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Stem cell impregnated nanofiber stent sleeve for on-stent production and intravascular delivery of paracrine factors

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ABSTRACT

Stem cell therapies for atherosclerotic diseases are promising, but benefits remain modest with present cell delivery devices in part due to cell washout and immune attack. Many stem cell effects are believed mediated by paracrine factors (PFs) secreted by the stem cells which potentiate tissue repair via activation and enhancement of intrinsic host repair mechanisms We therefore sought to create an "intravascular paracrine factor factory" by harnessing stem cells on a stent using a nanofiber (NF) stent sleeve, and thus providing a sheltered milieu for cells to continuously produce PFs on-stent. The NF sleeve acts as a substrate on which stem cells grow, and as a semi-permeable barrier that protects cells from washout and host immune response while allowing free outward passage of PFs. NF stent sleeves were created by covering stents with electrospun poly-lactic-co-glycolic acid nanofibers and were then uniformly coated with mesenchymal stem cells (MSCs). NF sleeves blocked cell passage but did not hamper MSC attachment or proliferation, and did not alter MSC morphology or surface markers. NF sleeve MSCs continued to secrete PFs that were biologically active and successfully induced tubulogenesis in human endothelial cells. NF stent sleeves seeded with allogeneic MSCs implanted in pigs remained patent at 7 days without thrombotic occlusion or immune rejection. Our results demonstrate the feasibility of creating an intravascular PF factory using a stem cell impregnated NF stent sleeve, and pave the way for animal studies to assess the efficacy of local PF production to treat ischemic artery disease.

1. Introduction

The final common pathway for most forms of coronary and peripheral artery diseases is damage to downstream tissue. Several clinical trials performed to investigate the utility of intravascular infusion and intramuscular injection of stem cells to repair muscle damage [1–5] demonstrated only modest beneficial effects [2,6–9]. Current methods of cell delivery are thought to be limited by rapid loss of viable cells due to washout into the bloodstream, host immune response, and exposure to an inflamed local environment [10–13]. However, growing evidence suggests that many of the beneficial effects of stem cells are actually mediated by paracrine processes. It is thought that in addition to tissue repair via direct engraftment, stem cells release paracrine factors (PFs) that activate host cells, promote homing of other cells to the site of injury and thus enhance the host's intrinsic repair mechanisms [14–20].

If the reparative capacity of stem cells derives to a significant extent from their secreted PFs, then prolonging exposure of the damaged tissues to these PFs would augment the reparative response. In this work, we sought to create an intravascular "paracrine factor factory" to locally produce and deliver PFs by harnessing stem cells within a sheltered environment attached to a stent. Using an electrospinning technique, we constructed a nanofiber (NF) stent sleeve which we impregnated with a large number of stem cells. The mesh-like NF sleeve acts as a substrate on which the stem cells grow, and as a protective semi-permeable barrier that prevents washout of the stem cells into the bloodstream, excludes host immune cells, and at the same time, allows free passage of PFs, nutrients and wastes as well as the host's

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signaling cytokines (Fig. 1). With the stem cells protected, the NF stent sleeve behaves as an intravascular factory that produces and releases PFs directly into the circulation. Inflammatory cytokines released by ischemic tissue [21], such as IL-1, IL-8, TNF-α, or TGF-β, could be sensed by NF sleeve stem cells, and PF production and release therefore tailored according to the evolving needs of the healing tissue.

In this study, we demonstrate the technical feasibility of creating an intravascular PF factory using a stem cell impregnated NF stent sleeve. We show that NF sleeves prevent cell passage but do not hamper MSC attachment or proliferation, and do not alter stem cell morphology or stem cell surface markers. The NF stent sleeve stem cells continue to produce relevant PFs, and these PFs have biological functionality. Finally, we successfully implanted an NF stent sleeve impregnated with allogeneic MSCs in the pig umbilical artery for 7 days and demonstrate biocompatibility and absence of immune rejection. These data pave the way for larger animal studies to assess the effectiveness of a local paracrine-based approach in reversing some of the consequences of coronary and peripheral artery diseases.

