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Development and evaluation of *in vivo* tissue engineered blood vessels in a porcine model

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ABSTRACT

Background: There's a large clinical need for novel vascular grafts. Tissue engineered blood vessels (TEBVs) have great potential to improve the outcome of vascular grafting procedures. Here, we present a novel approach to generate autologous TEBV *in vivo*. Polymer rods were engineered and implanted, evoking an inflammatory response that culminates in encapsulation by a fibrocellular capsule. We hypothesized that, after extrusion of the rod, the fibrocellular capsule differentiates into an adequate vascular conduit once grafted into the vasculature.

Methods and results: Rods were implanted subcutaneously in pigs. After 4 weeks, rods with tissue capsules grown around it were harvested. Tissue capsules were grafted bilaterally as carotid artery interposition. One and 4-week patency were evaluated by angiography whereupon pigs were sacrificed. Tissue capsules before and after grafting were evaluated on tissue remodeling using immunohistochemistry, RNA profiling and mechanical testing. Rods were encapsulated by thick, well-vascularized tissue capsules, composed of circumferentially aligned fibroblasts, collagen and few leukocytes, with adequate mechanical strength. Patency was 100% after 1 week and 87.5% after 4 weeks. After grafting, tissue capsules remodeled towards a vascular phenotype. Gene profiles of TEBVs gained more similarity with carotid artery. Wall thickness and αSMA-positive area significantly increased. Interestingly, a substantial portion of (myo)fibroblasts present before grafting expressed smooth muscle cell markers. While leukocytes were hardly present anymore, the lumen was largely covered with endothelial cells. Burst pressure remained stable after grafting.

Conclusions: Autologous TEBVs were created *in vivo* with sufficient mechanical strength enabling vascular grafting. Grafts differentiated towards a vascular phenotype upon grafting.

1. Introduction

Small caliber vascular graft failure is still a frequent complication in vascular surgery, involving substantial morbidity [1] and health care costs [2]. Autologous vessels are used as golden standard as they show superior patency when compared with synthetic grafts in various vascular settings, such as peripheral bypass grafts [3,4] and arteriovenous grafts for hemodialysis access [5,6]. The inferiority of synthetic grafts is attributed to the lack of compliance, thrombogenic surface and continuous presence of synthetic material in the vasculature [1,7]. Unfortunately, native vessels are frequently unavailable or unsuitable for surgical procedures [8,9]

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due to pre-existent vascular disease [10–12].

Tailor-made tissue engineered blood vessels (TEBVs) could offer a better alternative for prosthetic grafts. Most vascular tissue engineering approaches aim to develop off-the-shelf vascular grafts consisting of extracellular matrix (ECM) that in some cases is pre-seeded with vascular cells before implantation into the vasculature [13–15]. Recently, we described a method for *in situ* vascular tissue engineering that allowed us to generate autologous TEBVs *in vivo* in only a few weeks [16,17]. This approach utilizes the foreign body response (FBR) directed to a subcutaneously implanted polymer rod that culminates into the formation of a fibrocellular tissue capsule. Upon extrusion of the rod several weeks after implantation, the remaining tissue capsule is grafted into the vasculature whereupon it could differentiate towards a vascular phenotype. Indeed, flow and wall tension can enhance matrix synthesis [18,19] and vessel wall thickening [20], (myo)fibroblast to vascular smooth muscle cell (VSMC) differentiation [21] and luminal caliber remodeling to adapt the tissue to the present hemodynamic situation [20,22,23].

In previous *in vitro* [16] and *in vivo* [17] studies, we showed that the implant material's surface largely dictates the morphology and composition of the formed tissue. Based on these studies, we selected the optimal polymer compound and surface to generate a tissue capsule that forms a suitable basis for a TEBV. Our previous *in vivo* rat study revealed that the tissue capsules generated with this method were well vascularized capsules largely composed of circumferentially aligned collagen and (myo)fibroblasts. Here, we describe the development of TEBVs in a porcine model and the remodeling towards a vascular phenotype upon grafting as autologous carotid interposition graft.

