In vitro Biocompatibility of a Novel Composite Vascular Scaffold based on Protein/Poliglecaprone/Polycaprolactone Using Primary Human Aortic Endothelial Cells

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Statement of Purpose: Our lab has successfully fabricated a hybrid vascular scaffold composed of non-woven micro/nano fibers by co-electrospinning proteins gelatin B (G), and elastin (E) with polycaprolactone (PCL) and poliglecaprone (PGC). Mechanical characterizations demonstrate that this novel scaffold possesses comparable tensile properties to those associated with native arteries (1-2 MPa). Therefore, in this study, we proceed to evaluate the biocompatibility of the scaffold by growing primary human aortic endothelial cells (HAECs) on its luminal surface for up to 72 h. HAECs' adhesion and morphology were characterized by fluorescent staining.

Methods: A tubular scaffold was fabricated by electrospinning 0.3 mL solution of G (Sigma-Aldrich, St Louis, MO), E (Elastin Products Co., Inc., Owensville, MO), PCL (Absorbable Polymers, Birmingham, AL) and PGC (Advanced Inventory Management, Mokena, IL) (mass ratio = 1:2:1:3) dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) (Sigma-Aldrich, St Louis, MO), with a feeding rate of 3 mL/h, onto a stainless steel mandrel rotating at 400 rpm, which was distanced at 30 cm from the needle tip in the presence of a 30 kV voltage. Then each scaffold (1.1 cm^2) was installed on a cell crown, sterilized in 70% ethanol and coated overnight by fetal bovine serum (FBS) at 37 ºC in a 24-well plate. 5×10^5 HAECs (Lonza, Walkersville, MD) were added to each well on the luminal surface of the scaffold and subsequently incubated for up to 72 h at 37°C in 5% CO_2 atmosphere. Fluorescent staining, including phalloidin, 4',6-diamidino-2-phenylindole (DAPI) and ZO-1, was employed to characterize the morphology and formation of tight junctions of HAECs.

Results:

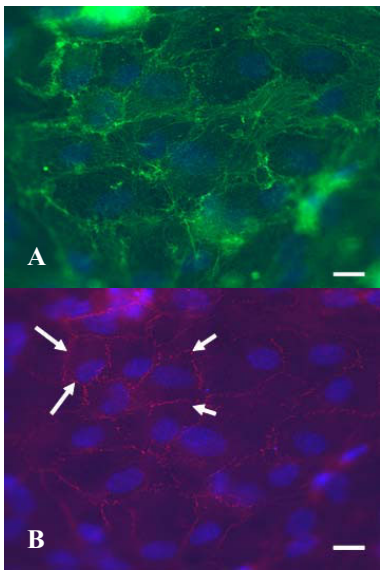

Fig. 1. Fluorescent microscopic images of , cells cultured on FBS-coated scaffolds for 72 h. (A) phalloidin (alexa 488-green) and DAPI (blue) staining illustrate the cellular skeleton and nucleus; (B) ZO-1(alexa 568-red) and DAPI (blue) staining demonstrate the formation of tight junctions and nucleus. Images were captured in the same region and merged. Scar bars are 50 μm. White arrows indicate tight junctions.

To help facilitate cell adhesion, scaffolds were pre-coated with fetal bovine serum (FBS), which deposits cell adhesion molecules such as fibronectin and vitronectin. Of note, this type of coating mimics what happens in the body when a vascular graft becomes exposed to blood. FBS-coated scaffolds exhibited superior biocompatibility, yielding 100% coverage with HAECs, over uncoated scaffolds (data now shown). HAECs successfully adhered to the scaffold and assumed their normal morphology within 24 h upon seeding. Fig. 1 shows that, in the last two days, HAECs underwent a cascade of biological events, like migration, leading to the establishment of a monolayer-like cell coverage. Positive ZO-1 staining reveals the prevalent formation of tight junctions between adjacent HAECs. Subsequent study found that a uniform monolayer of HAECs could be possibly engineered on the scaffold by optimizing the FBS density and the concentration of HAECs.

Conclusions: By adsorbing ligands onto the surface of scaffolds, cell adhesion was tremendously enhanced, leading to a complete and uniform coverage by HAECs within 24 h. The spread morphology of individual cells illustrates that the surface chemistry as well as the scaffold mechanics are favorable for biological activities of HAECs, such as the migration, proliferation and intercellular communications, etc. In addition, the viability and morphology of HAECs at 72 h reveal that the scaffolding environment is beneficial to engineer neo-tissues. Particularly, the formation of tight junctions promises the normal function of the engineered endothelium, like preventing blood fluid penetration through the wall of the tubular scaffold. These preliminary results uncover the great potential of the scaffold to induce the formation of a functional monolayer of endothelial cells.

In the future, biological and mechanical characterizations of the HAECs-scaffolds construct, including proliferation, apoptosis, anti-thrombogenesis, and capacity of withstanding shear stress will be performed.

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