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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 ± 9% and 67 ± 7%, respectively, after shear stress treatment. The distribution pattern of integrin β1 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.

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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