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Novel tissue-engineered vascular patches based on decellularized canine aortas and their recellularization in vitro

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ABSTRACT

Decellularized allo- or xenogeneic vascular grafts have been found to give more promising results than some biodegradable synthetic polymers. However, owing to absence of well-organized cells, especially confluent endothelial cells, their long-term patency is limited. Seeding vessel-originated cells on these grafts may overcome the deficiency. In this study, canine aortas were decellularized and cross-linked. 4′,6-Diamidino-2-phenylindole (DAPI) and Masson' trichrome staining showed complete removal of cell debris, while structure integrity of extracellular matrix (ECM) was remained. Human umbilical vein endothelial cells (HUVECs) and human umbilical artery smooth muscle cells (HUASMCs) were seeded on these decellularized aorta patches in three manners, ECs alone (EC/O), SMCs alone (SMC/O) and ECs on SMCs layer (EC/SMC). In EC/O and SMC/O, scanning electron microscopy (SEM) examination indicated both cells could form confluent layers on the decellularized patches when seeded at high density, but their morphology and alignment changed with seeding density. In EC/SMC, ECs could grow well on SMCs layer, but their morphology, alignment, and confluence degree were deeply influenced by the density of SMCs beneath.

1. Introduction

Decellularized allo- or xenogeneic vascular grafts have reached more promising results than various biodegradable synthetic polymers [1], while their structure integrity and vasoactivity are inferior to native vessels because of the absence of well-organized cells, which subsequently threatens their long-term patency [2,3].

It is generally accepted that an integral endothelium is effective in preventing thrombi formation on the surface of a tissue-engineered vascular graft (TEVG) [4,5]. We hypothesize that smooth muscle cells, another essential cell component in natural blood vessels, should play important roles in maintaining the long-term function of a TEVG too. Numerous co-culture experiments of SMCs and ECs have shown that SMCs could not only affect the morphology of ECs, but also their gene expression of growth factors, adhesion molecules and so on [6,7]. In addition, preseeding of SMCs on an ePTFE graft was found to improve retention rate of ECs under shear stress [8]. However, in the case of a decellularized graft, co-culture of SMCs and ECs was rare, and the reported results were fairly superficial. In this study, not only growth and morphology of SMCs and ECs on the decellularized canine aorta patches, but also ECs on SMCs were investigated in detail.

2. Experiments

2.1. Decellularization of canine aorta

Canines were anaesthetized with pentobarbital intraperitoneally, and then their aortas were excised. After removal of residual blood and adherent adipose tissues, these aortas were cut into 1 cm × 1 cm pieces and soon dipped in ion-free water for 24 h to disrupt the cells, followed by sonication to help cleaning the cell debris. Finally, 4% ethylene glycol diglycidyl ether (EX-810) was used to cross-link the extracellular matrix (ECM). Decellularizing efficiency was detected by 4′,6-diamidino-2-phenylindole (DAPI) and Masson's trichrome staining.

2.2. Cell isolation and culture

Human umbilical vein endothelial cells (HUVECs) were digested from intima of umbilical vein with collagenase II and cultured in M199 medium containing 15% fetal bovine serum,

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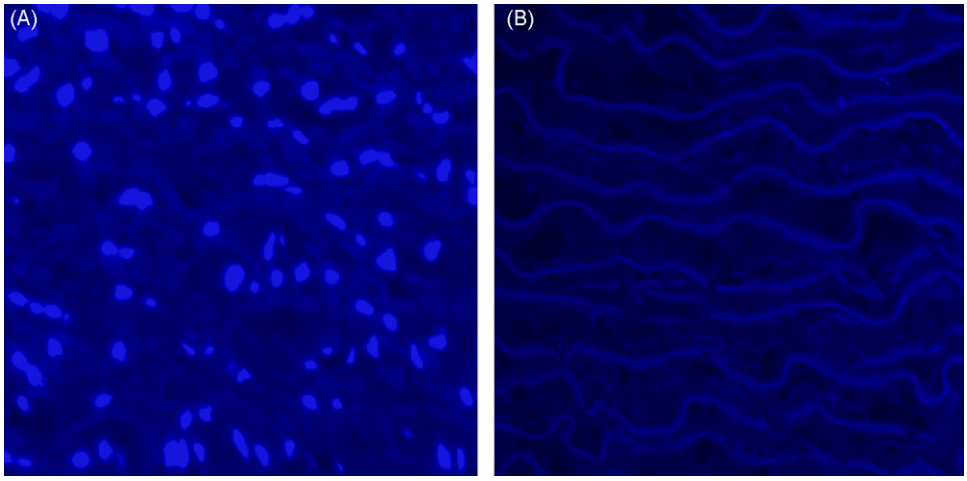

Fig. 1. DAPI staining micrographs for histological section of canine aorta patches by fluorescence microscope: (A) before and (B) after decellularization (200×).

