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A bio-inspired hybrid nanosack for graft vascularization at the omentum

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ABSTRACT

For three-dimensional tissue engineering scaffolds, the major challenges of hydrogels are poor mechanical integrity and difficulty in handling during implantation. In contrast, electrospun scaffolds provide tunable mechanical properties and high porosity; but, are limited in cell encapsulation. To overcome these limitations, we developed a "hybrid nanosack" by combination of a peptide amphiphile (PA) nanomatrix gel and an electrospun poly (ε-caprolactone) (ePCL) nanofiber sheet with porous crater-like structures. This hybrid nanosack design synergistically possessed the characteristics of both approaches. In this study, the hybrid nanosack was applied to enhance local angiogenesis in the omentum, which is required of tissue engineering scaffolds for graft survival. The ePCL sheet with porous crater-like structures improved cell and blood vessel penetration through the hybrid nanosack. The hybrid nanosack also provided multi-stage fibroblast growth factor-2 (FGF-2) release kinetics for stimulating local angiogenesis. The hybrid nanosack was implanted into rat omentum for 14 days and vascularization was analyzed by micro-CT and immunohistochemistry; the data clearly demonstrated that both FGF-2 delivery and porous crater-like structures work synergistically to enhance blood vessel formation within the hybrid nanosack. Therefore, the hybrid nanosack will provide a new strategy for engineering scaffolds to achieve graft survival in the omentum by stimulating local vascularization, thus overcoming the limitations of current strategies.

Statement of Significance

For three-dimensional tissue engineering scaffolds, the major challenges of hydrogels are poor mechanical integrity and difficulty in handling during implantation. In contrast, electrospun scaffolds provide tunable mechanical properties and high porosity; but, are limited in cell encapsulation. To overcome these limitations, we developed a "hybrid nanosack" by combination of a peptide amphiphile (PA) nanomatrix gel and an electrospun poly (ε-caprolactone) (ePCL) nanofiber sheet with porous crater-like structures.
This design synergistically possessed the characteristics of both approaches. In this study, the hybrid nanosack was applied to enhance local angiogenesis in the omentum, which is required of tissue engineering scaffolds for graft survival. The hybrid nanosack was implanted into rat omentum for 14 days and vascularization was analyzed by micro-CT and immunohistochemistry. We demonstrate that both FGF-2 delivery and porous crater-like structures work synergistically to enhance blood vessel formation within the hybrid nanosack. Therefore, the hybrid nanosack will provide a new strategy for engineering scaffolds to achieve graft survival in the omentum by stimulating local vascularization, thus overcoming the limitations of current strategies.

1. Introduction

Three dimensional (3-D) tissue engineering scaffolds necessitate blood supply to achieve their biofunctionality. Transplanted cells compete for oxygen and nutrients among themselves as well

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as with host cells; the survival of implanted cells is limited to the distance of oxygen diffusion (approximately 150 ~ 200 μm from a blood vessel) [1]. Thus, angiogenesis is a critical feature which defines the success or failure of constructed 3-D tissues. Besides angiogenesis, a biofunctional tissue engineering scaffold must also meet the following requirements: the scaffold needs to provide a nurturing cellular microenvironment with delivery of therapeutic molecules needed for initial function and stimulation of local angiogenesis; the scaffold should maintain mechanical integrity while simultaneously providing porosity for nutrient transport; finally, the scaffold must be biodegradable to facilitate long-term integration with native tissues. The above criteria are all imperative for the design of an ideal 3-D tissue engineering scaffold.

Currently, there are several approaches for the fabrication of synthetic tissue engineering scaffolds. Hydrogel-based scaffolds have been widely used as a tissue engineering scaffold, because gels can be produced from biocompatible components which facilitate the encapsulation of living cells into a 3-D cell nurturing environment [2–4]. Also, therapeutic molecules can be easily encapsulated within the gel and the controlled release of these molecules is well-established [5–7]. However, hydrogel-based scaffolds generally have poor mechanical properties with low tensile and shear strength. While it is possible to increase the mechanical integrity of hydrogel-based scaffolds via cross-linking, such an increase in mechanical properties comes at the cost of decreasing scaffold degradability and other desirable properties, such as a decrease in cell migratory freedom and impediment of angiogenesis. Furthermore, the hydrogel-based scaffolds are difficult to handle and load into the implant site. Besides hydrogels, electrospinning is also a popular approach in tissue engineering applications, because it allows for the fabrication of nano- or micro- fibers with high surface area to volume ratio and an interconnected porous structure [8,9]. Notably, highly interconnected electrospun nanofibers structurally mimic the extracellular matrix (ECM) fiber network [10]; thus, this structure can be reasonably well-defined to provide the architecture needed for adhesion, proliferation, and function of various cells, such as skin, bone, tendons, and neurons [11–14]. The mechanical strength is also tunable to match the native tissue properties [9]. While electrospun scaffolds can frequently be made porous, which aids angiogenesis and in transport of nutrients, these scaffolds have a limited capacity to encapsulate cells and to deliver therapeutic molecules. Thus, to produce an ideal synthetic tissue engineering implant, a novel approach is needed for a scaffold that combines the advantages of both hydrogel- and electrospun-based materials. Here, we describe the development and fabrication of a novel hybrid angiogenesis-inducing and cell-nurturing tissue engineered scaffold, called a "hybrid nanosack". This hybrid nanosack merges electrospinning technology with hydrogel-based controlled release of angiogenic growth factors. The hybrid nanosack consists of a self-assembled peptide amphiphile (PA) nanomatrix gel placed within a porous electrospun poly (ε-caprolactone) (ePCL) nanofiber sheet with crater-like structures (Fig. 1). We use the term hybrid "nanosack" because both PA nanomatrix gel and ePCL nanofiber sheet are composed of a basic nano-scale structure: self-assembled PA nanofibers (each fiber length: about 4 nm) and highly interconnected ePCL nanofibers (each fiber diameter: 703 ± 152 nm) respectively; these components interact with cell or tissue graft at nano-scale levels. The PA gel filling the ePCL sack is a novel approach to obtain the synergistic advantages of both materials for stimulation of local angiogenesis with a cell nurturing platform. The hybrid nanosack is designed to provide a multi-stage release of fibroblast growth factor-2 (FGF-2) to promote local angiogenesis. FGF-2 is a potent angiogenic growth factor that stimulates vein endothelial cell proliferation and migration *in vitro* and angiogenesis *in vivo* [15,16]. FGF-2 is coated on the outer surface of the ePCL sheet and is also encapsulated within the PA nanomatrix

