Multiple release of polyplexes of plasmids VEGF and bFGF from electrospun fibrous scaffolds towards regeneration of mature blood vessels

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\textbf{A B S T R A C T}

Key challenges associated with the outcomes of vascular grafting (for example, to fully vascularize engineered tissues and promptly regenerate blood vessel substitutes) remain unsolved. The local availability of angiogenic growth factors is highly desirable for tissue regeneration, and plasmid loading in scaffolds represents a powerful alternative for local production of tissue-inductive factors. No attempt has been made so far to clarify the efficacy of electrospun fibers with the loading of multiple plasmids to promote tissue regeneration. In the present study, core–sheath electrospun fibers with the encapsulation of polyplexes of basic fibroblast growth factor-encoding plasmid (pbFGF) and vascular endothelial growth factor-encoding plasmid (pVEGF) were evaluated to promote the generation of mature blood vessels. In vitro release indicated a sustained release of pDNA for approximately 4 weeks with as low as $\sim 10\%$ initial burst release, and the release patterns from the single and twofold plasmid-loaded systems coincided. In vitro investigations on human umbilical vein endothelial cells showed that the sustained release of pDNA from fibrous mats promoted cell attachment and viability, cell transfection and protein expression, and extracellular secretion of collagen IV and laminin. The acceleration of angiogenesis was assessed in vivo after subcutaneous implantation of fibrous scaffolds, and the explants were evaluated after 1, 2 and 4 weeks’ treatment by histological and immunohistochemical staining. Compared with pDNA polycation infiltrated fibrous mats, the pDNA polycation encapsulated fibers alleviated the inflammation reaction and enhanced the generation of microvessels. Although there was no significant difference in the total number of microvessels, the density of mature vessels was significantly enhanced by the combined treatment with both pbFGF and pVEGF compared with those incorporating individual pDNA. The integration of the core–sheath structure, DNA condensation and multiple delivery strategies provided a potential platform for scaffold fabrication to regenerate functional tissues.

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\textbf{1. Introduction}

Tissue engineering aims to overcome the limitations of organ transplantation by providing engineered tissues and organs to patients desperately in need of them [1]. Despite the success in thin layer tissues such as bioengineered skin, regeneration of large and more complex organs has met with more significant challenges. One of the most important issues is the proper vascularization of the constructs [2], since a tissue that is more than a few millimeters in size generally cannot survive by only the diffusion of nutrients and metabolic products [3]. Vascularization is also important in several pathological conditions, including ischemic heart disease [4] and diabetic ulcers [5]. The rapid formation of new blood capillaries is essential to alleviate the symptoms by supplying the necessary nutrients and oxygen to and removing waste products from cells [6]. However, key challenges associated with the outcomes of vascular grafting (for example, to get full vascularization in engineered tissues and promptly regenerate blood vessel substitutes) remain unsolved [7].

Angiogenic growth factors are major driving forces of vascularization, which makes directing these cues critical for the induction of new blood vessels [8]. For example, vascular endothelial growth factor (VEGF) stimulates cells to produce matrix metalloproteinases, which degrade the basement membrane and enhance the proliferation and migration of endothelial cells towards the interstitium to sprout new vascular networks [9]. Thus, local delivery of angiogenic growth factors is highly desirable for therapeutic angiogenesis. des Rieux et al. encapsulated VEGF into polyelectrolyte complexes of dextran sulfate and chitosan, which were embedded into tissue engineering scaffolds. The encapsulation of VEGF enhanced its efficiency by protection and controlled release from scaffolds, which sustained cell infiltration and organization and stimulated blood

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