## Poly(ε-Caprolactone)-Based Bilayered Scaffolds Prepared by Electrospinning for Tissue Engineering of Small-Diameter Vascular Grafts

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Abstract : Nowadays, there is an unmet clinical need for new small-diameter vascular grafts to overcome the drawbacks of traditional methods used for treatment of widespread cardiovascular diseases. Vascular tissue engineering (VTE) is a promising approach that can be utilized to develop viable vascular grafts by in vitro seeding of functional cells onto a scaffold allowing them to attach, proliferate and differentiate. To achieve this purpose, the scaffold should provide cells with the initial necessary extracellular matrix environment and structure until being able to reconstruct the required vascular tissue. Therefore, producing scaffolds with suitable features is crucial for guiding cells properly to develop the desired tissueengineered vascular grafts for clinical applications. The main objective of this work is fabrication and characterization of tubular small-diameter ( < 6 mm) bilayered scaffolds for VTE. The scaffolds were prepared via mixing electrospinning approach of biodegradable poly(ɛ-caprolactone) (PCL) polymer – due to its favorable physicochemical properties – to mimic the natural environment-extracellular matrix. Firstly, tubular nanofibrous construct with inner diameter of 3, 4 or 5 mm was electrospun as inner layer, and secondly, microfibrous construct was electrospun as outer layer directly on the first produced inner layer. To improve the biological properties of PCL, a group of the electrospun scaffolds was immersed in type-1 collagen solution. The morphology and structure of the resulting fibrous scaffolds were investigated by scanning electron microscope. The electrospun nanofibrous inner layer contained fibers measuring 219±35 nm in diameter, while the electrospun microfibrous outer layer contained fibers measuring 1011 ± 150 nm. Furthermore, mechanical, thermal and physical tests were conducted with both electrospun bilayered scaffold types where revealed improved properties. Biological investigations using endothelial, smooth muscle and fibroblast cell line showed good biocompatibility of both tested electrospun scaffolds. Better attachment and proliferation were obviously found when cells were cultured on the scaffolds immersed with collagen due to increasing the hydrophilicity of the PCL. The easy, inexpensive and versatile electrospinning approach used in this work was able to successfully produce double layered tubular elastic structures containing both nanofibers and microfibers to imitate the native vascular structure. The PCL - as a suitable and approved biomaterial for many biomedical and tissue engineering applications can ensure favorable mechanical properties of scaffolds used for VTE. The VTE approach using electrospun bilavered scaffolds offers optimal solutions and holds significant promises for treatment of many cardiovascular diseases.

Keywords : electrospinning,  $poly(\epsilon$ -caprolactone) (PCL), tissue-engineered vascular graft, tubular bilayered scaffolds, vascular cells

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