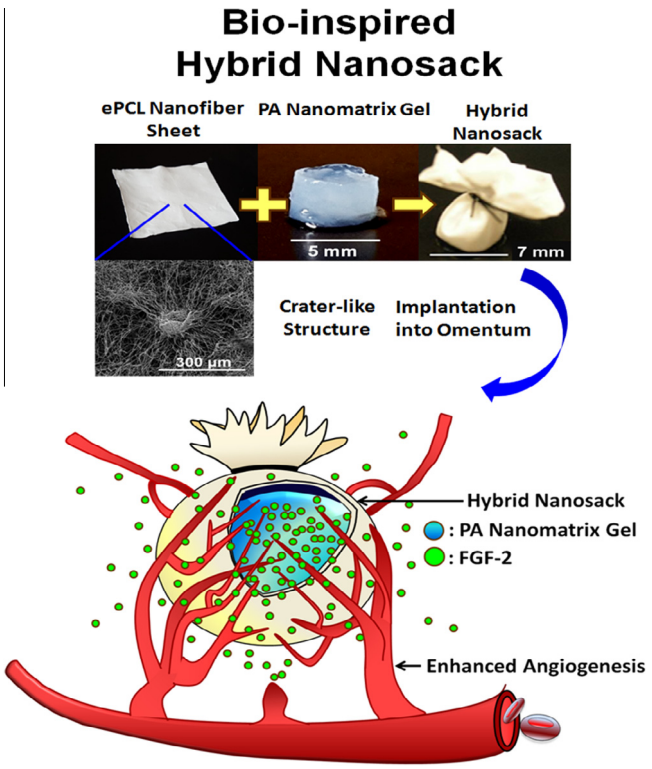

Fig. 1. Schematic figure of a bio-inspired hybrid nanosack. Creation of a bio-inspired hybrid nanosack for enhanced angiogenesis at the omentum.

gel. The ePCL sack delivers FGF-2 initially in a burst manner by simple diffusion, which rapidly recruits new blood vessels and promotes initiation of angiogenesis at the implantation area. In addition, the PA nanomatrix gel enables sustained delivery of FGF-2 as the gel slowly degrades, which allows formation of a stable vascular network. Importantly, porous crater-like structures, generated on the ePCL nanofiber sheet by gas foaming/salt leaching technique, enhance blood vessel infiltration and fast vascularization through the hybrid nanosack. PCL is a biodegradable and non-immunogenic polyester, and also provides specific mechanical and elastic properties; thus, it has been widely used for many biomedical applications, such as implantable drug delivery devices, wound dressings, sutures, and fixation devices [17–20]. In addition, degraded PCL products did not show toxic effect and some PCL applications have already been approved by the FDA [21,22]. The ePCL nanofiber sack with crater-like structures provides suitable mechanical stability for supporting the PA nanomatrix gel. The PA nanomatrix gel can encapsulate tissues as well as therapeutic molecules, and also provide a 3-D extracellular matrix (ECM)-mimicking microenvironment [23,24]. Therefore, by the combination of these two materials, the hybrid nanosack provides a 3-D nurturing and protective environment for the implanted tissue, multi-stage FGF-2 delivery for enhancing local angiogenesis, and crater-like structures for rapid vascularization. In addition, sack architecture allows the convenient surgical manipulation for implantation by using a simple suturing technique as well as transferability to various implantation sites, such as omentum, subcutaneous, intramuscular, and peritoneal sites.

One of the major issues of conventional electrospun scaffolds is that they have tightly deposited nanofiber layers with only a small porous network, which limits cell migration or blood vessel penetration through the scaffold [25,26]. Thus, to overcome this challenge, we used gas foaming/salt leaching technique to create porous spaces within the electrospun scaffold [27]. By this process,