Can a Peritoneal Conduit Become an Artery?

Petter Davik, Zuzana Chabado, Martin Altreuther, Ingeborg Leinan, Sashidar Bandaru, Levent M. Akyürek, Erney Mattsson

Objective: Current vascular grafts all have limitations. This study examined peritoneum as a potential graft material and the in vivo transfer of peritoneum into a functional artery like conduit after end to end anastomosis into the common carotid artery of sheep. The aim was to investigate whether implantation of a peritoneal tube into the arterial tree results in a structure with function, histological findings, and gene expression like an artery, and whether such arterialisation occurs through a conversion of the phenotype of peritoneal cells or from host cell migration into the implant.

Methods: Peritoneum with adherent rectus aponeurosis from sheep was used to form tubular vascular grafts that were implanted into the common carotid artery of six sheep, then removed after five months. Two sheep received allogenic peritoneal grafts and four sheep received autologous peritoneal grafts.

Results: One sheep died shortly after implantation, so five of the six sheep were followed. Five months after implantation, four of the five remaining grafts were patent. Three of four patent grafts were aneurysmal. The four patent grafts had developed an endothelial layer indistinguishable from that of the adjacent normal artery, and a medial layer with smooth muscle cells with a surrounding adventitia. The new conduit displayed vasomotor function not present at the time of implantation. DNA genotyping showed that the media in the new conduit consisted of recipient smooth muscle cells. Little difference in mRNA expression was demonstrated between the post-implantation conduit and normal artery.

Conclusion: During a five month implantation period in the arterial system, peritoneum converted into a tissue that histologically and functionally resembled a normal artery, with a functional genetic expression that resembled that of an artery. Single nucleotide polymorphism analysis indicated that this conversion occurs through host cell migration into the graft.

INTRODUCTION

All current vascular grafts have inherent limitations. Synthetic grafts generally lack anticoagulative properties and are at risk of infections and of developing intimal hyperplasia, while autologous grafts are present in limited supply.

Cells have previously been thought to retain little plasticity (ability to change phenotype) beyond a certain stage of differentiation and maturation. In recent in vitro studies, mature cells from all germinal cell layers have been converted into other cell types. For example, fibroblasts have been converted into cardiomyocytes, neurons, hepatocytes, and β cells by exposing them to specific transcription factors. It was hypothesised that cellular conversion may also occur because of an altered in vivo environment and that this could be exploited to form a new arterial graft.

Peritoneum is covered by mesothelium, a thin layer of cells with anticoagulative properties. Peritoneum and blood vessels both stem from the mesoderm germinal cell layer, which might facilitate a cellular conversion between the two. The internal abdominal rectus fascia provides strength to the abdominal wall and could possibly prevent a peritoneal vascular graft from rupturing, like the adventitia of a normal artery.

The aims of the study were: firstly, to investigate whether transplantation of a peritoneal tube into the arterial tree results in a structure with function, histological findings,
and gene expression like an artery, and secondly to investigate whether such arterialisation occurs through conversion of peritoneal cells or from host cell migration?

**METHODS**

Sheep of the Dalasau type, a Norwegian crossbred strain were used (n = 6; three male and three female; weight 30—42 kg). Each sheep underwent peritoneal arterial graft implantation and graft removal five months later. Sheep received pre-operative sedation with xylazine 0.2 mg/kg (Xysol vet., CP-Pharma Handelsges. mbH, Burgdorf, Germany) and ketamine 7.5 mg/kg (Ketalar, PfizerAS, Lysaker, Norway), and 2.5 mg/kg of low molecular weight heparin (Klexane, Sanofi-Aventis, Oslo, Norway) at the time of induction. General anaesthesia was induced by atropine 1 mg (Takeda AS, Asker, Norway) and ketamine 100 mg and maintained by isoflurane (Baxter AS, Oslo, Norway) inhalation anaesthesia with additional ketamine boluses of 50—100 mg.

