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Controlled release of heparin from poly(ε-caprolactone) electrospun fibers

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Abstract

Sustained delivery of heparin to the localized adventitial surface of grafted blood vessels has been shown to prevent the vascular smooth muscle cell (VSMC) proliferation that can lead to graft occlusion and failure. In this study heparin was incorporated into electrospun poly(ε-caprolactone) (PCL) fiber mats for assessment as a controlled delivery device. Fibers with smooth surfaces and no bead defects could be spun from polymer solutions with 8% w/v PCL in 7:3 dichloromethane:methanol. A significant decrease in fiber diameter was observed with increasing heparin concentration. Assessment of drug loading, and imaging of fluorescently labeled heparin showed homogenous distribution of heparin throughout the fiber mats. A total of approximately half of the encapsulated heparin was released by diffusional control from the heparin/PCL fibers after 14 days. The fibers did not induce an inflammatory response in macrophage cells in vitro and the released heparin was effective in preventing the proliferation of VSMCs in culture. These results suggest that electrospun PCL fibers are a promising candidate for delivery of heparin to the site of vascular injury.

Keywords: Polycaprolactone; Electrospinning; Drug release; Heparin; Pro-inflammatory response; Vascular smooth muscle cells

1. Introduction

Intimal hyperplasia occurring after vascular interventions such as coronary bypass surgery is a major clinical and economic problem; approximately 30% of all arterial bypass grafts [1] and 50% of vein grafts [2] fail due to this process. During the first month after a vein graft procedure, changes may occur within the graft such as endothelial denudation, platelet adherence, and leukocyte infiltration [3]. These changes can lead to the proliferation of vascular smooth muscle cells (VSMCs) in the vessel media and the subsequent migration of these cells into the intima causing arterial stenosis. In an uninjured blood vessel, heparan sulfate glycosaminoglycans present in the vessel wall help maintain VSMCs in a contractile, non-proliferative state [4]. After injury the normal biochemical balance is disrupted leading to the myoproliferative response.

Heparin has long been known to inhibit the proliferation of VSMCs both in vivo [5] and in vitro [6]. Several modes of action for the anti-proliferative effect of heparin have been suggested, including inhibition of immediate-early genes [7], inhibition of production of matrix-degrading proteases important for cell migration and proliferation [8], and inhibition of mitogen-activated protein kinase [9]. The local delivery of heparin to the site of vascular injury could be used to prevent the myoproliferative response whilst avoiding the associated problems of systemic drug delivery. Application of anti-proliferative agents to the localized adventitial surface of injured blood vessels has been

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previously shown to be effective in reducing stenosis. Edelman et al. [10] showed that heparin delivered to the perivascular space via an ethylene-vinyl acetate co-polymer (EVAc) matrix was more effective than intravenous or subcutaneous delivery routes for preventing restenosis in a rat balloon catheter injury model; it also allowed a dosage estimated to be 20-fold less than the systemic doses that would normally be applied. Rogers et al. [11] demonstrated that the same devices were able to reduce stent-induced hyperplasia by 54%. Edelman et al. [12] used a prolonged delivery device consisting of heparin loaded microcapsules sequestered in a calcium alginate membrane wrapped around a grafted blood vessel to reduce intimal hyperplasia in a rat vascular bypass graft model without inducing bleeding complications. They suggested that localized drug delivery and distribution may be more efficient if drug release is circumferentially symmetric around the graft. In the present study we propose that heparin-eluting electrospun fiber mats offer an interesting material for the prevention of VSMC proliferation around vascular grafts.

Electrospinning is a simple process for the production of fibers with diameters in the range from submicron to micron. An electric field is applied to the end of a capillary containing a polymer solution, inducing a charge on the surface of the liquid. When the voltage reaches a critical value where the charge overcomes the surface tension of the polymer droplet at the tip of the capillary, a jet is ejected. Acceleration through the electric field causes elongation, thinning of the polymer jet and evaporation of the solvent to produce fibers that can be collected onto a ground plate [13].

