maximum operating pressure of 300 mmHg allowed by the pressure transducer (P23XL, Statham, USA). We found that none of these vessels burst out under this condition. It appears that the AM-based TEBVs are capable of maintaining their integrity at the highest pressure tested, i.e., 300 mmHg, which is much higher than the arterial pressure normally encountered by native vessels.

2.2. Cell culture

Porcine aortas were obtained from a local slaughter house and placed in the ice-cold PBS containing $150 \mu\text{g ml}^{-1}$ penicillin and $150 \mu\text{g ml}^{-1}$ streptomycin. Endothelial cells were isolated as previously described [16]. Briefly, the aorta was cut open longitudinally with scissors and the inner surface was treated with 0.25% trypsin for 10 min at room temperature. The ECs were scraped off with a scalpel blade into a medium and then harvested following low speed centrifugation. The cells were resuspended in M199 containing 10% fetal bovine serum (FBS) (HyClone) and the aforementioned antibiotics. Cells were cultured under 5% $\text{CO}_2/95\%$ air at 37°C in a T25 flask and then split in a 1 to 3 ratio upon confluence. Primary cultures were designated as passage 0 and cells were used for experiments at passages 2 and 3.

2.3. Shear stress test of TEBVs

The lumen surface of the cross-linked AM tube was seeded with ECs at $2 \times 10^5 \text{ cm}^{-2}$, and was cultured for 2 days to form a stabilized monolayer (TEBV). The TEBV was then subjected to a shear stress test in a bioreactor system [17]. The system consisted of a

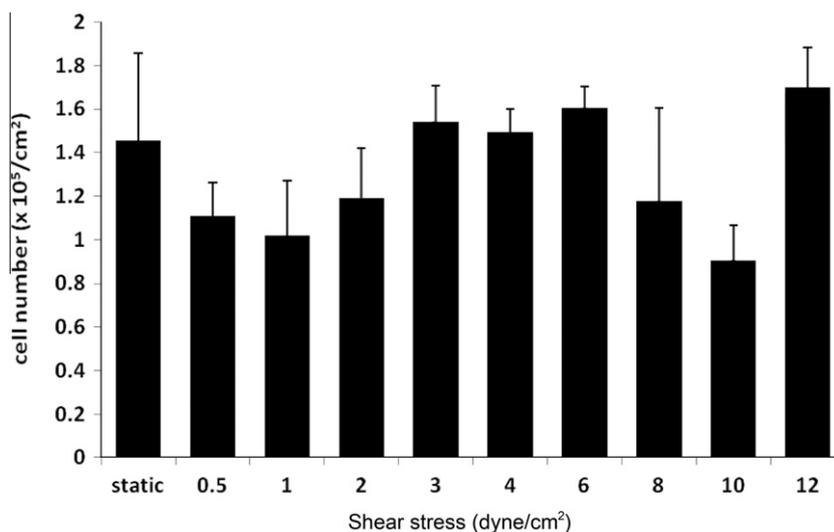


Fig. 2. The TEBV with or without shear stress treatment for 4 days. The cell density of the lumen endothelium was calculated and expressed as cell number cm^{-2} . The cell density remained unchanged among various levels of shear stress exposure. $n = 3$.

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