2. Materials & methods

2.1. Nanofiber stent sleeve

Fabrication: Nanofiber stent sleeves are electrospun onto the external surfaces of non-medicated cobalt chromium stents (Medtronic, Minneapolis, MN). Poly (L-lactide/glycolide) acid (PLGA) co-polymer (PLG 8531; PURAC, Lincolnshire, IL) was dissolved in a mixture of dichloromethane and methanol (7:3 *vol*:*vol*). The stents were balloon-expanded to their nominal diameters and removed from the balloon. The co-polymer was then electrospun onto the stent at 12 kV (Gamma High Voltage Research Model ES30P−5W/DAM, Ormond Beach, FL) using a syringe pump (NE-300 Infusion Pump; New Era Pump Systems, Farmingdale, NY) with a flow rate of 3.5 mL/h via an 22G × 1.5 inch blunt-tip needle at a distance of 9 cm from the collecting target for 25 min. To produce a uniform NF sleeve thickness, the stent was continuously rotated along its longitudinal axis during electrospinning at 130 revolutions per minute. The completed electrospun NF stents were sterilized in a solution of 70% ethanol for 12 h. Similar electrospinning techniques are described in the literature to fabricate covered stents [22,23] and vascular grafts [24].

Pore Size Characterization: NF sleeve morphology was imaged using a Hitachi S4700 scanning electron microscope (SEM) at baseline before and after crimping onto a 2.5 × 12 mm angioplasty balloon (Medtronic, Minneapolis, MN) using a radial compression stent crimper (Model RMC, Blockwise Engineering, Phoenix, AZ) and re-expanding to nominal diameter ($n = 3$). Pore diameters were measured using image processing tools in MATLAB (Mathworks, Natick, MA). Specifically, pixel intensity thresholds were used to define pores on the SEM images. Since pores are randomly oriented across the NF sleeve and are of different shapes and sizes, at least 500 individual samples of intensity profiles were taken across each SEM image, and the lengths of continuous segments of pixel intensities below the threshold defined as a pore were quantified, averaged, and taken to be the average pore diameter.

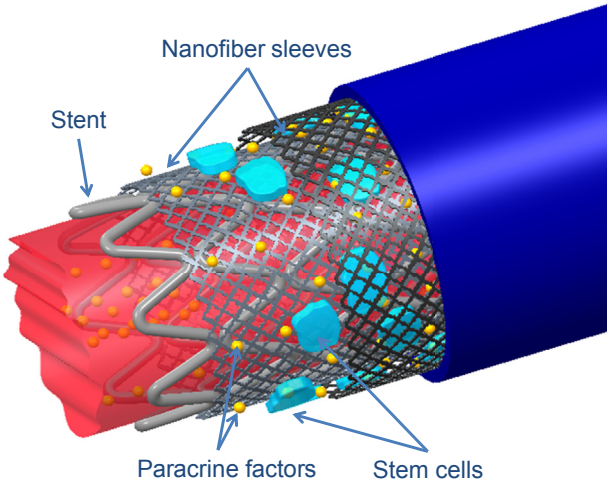

Fig. 1. Schematic of the structure of the stem cell impregnated nanofiber stent sleeve. An inner sleeve electrospun directly on the stent provides the primary barrier between stem cells (light blue) and the blood vessel lumen (red). An optional outer sleeve overcoat provides a barrier between stem cells and the blood vessel wall (dark blue). Paracrine factors (yellow) freely pass through the sleeve. Cells, being much larger than the sleeve pore size, do not pass through the sleeve. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cell Permeability: A NF stent sleeve was expanded to join two separate segments of tubing (Tygon S-50HL, 3/32″ inner diameter, Cole-Palmer). A gap of 0.5 cm was left between the two segments, so that part of the nanofiber stent sleeve remained not covered by the tubing. The construct was placed in a media bath, and a solution of 10^6 mesenchymal stem cells was placed into the tubing, filling the stent sleeve lumen. Outward stem cell transit from the NF stent sleeve lumen to the media bath through the exposed portion of the stent sleeve was quantified after 6 h and 60 h in culture.

