Combinatorial design of polymer patch to optimize delivery and differentiation of embryonic stem cells onto hypertrophic myocardium

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Statement of Purpose: Left ventricular hypertrophy (LVH) is a leading cause of mortal cardiac dysfunctions. Cell delivery to injured tissues offers a promising solution as a cell replacement therapy[1]. In particular, embryonic stem cells (ESCs) have been delivered to hypertrophic myocardium and showed effective therapeutic results from preclinical and clinical studies. However, the therapeutic efficacy of this approach is often rendered ineffective due to a rapid loss of delivered cells caused by heart beating [2]. Cell-seeded electrospun polymer scaffold is an alternate tissue engineering approach to deliver ESCs to hypertrophic myocardium[3] and to direct their differentiation. The goal of this study is to develop a multifunctional cardiac patch which can 1) deliver ESCs to hypertrophic ventricular myocardium; 2) control their differentiation to cardiomyocytes; and 3) release bioactive molecules (i.e., anti-inflammatory Ac-SDKP, anti-fibrotic thymosin β4, and pro-angiogenic C16Y) in response to pathogenic oxidative stimuli.

Methods: A series of combinatorial polymers containing different molar ratios of hydrophilic polyethylene glycol (PEG, Mw = 5,000), hydrophobic poly(ε-caprolactone) (PCL) and negatively-charged, carboxylated PCL (CPCL) was synthesized by ring opening polymerization (Figure 1) to tune chemical, mechanical, and biological properties of polymers. A subset of the combinatorial library was tested in terms of their biocompatible, bioactive, and instructive effects on ESCs and their differentiation to cardiomyocytes. The test polymers include (i) PCL, (ii) 4%PEG-96%PCL, (iii) 8%PEG-92%PCL, (iv) PCL90%-10%CPCL (v) 4%PEG-86% PCL-10%CPCL, and (vi) 8% PEG-82%PCL-10%CPCL.

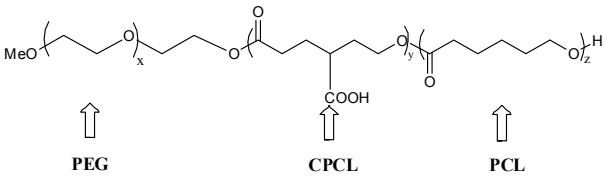

Figure 1. Schematic representation of combinatorial polymers (**x%PEG-*co*-y%CPCL-*co*-z%PCL**), where x, y, and z represent molar % of the corresponding units.

The synthesized polymers were used to prepare electrospun scaffolds with a high degree of control over their structure, thereby creating highly porous scaffolds of ultrafine fibers that resemble the extracellular matrix. The diameter and orientation of fibers was measured by scanning electron microscopy. Mouse embryonic stem cells (CGR8 cell line) were cultured by hanging drop method. The cultured embryonic bodies (EBs) were placed over electrospun polymeric scaffolds. *In vitro* cell viability and intracellular reactive oxygen species (ROS) level were measured over electrospun fiber scaffolds and compared to control without polymer fiber. The differentiation of ESCs to cardiomyocytes was assessed in terms of α-and β-myosin heavy chain expression and Ca^{2+} signaling-based contractility.

Results: Embryoid bodies (EBs) demonstrate different abilities to attach and differentiation into cardiomyocytes over electrospun polymer scaffolds of different chemical compositions and mechanical properties. All the polymer

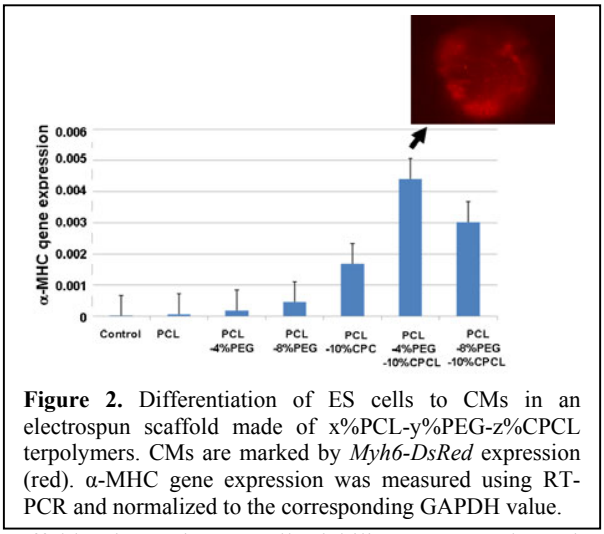

Figure 2. Differentiation of ES cells to CMs in an electrospun scaffold made of x%PCL-y%PEG-z%CPCL terpolymers. CMs are marked by *Myh6-DsRed* expression (red). α-MHC gene expression was measured using RT-PCR and normalized to the corresponding GAPDH value.

scaffolds shows better cell viability, compared to the control. As shown in Figure 2, ESCs, stably transfected with the nuclear red fluorescent protein gene under the cardiac-specific α-MHC or Myh6 promoter (*Myh6-DsRed*), attached well to test scaffolds. Interestingly, a terpolymer type, 4%PEG-86%PCL-10%CPCL induced α-MHC expression of ES cells more than the other polymers as quantified by Q-PCR using α-MHC gene primers. These results were further supported by protein expression of α-MHC and Ca^{2+} signaling-based contractility. The detailed results from characterization of polymer and patch properties as well as a series of studies on *in vitro* ESC differentiation will be presented.

Conclusions: It was found that ESCs over 4%PEG-86% PCL-10%CPCL exhibit better cardiac signals compared to the other test conditions, which indicates that other material properties (e.g., geometry and mechanical property of electrospun fibers) can be further optimized.

References:

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