An anterolateral laparotomy was made and a sheet (6 × 8 cm) of peritoneum with adherent rectus aponeurosis was collected. The tissue defect and laparotomy incision were closed with absorbable sutures (Vicryl 2.0 and 3.0, Ethicon Inc., Bridgewater, NJ, USA). The diameter of the common carotid artery was estimated by Doppler ultrasound (range Inc., Bridgewater, NJ, USA). The diameter of the common carotid artery was clamped. The artery was closed with absorbable sutures (Vicryl 2.0 and 3.0, Ethicon

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Five months after implantation, a 2 cm length removed. Arterial samples were

Figure 1. Left: Peritoneal graft ready for implantation. Sheath of peritoneum with rectus aponeurosis placed around a piece of sterile plastic tubing 6 mm in diameter and sutured with a single, longitudinal row of interrupted, non-absorbable 6–0 sutures. Right: Peritoneal graft implanted into the transected common carotid artery with continuous 6–0 non-absorbable suture. C = common carotid artery; P = peritoneal graft.
collected and frozen (−80°C). Samples for mRNA sequencing were collected from all harvested grafts and from arterial tissue and immediately frozen (−80°C). Samples for immunohistochemical analyses were collected from all grafts and arterial tissues at harvesting and placed in formalin.

**Immunohistochemistry**
Sections of peritoneum, native arterial tissue, and post-implantation grafts were embedded in paraffin after formalin fixation. Slides were stained with haematoxylin and eosin (H&E). Immunohistochemical staining was performed for α-actin (Alfa-actin Anti-alpha smooth muscle Actin antibody ab5694, Abcam, Cambridge, UK) as a marker for arterial smooth muscle cells and von Willebrand factor (Anti-von Willebrand Factor F3520, Sigma–Aldrich, Saint-Louis, MO, USA) as a marker of endothelium. Sheep arteries were used as positive controls, and the primary antibody was omitted as a negative control (see Supplementary material, Methods for details). Conduit collagen subtype analysis was attempted but unsuccessful, as no working primary antibodies for sheep could be found.

**Vasomotor activity**
Vasomotor analysis was performed on the four patent grafts using wire myographs (Danish Myo Technology, Hinnerup, Denmark), where static contractile forces in cross sections of vascular grafts, and sections of normal sheep artery as control, were measured before and after the five month implantation period. Sections were threaded onto myograph wires and submerged in continuously aerated Krebs buffer solution throughout the analyses. After reaching a stable resting tension, potassium and then incremental concentrations of phenylephrine (Abcur AB, Helsingborg, Sweden), acetylcholine (Novartis Norway AS, Oslo, Norway), and nitroprusside (Pfizer AS, Oslo, Norway) were added, while the vasomotor responses were recorded.

**Laser capture microdissection and DNA extraction**
Two sheep received allogenic transplants. Tissue was isolated from the “media” of the graft to determine whether arterialisation had developed through cellular conversion of transplanted peritoneal cells or cell migration into grafts from the recipient. The smooth muscle tissue on the luminal side of the external layer was prioritised, which invariably stained positive for α-actin, as this was deemed the most likely to yield a representative genotype of the post-implantation conduit. Fresh frozen samples of post-implantation grafts were cryosected in thin serial sections (10 μm). Every second section was stained with haematoxylin and used as a map to identify the region of interest in the adjacent unstained section (Fig. 2). The unstained sections were washed in Milli-Q (Merck-Millipore Corporation, Burlington, MA, USA) water and then refrozen (−80°C) until laser capture microdissection could be performed. The microdissected tissue was refrozen and genomic DNA extracted for subsequent SNP analysis (see Supplementary material, Methods for details).

**SNP genotyping**
SNPs may be unique to an individual and may be used to differentiate between individuals. SNP genotyping was performed on the microdissected tissue from the peritoneal conduits, on arterial tissue from transplant recipients, and on peritoneum from donors (see Supplementary material, Methods for details).

**RNA extraction and purification method, mRNA sequencing**
Samples for RNA sequencing were frozen at −80°C immediately after collection and kept frozen until extraction and purification (see Supplementary material, Methods for details).

**Ethics statement**
Ethics approval was granted by the Norwegian Animal Research Authority (NARA)/Forsøksdyrutvalget (FDU). Animal welfare was ensured by strict adherence to the approved study protocol and continuous supervision by veterinary staff at the St Olav Hospital animal research facility (reference 2012/119557).

**RESULTS**

**Graft patency and integrity**
One sheep developed an early pseudo-aneurysm and was euthanised shortly after implantation (female sheep, autologous graft). On ultrasound examination three months
after implantation, patency was uncertain in one graft. At removal, this graft was occluded with old thrombus (male sheep, autologous graft). The remaining four grafts were patent without thrombosis (two autologous, two allogenic), but three of four had fusiform aneurysms. Two male sheep with allogeneic grafts from female sheep had aneurysms of 1.3 cm and 1.9 cm. One female sheep with an autologous graft had a 2.5 cm aneurysm. The remaining autologous graft, in a female sheep, was patent and without aneurysm, diameter 9 mm. There were no ruptures or dissections (Table 1).

