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Improved endothelialization and reduced thrombosis by coating a synthetic vascular graft with fibronectin and stem cell homing factor SDF-1α

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ABSTRACT

Failure of synthetic small-diameter vascular grafts is determined mainly by the lack of endothelial cells, as these cells inhibit thrombosis and intimal hyperplasia. Coating of graft material with homing factors for circulating stem cells has the potential to improve endogenous endothelialization of these grafts and to reduce graft failure. Synthetic knitted polyester grafts (6 mm diameter) were coated with FN and SDF-1α before surgical interposition in the carotid artery of sheep. Similar uncoated vascular grafts were implanted in the contralateral side as internal controls. To study the early attraction of stem cells, grafts were implanted in a first series of nine sheep and explanted after 1 or 3 days. In coated grafts, four times higher fractions of $CD34^+$ and three to four times higher fractions of $CD117^+$ cells adhering to the vessel walls were found than in control grafts ($P < 0.05$). When such coated and non-coated grafts were implanted in 12 other sheep and explanted after 3 months, all coated grafts were patent, while one control graft was occluded. EcNOS staining revealed that FN-SDF-1α coating significantly increased coverage with endothelial cells from 27 ± 4% of the graft to 48 ± 4% compared with the controls ($P = 0.001$). This was associated with a significant reduction of intimal hyperplasia (average thickness 1.03 ± 0.09 mm in controls vs. 0.69 ± 0.04 mm in coated grafts; $P = 0.009$) and significantly less adhesion of thrombotic material in the middle part of the graft ($P = 0.029$). FN-SDF-1α coating of synthetic small-caliber vascular grafts stimulated the attraction of stem cells and was associated with improved endothelialization and reduced intimal hyperplasia and thrombosis.

1. Introduction

Synthetic grafts show outstanding results after large vessel replacement. However, these grafts are much less suitable for small-caliber vessel grafting, where autologous artery or vein grafts have become the "gold standard" [1]. Patency rates are also remarkably higher for these native grafts than for prosthetic grafts [2–4]. The patency of prosthetic vascular grafts is impaired by intimal hyperplasia near the anastomotic regions, which ultimately leads to graft thrombosis [5]. The absence of viable endothelial cells (EC) on the luminal surface of prosthetic grafts induces intimal hyperplasia formation [6]. Endothelial cells also actively inhibit thrombosis and form an anticoagulant surface [7]. Several approaches have been used to seed vascular grafts with EC using harvested venous or microvascular EC, mesothelial cells or endothelial progenitor cells (EPC) and the introduction of *in vitro* maturation steps resulted in an improvement of the patency [8–12]. However, these methods are also associated with serious drawbacks, such as the supplemental procedure to harvest the cells (in the case of autologous cell use), risk of infections, long culture times and the associated high cost of the procedure.

To circumvent these problems, this study advocates a completely different approach of tissue engineering using the concept of endogenous attraction and selective seeding of progenitor cells [13]. In a previous study, a fibronectin and stromal cell derived factor 1 alpha (FN-SDF-1α) coating was used as a recellularization paradigm for heart valves and showed endothelialization after 5 months of implantation in the pulmonary position in sheep [14]. SDF-1α, a CXC chemokine, acts as a chemoattractant for hematopoietic stem cells (HSC) and can even induce the recruitment of EPC [14–16]. HSC homing mediated by SDF-1α/CXCR4 can be taken over by the α4-integrin-VCAM1/FN axis [17]. FN can present SDF-1α to cells and facilitates binding of SDF-1α to matrices [14,18].

Protein coating of vascular grafts to improve endothelialization has already been suggested by several groups [19–23]. The present authors hypothesized that impregnation of small-caliber vascular grafts with FN-SDF-1α might be useful in the *in vivo* seeding to improve endothelialization and graft function.

This study specifically looked at two types of commercially available knitted polyester grafts which are classically pre-coated

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with collagen for hemostatic purposes. The presence of collagen facilitates FN-SDF-1α coating. The only difference between these two graft types is the fact that this collagen pre-coating is either glutaraldehyde cross-linked (Gelsoft) or not cross-linked (Polymaille). These two graft types were included because cross-linking of the collagen pre-coating can have negative effects on the required recellularization phenomenon.

Recellularization was studied in a large animal model using carotid artery graft interposition, an FN-SDF-1α coated graft in one carotid artery and a control graft in the contralateral artery.

2. Materials and methods

2.1. Animal model

Twenty-one adult, healthy female "Lovenaar" sheep with an average weight of 40.3 ± 1.1 kg were selected and cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-23, revised 1985). The study was approved by the ethics committee for animal experiments of the Catholic University of Leuven, Belgium.

2.2. Grafts

Two types of clinically available small-caliber vascular grafts (6 mm diameter) were used: Gelsoft™ (Vascutek Ltd., Inchinnan, UK) and Polymaille®C (Pérouse Laboratoires, Ivry le Temple, France).

