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Chondroitin sulfate-oriented epidermal growth factor (EGF) coating for random and aligned electrospun vascular grafts

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Introduction: Primary requirements for functional synthetic small-diameter vascular grafts (SDVG) or tissue engineered blood vessels are hemocompatibility and favorable compliance [1]. Particularly critical is the formation of a continuous, stable monolayer of endothelial cells (HUVEC) on the lumen under physiological shear stress. On the other hand, the media layer of native vessels contains vascular smooth muscle cells (VSMC) and circumferentially-aligned collagen fibres, which are essential for contraction, dilation and blood pressure control [1]. In this research, we fabricated random (R) and aligned (A) electrospun PET mats as scaffolds for luminal and media layers, respectively. Electrospinning enables one to enhance mechanical compliance and to mimic the morphology of tissues, but cell behaviour (e.g. adhesion, growth, survival and retention) remains a problem. The objective of this work has been to investigate electrospun mats with coatings that improve cell behaviour, said coatings being based on primary amine-rich plasma polymers (PP) and chondroitin sulfate (CS), with or without tethered epidermal growth factor (EGF). On the lumen, CS was immobilized on (R) or (A) mats, and its effect on HUVEC adhesion and growth under flow was studied. For the media layer, a CS-EGF coating was created on (A) scaffolds presenting higher porosity, and then tested for increased cell infiltration into the pores.

Materials and Methods: (R) and (A) mats were prepared by electrospinning on a rotating mandrel. Parameters were adjusted to fine-tune the mats' morphology for HUVEC and VSMC culture, respectively [2]. The substrates were then coated with a thin amine-rich plasma-polymer layer in a low-pressure (LP) plasma reactor [3]. CS was covalently attached via carbodiimide chemistry, following which EGF was tethered via coiled-coil peptide interactions [4],[5]. The mats were characterized by SEM, porosimetry and XPS measurements; EGF surface density and bioactivity was confirmed by ELISA. The metabolic activity of HUVEC and VSMC on (R) and (A) mats was assayed at different culture times. Cell morphology, infiltration (VSMC) and distribution on the mats were tracked using immunofluorescent staining, followed by Confocal Microscopy (CM) and SEM. EC resistance to shear was also studied in a parallel-flow chamber.

Results and Discussion: SEM images confirm that (R) and (A) were highly porous, with optimized porosity and pore parameters (Fig. 1). Significant increase of HUVEC metabolic activity was observed on bioactive coatings compared with bare mat, LP+CS being an excellent pre-treatment to promote HUVEC metabolic activity (Fig. 2). These cells formed a confluent monolayer (Fig. 1), and their retention was greatly improved on both LP- and LP+CS-coated mats, both (R) and (A), results being better for (A). For the media layer, ELISA confirmed that EGF was successfully grafted on the LP-CS layer on (A) mats. CS+EGF coatings led to dramatically increased VSMC -growth, -infiltration and -survival in serum-free medium.

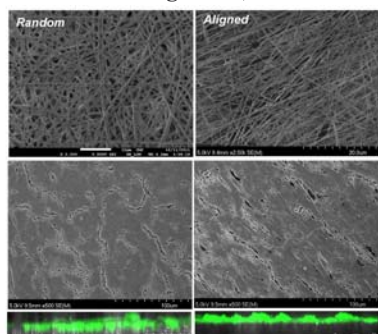


Fig. 1. SEM and z-stack images of random (R) and aligned (A) PET mats with confluent HUVECs for luminal layer

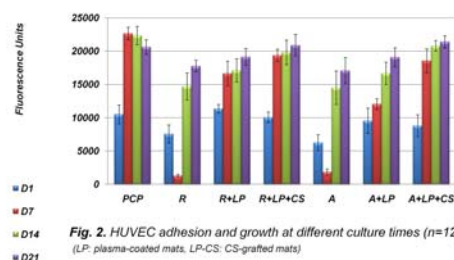


Fig. 2. HUVEC adhesion and growth at different culture times (n=12) (LP: plasma-coated mats, LP-CS: CS-grafted mats)

Conclusion: Combinations of bioactive coatings (CS+EGF) with (R) and (A) electrospun nanofiber mats can provide promising scaffolds for luminal and media layers of SDVGs, ones that greatly improve cell growth, VSMC-infiltration, and HUVEC-resistance to shear.

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Keywords: blood vessel, Surface modification, endothelialization, bioactive interface

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