A broad range of applications for electrospun fibers have been suggested [14] including drug delivery, which has been demonstrated from biodegradable polymeric fibers for antibiotics [15–19], and DNA for gene therapy [20]. The electrospinning process allows for control over the morphology of the fibers and utilization of a wide variety of polymers. The small fiber diameters produced by electrospinning have the advantage of a large surface-to-volume ratio, as well as a high permeability and interconnecting pore structure, both of which are desirable in a biological setting.

Poly(ε-caprolactone) (PCL) is a semi-crystalline aliphatic polyester well known for its slow biodegradability, high biocompatibility, and good drug permeability [21]. PCL and some of its co-polymers have been investigated for the delivery of heparin in a number of different formats, including the use of solvent casting to coat catheters with a PCL-polyethylene glycol film containing heparin in order to improve blood compatibility [22], and the encapsulation of heparin in PCL microcapsules [23] and nanocapsules [24] using the oil-in water-in oil emulsion technique for oral heparin delivery. Unlike commonly used biodegradable polymers such as poly(D,L-lactic-co-glycolic acid), PCL does not produce a local acidic environment as it degrades [25]. This, along with its good mechanical properties and comparatively lower cost, renders PCL an attractive biomedical polymer. Electrospun fibers made from PCL and its co-polymers have been characterized for a variety of spinning solutions and conditions. Various solvents have been used for the electrospinning of PCL including 2,2,2-trifluoroethanol [26], chloroform [17,27,28], methylene chloride and methylene chloride/DMF mixtures [29,30], and chloroform/DMF mixtures [31]. One study systematically investigated the useable range of PCL concentrations and molecular weight [32]. However, most researchers use PCL of molecular weight of 80 000 at concentrations in the range of 8–15% w/v.

In this study, fiber mats were electrospun from PCL solutions containing heparin at two concentrations. The effect of heparin incorporation on fiber morphology was studied together with heparin distribution and release rates. In order to assess the efficacy of the fibers as an implantable drug delivery device, inflammatory response to the fibers was assessed through in vitro macrophage assays and the ability of the heparin released to inhibit the proliferation of VSMCs was studied in culture. We hypothesized that heparin would retain its biological activity during the fabrication process and would, through incorporation into PCL fibers, achieve sustained release over an appropriate time frame for the treatment of vascular injury.

2. Methods

2.1. Electrospinning

Fibers were electrospun from PCL (Mn 80 000, Aldrich) solutions containing heparin. Polymer solutions containing 8% w/v PCL alone or 8% w/v PCL with heparin (Sigma, sodium salt, average MW approx 13 kDa) at 0.5 or 0.05 wt% of PCL were prepared in a 7:3 dichloromethane (DCM):methanol solvent mixture. To homogeneously incorporate heparin into the polymer solution, the desired amount of heparin was dissolved in 20 μl water and mixed with approximately 400 μl methanol. This heparin solution was added to DCM and the resultant solution used to dissolve the polymer. No heparin precipitate was apparent in the polymer solution. The solution was transferred to a 1 ml syringe attached to a blunt end metal capillary with a 0.4 mm internal diameter by a 10 cm length of Teflon tubing. A steady flow of the solution from the capillary outlet was achieved using a syringe pump (KD Scientific, USA), operating at a flow rate of 0.5 ml/h. A high voltage power supply (Gamma High Voltage Research, USA) was used to create an electric field strength of 0.8 kV/cm between the capillary and a metal collection plate. Fibers were collected onto glass microscope slides and the resulting fiber membranes dried in a desiccator. Fibers were sterilized by soaking in 70% ethanol for 15 min followed by 3 × 5 min washes in cell culture media.

2.2. Fluorescent labeling of heparin

Heparin was labeled with Alexa Fluor 488 (A488, Molecular probes) by a method modified from Osmond et al. [33]. A 10% 1-ethyl-3-(3-dimethylaminopropryl)carbodiimide hydrochloride (EDC) solution (50 μl) in 0.1 M 4-morpholinoethanesulfonic acid (MES) buffer (pH 4.5) was added to 300 μl of a 1% solution of heparin in 0.1 M MES buffer. A 1% A488 solution (50 μl) in 0.1 M MES buffer was then added to the heparin/EDC mixture. The reaction tube was protected from light and incubated overnight at room temperature. The A488-heparin product was purified using an Amersham PD10 desalting column. The labeling efficiency was approximately 1.3 mol A488/mol heparin.