**Macroscopic findings**

During the implantation period peritoneum with rectus aponeurosis changed from a tissue only a few cell layers in thickness into a fibromuscular tube with wall thickness up to two mm, similar to the adjacent normal artery.

**Histological findings**

The inner surfaces of the four patent post-implantation grafts had a luminal layer indistinguishable from the endothelium of adjacent native artery. The cells stained positive for von Willebrand factor (Fig. 3), suggesting the presence of endothelial cells. External to this, in all patent post-implantation grafts, a layer of cells with a positive α-actin stain organised in a stratified circular pattern was seen, suggesting the presence of smooth muscle cells. Most externally, a layer resembling an adventitia was seen (Figs 2 and 5). Pre-implantation peritoneal grafts were almost acellular (Fig. 4). Post-implantation grafts displayed dense cellularity (Fig. 5). For comparison, a normal artery is shown in Fig. 6.

**Vasomotor analysis**

The peritoneal grafts had no vasomotor activity on implantation. After the implantation period the conduits were able to vasoconstrict when exposed to potassium and phenylephrine and to vasodilate in response to nitroprusside. No endothelium dependent vasodilation was found with acetylcholine. The responses were weaker than with the adjacent artery (Fig. 7) (combined vasomotor data given in Supplementary material, Results).

**SNP genotyping of allogenic transplant grafts**

To determine whether the “media” cells in the two cases of allogenic transplant grafts originated from the graft donor or recipient, SNP analysis was performed. Eighty SNPs were genotyped from peritoneum from donors, from arterial tissue from recipients, and from the “media” tissue obtained from laser capture microdissection. In both transplants 27 SNP loci genotypes could not be identified because of insufficient sample DNA or because of a deletion of one or both alleles. The same 53 SNPs remained for analysis from all samples. Donor and recipient genotypes were identical in 26 SNP loci for the first transplant, and in 27 SNP loci for the second transplant, and therefore could not be used to determine the origin of the “media” tissue.

**Table 1. Summary of results/findings.**

|                        | Graft patency | Vasomotor activity | Smooth muscle cells | Endothelial lining | “Media” SNP genotype |
|------------------------|---------------|--------------------|---------------------|--------------------|----------------------|
| Peritoneum prior to implantation (n=6) | N/A | Present in 0/6 | Present in 0/6 | Present in 0/6 | N/A |
| Post-implantation - autologous graft (n=3) | 2/3 grafts patent (1 graft thrombosed) | Present in 2/3 | Present in 2/3 | Present in 2/3 | Graft recipient genotype |
| Post-implantation - allogenic graft (n=2) | 2/2 patent | Present in 2/2 | Present in 2/2 | Present in 2/2 |  

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Figure 3. Luminal layer. Post-implantation graft with a positive cytoplasmic staining for von Willebrand factor indicating a luminal layer comprising endothelial cells. L = lumen; E = endothelial cell.

Figure 4. Pre-implantation peritoneal graft. Cross section of peritoneal graft with α-actin stain before implantation. This figure shows the absence of smooth muscle cells in the pre-implantation peritoneal tissue. L = lumen; P = peritoneum; R = internal abdominal aponeurosis.
In remaining SNPs, loci genotypes were either homozygously different, for example AA and GG (in five and six loci), or heterozygously different, for example AA and GA, (in 22 and 20 loci). In all SNPs where donor and recipient genotypes differed, the post-implantation conduit “media” tissue genotype corresponded with that of the graft recipient, and not the donor (for SNP genotyping data, see Supplementary material, SNP sequences and map).

**mRNA sequencing results**

mRNA sequences were prepared from all post-implantation grafts as well as from normal arteries. On comparison there was very little difference between the post-implantation conduits and normal arteries. Only four to seven in ~20,000 (0.025%) of sequenced mRNAs had significantly different expression (padj < .05). This suggests only small functional genetic differences between the two tissues. Because of a low number of samples, further conclusions could not be drawn.

