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Magnetizable stent-grafts enable endothelial cell capture

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ABSTRACT

Emerging nanotechnologies have enabled the use of magnetic forces to guide the movement of magnetically-labeled cells, drugs, and other therapeutic agents. Endothelial cells labeled with superparamagnetic iron oxide nanoparticles (SPION) have previously been captured on the surface of magnetizable 2205 duplex stainless steel stents in a porcine coronary implantation model. Recently, we have coated these stents with electrospun polyurethane nanofibers to fabricate prototype stent-grafts. Facilitated endothelialization may help improve the healing of arteries treated with stent-grafts, reduce the risk of thrombosis and restenosis, and enable small-caliber applications. When placed in a SPION-labeled endothelial cell suspension in the presence of an external magnetic field, magnetized stent-grafts successfully captured cells to the surface regions adjacent to the stent struts. Implantation within the coronary circulation of pigs (n=13) followed immediately by SPION-labeled autologous endothelial cell delivery resulted in widely patent devices with a thin, uniform neointima and no signs of thrombosis or inflammation at 7 days. Furthermore, the magnetized stent-grafts successfully captured and retained SPION-labeled endothelial cells to select regions adjacent to stent struts and between stent struts, whereas the non-magnetized control stent-grafts did not. Early results with these prototype devices are encouraging and further refinements will be necessary in order to achieve more uniform cell capture and complete endothelialization. Once optimized, this approach may lead to more rapid and complete healing of vascular stent-grafts with a concomitant improvement in long-term device performance.

1. Introduction

Stent-grafts, also known as covered stents, are endovascular devices that can be implanted within a blood vessel using a minimally invasive procedure to create an artificial conduit for blood flow [1]. Common indications for stent-grafts include aortic coarctation, aneurysm, arteriovenous fistula, vein graft degeneration, dissection, perforation, traumatic injury, and hemodialysis access. The synthetic materials present on the blood contacting surface of these devices stimulate thrombosis and neointimal hyperplasia, both of which reduce device patency over time [2,3]. This is especially problematic in small-caliber applications and stent-grafts are therefore currently limited to routine use in medium- and large-caliber applications.

An effective strategy for improving performance in large- and medium-caliber applications and enabling small-caliber applications is to rapidly heal the stent-graft by establishing a confluent and robust endothelium on the blood contacting surface [1]. Endothelial cells serve as a natural barrier between circulating blood elements and underlying tissues and inhibit both thrombosis and neointimal hyperplasia. A variety of approaches for coating the blood contacting surface of implantable devices with endothelial cells have been studied [4]; however, poor cell retention upon exposure to the shear stress of circulating blood remains problematic [5].

Our group has developed an approach to rapidly and stably target endothelial cells to implantable devices using magnetic forces. We have developed a process for synthesizing superparamagnetic iron oxide nanoparticles (SPION) and using them to label blood outgrowth endothelial cells (BOEC) [6]. Furthermore, we have demonstrated that SPION-labeled BOECs can be targeted to magnetic devices including vascular grafts [7] and stents [8–10]. In this study, we extend our magnetic cell targeting approach to a novel ferromagnetic stent-graft design and demonstrate successful cell capture and retention after 7 days of implantation in porcine coronary circulation.

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2. Methods and materials

2.1. SPION synthesis

SPIONs were synthesized by coating magnetite (Fe_3O_4) with poly(lactic-co-glycolic acid) (PLGA) as previously described [6]. Briefly, magnetite nanoparticles approximately 10 nm in diameter were synthesized by stirring an aqueous solution of iron(II) chloride tetrahydrate and iron(III) chloride at 1000 RPM and 50 °C. The magnetite nanoparticles were then precipitated using ammonium hydroxide, coated with oleic acid to form a gel, and purified. Next, the magnetite nanoparticles were coated with a shell of PLGA approximately 50 nm thick by high speed oil-in-water emulsification (1:15 ratio of magnetite to PLGA by mass) using Pluronic F-127 as a stabilizer. Finally, the PLGA-magnetite SPIONs were washed and lyophilized prior to storage at −20 °C.

2.2. BOEC culture

BOECs were generated from porcine peripheral blood mononuclear cells as previously described [11]. Briefly, 50–200 mL of peripheral blood was collected from an anesthetized pig and immediately mixed with approximately 10 units/mL of heparin to prevent coagulation. The heparinized blood was diluted 1:1 with phosphate buffered saline (PBS) and the mononuclear cell fraction was isolated by density gradient centrifugation (Histopaque 1077, GE Healthcare Life Sciences, Piscataway, NJ). The mononuclear cells were washed and residual erythrocytes were lysed using a 0.6% ammonium chloride solution. Next, the mononuclear cells were washed twice more and cultured in fibronectin-coated wells with EGM-2 cell culture medium (Lonza, Basel, Switzerland). BOEC colonies typically formed within 10–14 days and millions of highly proliferative and phenotypically homogenous BOECs were typically generated within 2–3 weeks. Our group has previously characterized these cells when derived from human [11] and pig [7] blood including positive expression of von Willebrand factor and lectin uptake, which are indicative of endothelial cell phenotype.

