Growth Factor-encapsulated and Cell-laden Nanofibrous Bilayer Scaffolds for Vascular Regeneration

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Introduction: Tissue loss or dysfunction caused by trauma or disease is a major medical problem. Tissue engineering offers promising means for human body tissue repair. Significant advances have been made for regenerating simple body tissues. But the regeneration of complex tissues is difficult. For regenerating complex tissues such as blood vessels, 3D cell-scaffold constructs are desirable [Pedde RD, *et al., Adv Mater*, 2017, 29:1-27.]. Furthermore, growth factors (GFs) can promote tissue regeneration. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) enhance the formation and maturation of blood vessels. Electrospun nanofibrous scaffolds mimic natural extracellular matrix and have many advantages for tissue regeneration. GFs can be encapsulated in nanofibrous scaffolds through emulsion electrospinning (E-ES) [Wang C., *et al., Adv Mater Res*, 2012, 410:118-121.]. Coaxial electrospray (ESpray) produces core-shell structured microspheres for cell delivery [Zhou Y., *et al., Proc. 10th WBC*, Montreal, Canada, 2016]. In this study, GF-encapsulated and cell-laden bilayer scaffolds were fabricated by using a novel concurrent E-ES and ESpray technique. They were subsequently assessed.

Methods: Biopolymers PLGA50/50 and PLGA75/25 were used for making the bottom layer and the top layer, respectively, of nanofibrous bilayer scaffolds. VEGF was encapsulated in PLGA50/50 fibers and PDGF was encapsulated in PLGA75/25 fibers. Human vein umbilical endothelial cells (HUVECs) and human aortic smooth muscle cells (HASMCs) were encapsulated in sodium alginate (SA) microspheres which were lightly crosslinked by a $CaCl_2$ solution. GF-encapsulated and cell-laden nanofibrous bilayer scaffolds were made via concurrent E-ES and ESpray. VEGF-encapsulated PLGA50/50 fibers with HUVECs-containing microspheres and PDGF-encapsulated PLGA75/25 fibers with HASMCs-containing microspheres formed the bottom and top layers of bilayer scaffolds. Scaffolds without or with cells were subsequently studied using various techniques. *In vitro* cell release could be achieved by a weak sodium citrate solution (0.055M). Distributions of released HUVECs and HASMCs in bilayer scaffolds were evaluated using confocal laser scanning microscopy.

Results: By using a rotating drum collector, bilayer scaffolds consisting of aligned fibers were made (Fig.1). Fibers in the bottom layer were perpendicular to fibers in the top layer (Fig.1b). The bilayer structure of scaffolds mimics the ECM structures of some body tissues such as blood vessels. VEGF-encapsulated PLGA50/50 fibers and PDGF-encapsulated PLGA75/25 fibers exhibited mostly continuous core-shell structures under TEM. The encapsulation efficiency of VEGF and PDGF in E-ES fibers was 57.3±5.0% and 51.8±2.4%, respectively. During *in vitro* release tests, in the first 24 hours, slightly quick release of VEGF and PDGF was observed (Fig.2a). Afterwards, the released amounts of VEGF and PDGF were nearly the same every day for the remaining test duration (3 weeks), exhibiting the sustained release behavior. The VEGF release was faster than PDGF release due to the different degradation rates of PLGA50/50 and PLGA75/25 polymers. The sequential release of VEGF (firstly) and PDGF (secondly) could assist the regeneration of blood vessels. After *in vitro* release of HUVECs and HASMCs in bilayer scaffolds, their viability and distributions were studied. The viability of cells, as tested by the live/dead assay after staining, were high, indicating the materials and process for constructing the GF-encapsulated and cell-laden bilayer scaffolds with aligned nanofibers were suitable for HUVECs and HASMCs. For studying distributions of HUVECs and HASMCs in bilayer scaffolds, HUVECs were stained by calceim AM (green color) and HASMCs were stained by cell tracker orange (red color). The distributions of the two types of cells in a bilayer scaffold (cross-sectional view) are shown in Fig.2b. Distinctive layers with live cells were observed under CLSM for the bilayer scaffolds. HUVECs were basically located in the bottom layer and HASMCs were located in the top layer of the scaffolds. Only a few HASMCs were seen to migrate to the bottom layer. Locally delivered VEGF and PDGF in respective layers promoted cell growth and proliferation of HUVECs and HASMCs. Fiber alignment in respective layers also had effects on cells.

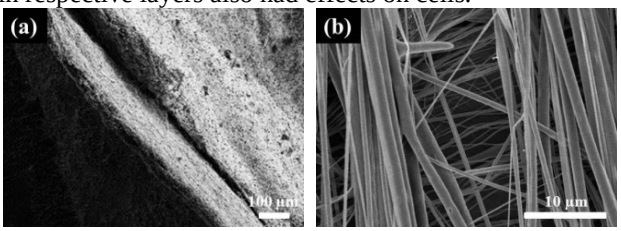

Fig. 1. Bilayer scaffolds: (a) General view of a bilayer scaffold, (b) Architecture of the aligned-fiber scaffold

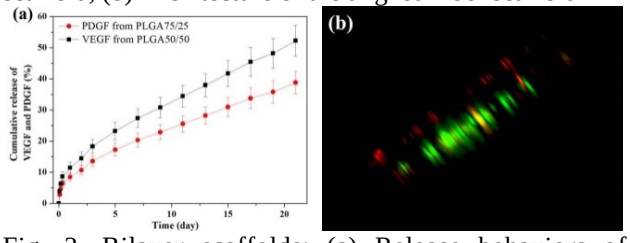

Fig. 2. Bilayer scaffolds: (a) Release behaviors of VEGF and PDGF, (b) CLSM image of cells in a bilayer cell-laden scaffold (cross-sectional view)

Conclusions: Complex GF-encapsulated and cell-laden bilayer scaffolds with aligned nanofibers could be successfully made. The two types of cells in scaffolds had high viability and the two types of encapsulated GFs exhibited sustained release. Released GFs in respective layers stimulated proliferation of respective type of cells. Aligned fibers in scaffolds provided contact guidance.