2. Materials and methods

2.1. Study design

Experiments were approved by the Animal Care Committee of the University Medical Center Utrecht and performed according to Dutch legislation, using 4 female Landrace pigs weighing approximately 40 kg. Cylindrical shaped polymer rods as described below more detailed were implanted subcutaneously. Four weeks later, rods with tissue capsules grown around it were harvested. Per pig, 2 tissue capsules were used as autologous vascular graft for bilateral carotid artery interposition. Thus, in total 8 tissue capsules were bilaterally grafted. Remaining tissue capsules were used for histology, RNA isolation and mechanical assessment. A biodegradable, elastic sheet was wrapped around the vascular graft and anastomosis for temporal external support during the first period of tissue capsule remodeling after exposure to flow. As standard antiplatelet therapy [24], pigs received 80 mg/d acetylsalicylic acid starting 6 days before vascular surgery until termination and 225 mg clopidogrel one day before vascular surgery, continued at 75 mg/d until termination. Patency was assessed by angiography 1 week and 4 weeks after vascular surgery whereupon pigs were sacrificed and tissue capsules were harvested. Tissue capsules before vascular grafting were compared to tissue capsules 4 weeks after vascular grafting to evaluate tissue remodeling after exposure to flow and strain.

2.2. Implant material

Solid cylindrical rods of 8 cm length and 4.2 mm diameter were fabricated using a stainless steel compression molding device. Rods were composed of the co-polymer poly(ethylene oxide terephthalate)–poly(butylene terephthalate) (PEOT/PBT, Polyvation, The Netherlands), with a PEOT/PBT weight percentage of 55/45 and 300 g/mol molecular weight of the initial polyethylene glycol used for the copolymer reaction. The implant surface was modified by etching with chloroform as previously described [16,17]. Surface topography of all modified rods was evaluated using SEM. In addition, surface roughness (Rq) of unmodified and chloroform etched rods was measured with AFM, using Tapping 1 Mode (PicoScan Controller 2500, Molecular Imaging, USA) with a sharp TESP cantilever: 42 N/m, 320 kHz, 2–5 nm ROC, No Coatings (Bruker AFM Probes). For unmodified and modified rods, 4 rods were analyzed, using 4 spots of 25 μm^2 per rod. Roughness was assessed with Scanning Probe Image Processor, SPIPTM, version 4.2.2.0 software.

External electrospun sheets were composed of poly-ε-caprolactone (PCL) (Purac Biomaterials, The Netherlands). Rods and sheets were sterilized using gamma-radiation of >25 kGy (Synergy Health, The Netherlands). The effect of gamma-radiation on the surface was evaluated using SEM.

2.3. Surgical procedures

Per pig, 6 rods were implanted subcutaneously in the abdominal area. A small incision was made and a pocket of 8 cm length was bluntly prepared. Rods were inserted in the pocket and attached to the abdominal wall using 4-0 prolene sutures. The skin was closed intracutaneously. Four weeks after insertion of the rods, tissue capsules with the rods were harvested. In short, a longitudinal incision lateral to the rod was made and the tissue capsule was gently removed from the surrounding tissue. After harvesting, rods could easily be extruded from the tissue capsule. Subsequently, tissue capsules were bilaterally inserted as autologous carotid artery interposition. Briefly, a midline incision in the neck was made and the carotid artery was dissected. After infusion of 100 IU/kg heparin intravenously, the carotid artery was clamped proximally and distally of the anastomosis site. A 3 cm segment of the carotid artery was excised and replaced by 4 cm tissue capsule using 2 end-to-end anastomoses. Subsequently, the flow was measured using a perivascular flow probe. Next, the external sheet was wrapped around the tissue capsule and covered 1 cm carotid artery adjacent to each anastomosis. After wrapping around the graft, the 2 neighboring ends of the sheet were interconnected using 6-0 sutures as illustrated in Fig. 1D. To assess patency, an angiography was performed 1 week after vascular grafting and before termination at 4 weeks after grafting. The excised carotid artery segment was used for histological assessment and RNA isolation.

2.4. Cannulation of tissue capsule

In each pig, one of two grafts was cannulated with a 16G dialysis needle. After removal of the needle, the cannulation site was digitally compressed for at least 3 min or until hemostasis was reached. Time to hemostasis was recorded.

2.5. Tissue capsule analysis

Tissue capsules before and after vascular grafting were processed and paraffin-embedded. Serial 5 μm cross-sections of the middle of the tissue capsules before and after grafting were made for morphometric and (immuno)histochemical analysis. Moreover, RNA was isolated from tissue capsules before and after vascular implantation and from a non-operated carotid artery. RNA was profiled (AROS, Danmark) using a porcine microarray (Affymetrix, Germany). A heatmap of all 3 groups was generated. Subsequently, gene profiles of tissue capsules before grafting were compared to tissue capsules after grafting. Genes with ≥8-fold difference and $p \leq 0.0001$ were selected. In addition, genes associated with ECM