Twenty-four segments of Polymaille and 12 segments of Gelsoft grafts 5 cm in length were prepared under sterile conditions. Twelve Gelsoft graft segments and six Polymaille graft segments were first coated with human FN (32 μg cm^{-2}, Biomedical Technologies) by immersion in a sterile phosphate buffered saline (PBS) solution and then with SDF-1α (3.2 μg cm^{-2}, Miltenyi Biotec) in PBS (each 24 h, 4 °C), as already described [14]. As control, the remaining uncoated graft segments were submerged in PBS, the coating solvent, during 48 h.

SDF-1α binding efficiency was assessed in FN-SDF-1α-coated synthetic vascular grafts (FN, 1.6, 8, 16 or 32 μg cm^{-2}; and SDF-1α, 0.16, 0.8, 1.6 or 3.2 μg cm^{-2}) with a modified ELISA protocol, as described before [14].

2.3. Study design

Two implantation designs were used for short-term implants. Gelsoft™ grafts (three control and three FN-SDF-1α coated grafts) were implanted as a unilateral carotid interposition in six sheep while, in three other sheep, six Gelsoft™ grafts (three control and three FN-SDF-1α coated grafts) were bilaterally implanted as a carotid interposition, a control at one side and a coated graft at the other.

Twelve sheep were selected for long-term implants (3 months) of Gelsoft™ ($n = 6$) and Polymaille®C ($n = 6$) grafts. In each animal, a segment of the native carotid artery was resected, a control graft (commercial available Gelsoft™ or Polymaille®C) was implanted as a carotid interposition at one side and a coated graft (Gelsoft™ or Polymaille®C coated with FN-SDF-1α) at the other side.

2.4. Graft implantation

The animals were pre-medicated with ketamine (10–20 mg kg^{-1} intramuscularly) and anesthesia was induced with increasing concentrations of isoflurane in oxygen. Anesthesia was maintained with isoflurane in 5 L min^{-1} O_2. A small incision in the region of the lateral portion superficial to the neck was made. The carotid artery was clamped at each end after administration of a minimal dose of heparin (250 IU kg^{-1}) to avoid any additional bleeding. The graft was sutured into the circulation with 8–0 silk sutures. The animals received anticoagulation (40 mg enoxaparin day^{-1}) during the first 3 days after vascular grafting, to prevent early occlusion due to early thrombotic events.

2.5. Explantation

Six short-term implants were explanted after 24 h, while the other grafts were explanted after 3 days of implantation. The long-term implants were terminated after 3 months of implantation.

Animals were heparinized (250 IU kg^{-1}) and sacrificed with an overdose of pentobarbital (Nembutal; Ovation Pharmaceuticals Inc.) and KCl intravenously. Intimal hyperplasia was macroscopically quantified.

2.6. Histology and immunohistochemistry

2.6.1. Unimplanted grafts

Impregnated samples (unimplanted) and explant samples were fixed in 4% paraformaldehyde, snap-frozen in Tissue Freezing medium (Leica) with liquid nitrogen and seven micrometer cryosections were made on a Microm HM500 OM cryostat (Prosan). Stainings were performed, as described before [24]. Sirius red staining was used to visualize collagen and its organization.

2.6.2. Short-term explants

Impregnation of the vascular grafts was confirmed by immunohistochemistry with the antibody to SDF-1α (polyclonal, Abcam). To study primitive cell attraction, sections from 24-h and 3-day implants were immunohistochemically stained for CD34 (clone: QBEnd 10, Serotec) and CD117 (ab5633, Abcam), both increased in FN-SDF-1α coated vascular graft implants in rats [14]. Immunostaining with CD34 and CD117 was visualized by a 3-amino-9-ethyl-carbazole (AEC, Sigma–Aldrich), after using the tyramide signal amplification biotin system [24].

2.6.3. Chronic explants

In the chronic explant samples, seven transversal sections of the graft were made from inflow to outflow (see Fig. 1). At each of these seven sections, two slices were cut, one for a standard hematoxylin and eosin (H&E) stain and one for immunohistochemistry. The neointima was quantified on H&E stained sections. Therefore, on each slice, thickness of the neointima was measured on three standardized positions and averaged to obtain an average thickness pro section.

Second, endothelialization was quantified on sections stained for endothelial nitric oxide synthase (EcNOS) with an EcNOS antibody (ecNOS, clone: 3, BD Pharmingen), which visualized the EC on the luminal surface of the implanted grafts. Endothelialization was not always completely circumferential, but presented itself as interrupted monolayers of $EcNOS^+$ cells. Therefore, the endothelialization was quantified by adding the length of all these EcNOS positive lines and dividing this sum by the length of the luminal circumference of the graft in this section. So a percentage of

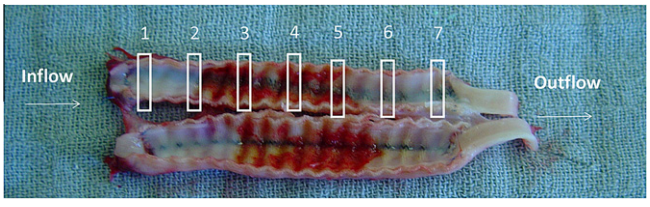

Fig. 1. Explanted vascular graft. Macroscopic aspect of a longitudinally sectioned control graft. The markers indicate the seven consecutive sections were taken from the inflow to the outflow side.

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