**DISCUSSION**

Four of six peritoneal grafts remained patent, with no evidence of thrombosis at removal after five months. Grafts
were harvested after five months as this was deemed sufficient time for any adaptive changes to have become apparent. Post-implantation grafts displayed markedly increased cellularity and wall thickness, as well as organisation into an endothelial layer, a medial layer with smooth muscle cells, and an outer adventitial layer, much like an artery (Figs 5 and 6). Grafts gained the ability to vasocostrict and to vasodilate in response to endothelium-independent vasoactive substances, while endothelium dependent vasodilatation was absent. This absence could be because of reduced function of the endothelial layer, of the remaining graft wall, or of both. The abdominal apononeurosis provided insufficient support to prevent aneurysmal dilatation but prevented implanted grafts from rupturing during the five month observation period.

In previous studies, arterial grafts have been made by implanting silastic tubing into the peritoneal cavity of rats and rabbits, and sheep. This initiated a foreign body response, which over some weeks resulted in tubes of granulation tissue covered by an antithrombotic mesothelium. The tubes were harvested, inverted, and implanted into arteries with the mesothelial surface towards the lumen, and remained patent after four months. The abandoned Sparks’ Mandril graft was made by subcutaneously implanting a 5.1 mm silicone mandril covered by a polyethylene mesh (Dacron) into patients. After 5–12 weeks this resulted in Dacron supported fibrous conduits, which were implanted as arterial grafts and followed. Early thrombotic occlusion occurred in 17% of cases, and late occlusion from neo-intimal hyperplasia occurred in 44%, while 20% of grafts became aneurysmal.

These methods are quite different to those used in the present study. The de novo created granulation and fibrous tissue tubes represent immature and reactive, rather than a mature, differentiated tissue. The clinical applicability of these methods is limited as the foreign bodies must be implanted, and several weeks are required for the foreign body reaction to take place. Peritoneum and adjacent internal abdominal fascia are immediately and easily available at laparotomy.

Peritoneum with an adjacent internal abdominal fascial layer has been used in humans to replace the inferior vena cava after resection, and in dogs for iliac artery replacement. The autologous peritoneal arterial grafts in dogs had high patency rates (37 out of 41 grafts) and showed signs of arterialisation at three and six months; however, neither vasomotor activity, nor the question of how arterialisation occurred were investigated in that study. In the present study, aneurysms developed in three of four arterial interponates (40% rupture at three months in sheep). The underlying mechanism for aneurysm development in the peritoneal arterial graft is not understood, but the allogenic transplant seems to provide a reproducible model for creating fusiform large artery aneurysms in a large mammal with low risk of rupture and of occlusion from thrombosis. The fate of established aneurysms beyond five months’ implantation was not elucidated in the present study.

Over five months peritoneal grafts transformed into conduits that functionally and histologically came to resemble arteries much more than they resembled the implanted peritoneum. Autologous and allogenic transplants had the same post-implantation histology and vasomotor findings. SNP analysis of the DNA from the new “media” layer in the two allotransplanted grafts corresponded with graft recipient genotypes, not that of graft donors. The “media” cells thus originated from the recipient, and migration of host cells into the grafts had taken place. The nature of these host cells and the mechanisms that lead to their migration, as well as how they contribute to arterialisation and perhaps to aneurysm formation, remain unclear. Little difference in the functional genetic expression was demonstrated between the post-implantation conduits and normal arteries. The present study shows that mechanisms exist whereby the structure and function of a mature peritoneal conduit can be altered to resemble that of an artery, simply by placing it in the arterial system.

**Limitations**

All grafts were harvested after five months. It is not known how implanted peritoneal grafts would have evolved beyond this time point. They could have gained or lost vasomotor function, and become more, or even less, aneurysmal. Therefore, it is a limitation of this study that the observation period was not staged, and prolonged. Graft analyses were not performed at different distances from the anastomoses, and therefore it is not known whether host cell migration into grafts occurs from the adjacent artery or from elsewhere. It is not known whether similar biological mechanisms exist in humans.

**Conclusions**

Implantation of peritoneum as an arterial graft in sheep over the course of five months resulted in a conduit that
histologically and functionally resembled an artery more than it resembled peritoneum. This process of arterialis-
tion seems to occur by a migration of host cells into the
peritoneal transplant.

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CONFLICT OF INTEREST
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APPENDIX A. SUPPLEMENTARY DATA
Supplementary data related to this article can be found at
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