100 μg/ml heparin and 20 ng/ml VEGF. Human umbilical artery smooth muscle cells (HUASMCs) were obtained by outgrowth of cells from discarded pieces of umbilical artery media, and cultured in DMEM/F12 medium with 10% fetal bovine serum.

2.3. Reseeding procedure

In EC/O or SMC/O, cells were seeded on the decellularized aorta patches at low (5×10^2 cells/cm^2) and high density (5×10^4 cells/cm^2). In EC/SMC, SMCs were seeded at low and high density for 4 days, then high-density ECs were seeded upon the SMC layer. Growth and morphology of both ECs and SMCs were detected by scanning electron microscopy (SEM).

3. Results and discussion

Regarding an allo- or xenogeneic graft, complete removal of cell components is the premises for its in vivo implantation [9]. In this study, canine aortas were decellularized and cross-linked. DAPI staining showed absolute removal of double-chained DNA (Fig. 1). Masson' trichrome staining illustrated complete removal of muscular cell component (red-stained), while collagen component (blue-stained) and ECM integrity were maintained (Fig. 2). The above results indicated that our decellularization technique can effectively reduce the immunogenicity of the canine aorta without visible damage to ECM integrity.

In EC/O, we found that ECs showed polygonal morphology when seeded at low density, whereas cobble-like morphology at high density (Fig. 3). In SMC/O, SMCs presented the same density-dependent morphology change. When seeded at low density, SMCs displayed a polygonal appearance; but a shuttle-like appearance at high-seeding density (Fig. 4). Cell density can influence cell morphology, which has been widely verified. These morphology changes were due to regulation of actins gene expression by cell density [10,11].

Cell alignment is worth mentioning in our study, because just SMCs but not ECs displayed regular alignment with main axes parallel to that of decellularized canine aortas (Fig. 4). On the inner surface of the decellularized canine aorta, some peak/valley structure was easily identified, which seemed like artificial microgrooves. Numerous researches have demonstrated the surface topography could influence the shape and alignment of the cells growing upon [12]. Microgrooves etched for researches on SMCs and ECs' response to surface topography were usually different in scale. For example, microgrooves for SMCs could be 15–70 μm, but only few micrometers for ECs instead [13,14]. The theoretical basis on which to choose the size of microgrooves was not mentioned. Maybe a certain kind of cells has alignment response only to microgrooves with special dimension extent. For a

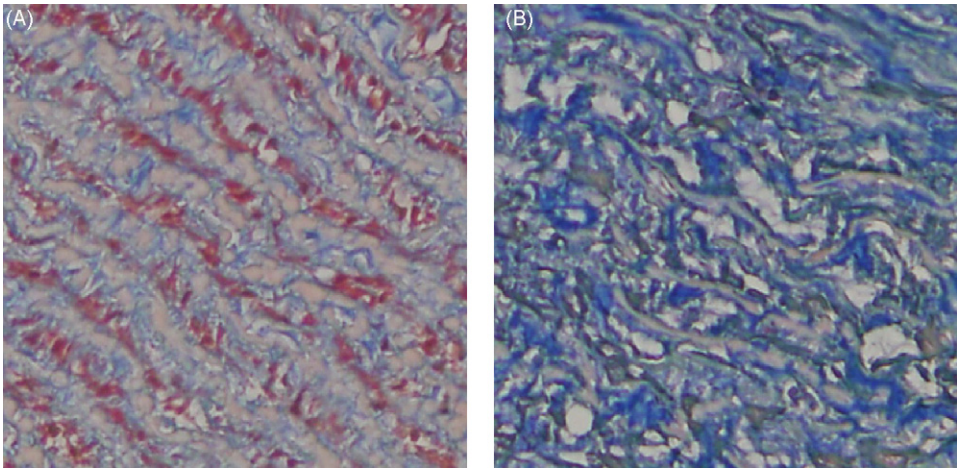

Fig. 2. Masson's trichrome staining micrographs for histological section of canine aorta patches: (A) before and (B) after decellularization (200×).