2.2. NF stent sleeve impregnation, overcoating, cell coverage and cell viability

2.2.1. NF sleeve impregnation and overcoating

Pig mesenchymal stem cells (pMSCs) were isolated and expanded from bone marrow biopsies of Yorkshire farm pigs using methods detailed previously [25,26]. For NF impregnation, pMSCs at passages 4–6 were harvested using 0.25% Trypsin-EDTA (Gibco), and re-suspended in culture medium at a concentration of 3×10^6 pMSCs/mL. NF stent sleeves were then placed in the pMSC suspension in a 1.5 mL Eppendorf tube which was gently rotated for 30 min at 37 °C to ensure uniform cell adhesion. Excess non-adherent stem cells were washed off with fresh medium. The cell-impregnated NF stent sleeves were then incubated in cell culture medium (Alpha-MEM, Life Technologies) supplemented with fetal bovine serum for at least an additional 24 h prior to use. Cell-impregnated NF stent sleeves can be overcoated with an additional NF sleeve layer outside the cell layer. Cell-impregnated NF stent sleeves for overcoating were cultured for 7 days prior to electrospinning under sterile conditions with another layer of NF for 12 min, and were cultured after overcoating for 3–6 days prior to use.

2.2.2. Cell coverage and viability

Cell coverage and stem cell viability on the NF stent sleeve were evaluated on days 1 and 8 after cell impregnation using a Calcein-AM/Ethidium homodimer assay (LIVE/DEAD Viability Assay, Life Technologies). Cell-impregnated NF stent sleeves were incubated in 2 μM Calcein-AM and 4 μM Ethidium homodimer solution for 30 min. The NF stent sleeves were briefly washed in phosphate buffered saline and then imaged using confocal fluorescence microscopy (Zeiss AxioObserver with 710NLO-Meta confocal module) to assess NF sleeve surface cell coverage and any cell infiltration into the sleeve matrix.

2.3. Flow cytometry

To confirm that the NF sleeve does not induce differentiation of impregnated cells, pMSCs were collected and analyzed for expression of MSC-specific surface markers by flow cytometry seven days after incorporation in the NF sleeve. Cells were analyzed for CD90, CD44, and CD34 expressions using commercially available antibodies (BD Bioscience) and samples were analyzed using FACS LSR-II (BD Bioscience). Data were gated, analyzed and compensated using FlowJo software (Tree Star, Ashland OR). 7-Aminoactinomycin-D (7-AAD) stained non-viable cells and cell aggregates were excluded during the analysis. To reliably distinguish positive and negative staining populations, standard isotype controls (BD Bioscience) were used as negative controls.

2.4. Paracrine factor production and In vitro tubulogenesis assays

2.4.1. Paracrine factor production

Human (hMSCs) were obtained from Lonza (Walkersville, Maryland) and expanded using standard methods [27]. hMSCs were used for the paracrine factor production assays because ELISA kits available commercially for hMSCs have a superior dynamic range and better reproducibility than do those available for pMSCs. The hMSCs were embedded into 1×1 cm^2 NF stent sleeve patches by incubating the sleeves in a 0.5 mL solution of 2×10^5 hMSCs (passage number 6) for 90 min, and then washing off the excess unattached hMSCs. Conditioned media was collected 1, 3, and 7 days after stem cell seeding. Secreted vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) were analyzed using enzyme-linked immunosorbent assays (Quantikine ELISA kits, R&D Systems; Minneapolis, MN).

2.4.2. In vitro tubulogenesis

For the Human Umbilical Vein Endothelial Cell (HUVEC) tubule formation assay, 6-well plates were coated with 250 μL of Geltrex (Invitrogen) and seeded with 1.8×10^5 HUVECs suspended in 2.5 mL of Medium 200PRF (Invitrogen). 1×1 cm^2 NF sleeves ($n = 4$) were seeded with hMSCs and incubated for 7 days before being placed on top of the HUVECs in each well. NF sleeves with an NF overcoat ($n = 3$) were incubated for an additional 3 days after the overcoating