BOECs in passages 1–2 were used for experiments. One day prior to use, BOECs were labeled with SPIONs by adding to cell culture medium at a concentration of 200 μg/mL and incubating overnight at 37 °C as previously described [6]. BOECs endocytose the SPION particles and store them within cytoplasmic endosomes as we have shown previously [9]. Just prior to use, BOECs were stained with a fluorescent marker (CM-DiI, ThermoFisher Scientific, Waltham, MA) by incubating at a concentration of 5 μL/mL in cell culture medium for 30 min at 37 °C.

2.3. Stent-graft fabrication

Stent-grafts were fabricated by coating stents made from magnetic 2205 duplex stainless steel (2205 SS) or non-magnetic 316L stainless steel (316L SS) [12] with electrospun polyurethane nanofibers as previously described [13]. Briefly, a 15% polyurethane in dimethylacetamide (DMA) solution (DSM Biomedical, Exton, PA) was loaded into a 5 mL glass syringe and a blunt needle was attached. A syringe pump was used to extrude the polyurethane solution at a rate of 0.01 mL/min while a power supply was used to provide 20 kV of potential to the blunt needle. A 3 mm diameter stainless steel mandrel coated with a food-grade support material (EnvisionTEC, Dearborn, MI) was placed 20 cm from the blunt needle, grounded, and rotated at 50 RPM. After 2 h of nanofiber collection on the rotating mandrel, a stent was crimped onto the mandrel, and nanofibers were collected for a further 3 h. After trimming the excess polyurethane, dissolving the support material in water, and removing from the mandrel, the resulting stent-graft consisted of a stent sandwiched between a 100 μm thick inner layer and a 150 μm thick outer layer of polyurethane nanofibers. Stent-grafts were carefully inspected prior to sterilization with ethylene oxide gas.

2.4. In vitro cell capture studies

SPION-labeled BOECs were suspended in PBS at a concentration of 1×10^6 cells/mL. Fluorescence microscopy was used to visualize the capture of cells as the cell suspension was pipetted over a magnetized stent-graft in the presence of an external magnetic field. The external magnetic field was generated by two 1.0 T rare earth magnets positioned on opposite sides of the device with opposing poles facing inward. One magnet was placed 8 cm above the device and the other was placed 19 cm below at an angle of 45° from the vertical to simulate the positioning of the magnets during animal implantation studies.

2.5. Porcine implantation studies

All animal procedures were performed in accordance with Mayo Clinic Institutional Animal Care and Use Committee (IACUC) policy. Domestic Yorkshire pigs (n=13) weighing 40–50 kg were anesthetized using an intramuscular injection of 2–3 mg/kg Xylazine, 5 mg/kg Telazol, and 0.05 mg/kg atropine. Continuous inhalation of 1.2–1.5% isoflurane was used to maintain anesthesia during the procedure. Carotid access was used to cannulate the right or left coronary artery with a 9F guide catheter. A stent-graft sterilized by ethylene oxide gas was crimped onto an over-the-wire balloon catheter and deployed within a segment of the right coronary artery or the circumflex artery. Each pig received 1–2 stent-grafts for a total of n=14 magnetic 2205 SS stent-grafts and n=10 non-magnetic 316L SS stent-grafts. Approximately $2–4\times10^6$ autologous BOECs labeled with SPIONs were delivered to the implanted 2205 SS stent-grafts using the balloon catheter over a period of 4 min of blood flow occlusion. The number of delivered cells is approximately an order of magnitude larger than the 2.8×10^5 cells needed to completely cover the inner surface of the stent-graft at an optimal seeding density of 2×10^5 cells/cm^2 [14].

An external magnetic field was applied to the stent-graft during cell delivery and for 1–2 h afterwards by strapping one 1.0 T rare earth magnet above the sternum and another on the right lateral side of the chest wall with opposing poles facing inward. Using magnetic field measurements of an in vitro phantom, we estimated this arrangement of magnets provided a uniform magnetic field of approximately 120 G at the location of the implant. With the stent-graft at that location, the magnetic field remained approximately 120 G but local field gradients allowed for generation of magnetic force. The magnets were left strapped to the pigs as long as possible in order to maximize cell retention, but they were promptly removed once the pigs exhibited sternal recumbency and semi-consciousness due to animal safety considerations. Pigs were allowed to recover and were administered 75 mg clopidogrel and 325 mg aspirin daily beginning 3 days prior to the procedure and continuing until sacrifice.

Pigs were sacrificed after 7 days and the treated arteries were carefully dissected, cleaned, and fixed in 10% formalin solution. Selected samples were sectioned longitudinally, splayed open, and assessed using light microscopy, fluorescence microscopy, and scanning electron microscopy (SEM). Remaining samples were analyzed histologically by embedding in plastic, cross-sectioning, and staining with H & E, Movat's pentachrome, or Prussian blue for iron according to established protocols [15] (CVPath Institute, Gaithersburg, MD).

3. Results

When delivered to a 2205 SS stent-graft in the presence of an external magnetic field in vitro, SPION-labeled BOECs were captured to the polyurethane cover at regions adjacent to underlying stent struts (Fig. 1). Cell capture was not evident at the regions between stent struts. This confirmed that the 100–150 μm thick polyurethane cover