Tissue-Engineered Blood Vessels

Alternative to Autologous Grafts?

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Abstract—Although vascular bypass grafting remains the mainstay for revascularization for ischemic heart disease and peripheral vascular disease, many patients do not have healthy vessels suitable for harvest. Thus, prosthetic grafts made of synthetic polymers were developed, but their use is limited to high-flow/low-resistance conditions because of poor elasticity, low compliance, and thrombogenicity of their synthetic surfaces. To fill this need, several laboratories have produced in vivo or in vitro tissue-engineered blood vessels using molds or prosthetic or biodegradable scaffolds, but each artificial graft has significant problems. Recently, conduits have been grown in the peritoneal cavity of the same animals in which they will be grafted, ensuring no rejection, in the short time of 2 to 3 weeks. Remodeling occurs after grafting such that the tissue is almost indistinguishable from native vessels. This conduit is derived from cells of bone marrow origin, opening new possibilities in vascular modeling and remodeling. (Arterioscler Thromb Vasc Biol. 2005; 25:1128-1134.)

Key Words: artificial arteries ■ bypass grafts ■ myofibroblasts ■ tissue engineering ■ tissue scaffolds

Vascular bypass grafting is the mainstay of revascularization for ischemic heart disease and peripheral vascular disease, and in the US alone 1.4 million arterial bypass operations are performed annually. However, ~100 000 patients have no suitable autologous arteries or veins.1 Synthetic materials (mainly Dacron and polytetrafluoroethylene; PTFE) are frequently used for treatment of peripheral vascular disease, but they are limited to high-flow/low-resistance conditions2,3 because of poor elasticity,4 low compliance, and thrombogenicity of synthetic surfaces.5 Autologous grafts for treatment of coronary artery disease include the saphenous vein, internal mammary artery, and radial artery.6–8 In contrast to venous grafts, which are prone to thrombosis, occlusion, and aneurysm,9,10 arterial grafts have superior clinical and angiographic outcomes attributed to greater prostacyclin production,11 better blood supply via vasa vasorum,12 and better concordance with native coronary artery diameter. It is on this basis that several groups have used gene therapy to engineer the desired end product. Herein, we describe several in vitro and in vivo methods used to produce artificial arteries, including our own in vivo intraperitoneal model.

Engineering Artificial Arteries In Vivo

Improving Prosthetic Vascular Grafts

Attempts to improve synthetic polymer grafts have included embedding them with antithrombotic drugs, seeding with endothelial cells, or developing new biomaterials. Although heparin-coated grafts have had better results than standard prostheses, improvements have in general been marginal,14 and heparin is rapidly lost to plasma.15 Extensions of this approach have included embedding grafts with dipyridamole,16 hirudin,17 tissue factor pathway inhibitor,18 or non-thrombogenic phospholipid polymer.19 The surface texture of prostheses has also been altered in an attempt to increase patency and promote endothelialization.20

In other studies, prosthetic grafts have been seeded with endothelial cells, with a view to reducing thrombotic occlusions.21 Sources of endothelial cells include veins, adipose tissue capillaries, blood-borne cells, or circulating CD34+ stem cells.22 Because attachment of endothelial cells to naked prosthetic surfaces is poor,23 graft material is often altered by coating with RGD-sequence peptides,24 matrix proteins such as fibronectin,25 growth factors such as fibroblast growth factor26 or endothelial cell growth factor27 or by combination coatings28 to enhance retention. Because seeding cells onto the graft surface at the time of implantation does not appear to improve graft patency in humans,29 a complex 2-stage operation is required that involves harvesting the endothelial cells, seeding them onto the graft material, and culturing to confluence before grafting. This technique has had some success when used for synthetic grafts in peripheral30 and coronary revascularization.31 Other cell types that have been seeded onto prosthetic grafts include mesothelial32 and bone marrow33 cells.

Polyurethanes have been investigated as alternative graft material because they are more compliant than Dacron and

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PTFE, and thus their mechanical and flow parameters are better matched to those of the native vasculature. Early attempts using polyurethane led to high rates of aneurysm formation and thrombosis compared with conventional prosthetic grafts. However, there has been renewed interest in using modified forms of polyurethane grafts that are bio-

Natural Scaffolds From Tubular Organs

Visceral smooth muscle organs contain similar extracellular matrix proteins to blood vessels, and decellularized scaffolds from these organs have been used extensively as vascular grafts without the need for ex vivo bioreactors. However, the methods used to produce these scaffolds can be problematic, and treatment with glutaraldehyde to reduce antigenicity has been associated with poor outcomes. Despite this, Lantz found that 28 days after grafting dog jejunal submucosa as an autologous vascular graft, the tissue was covered by a layer of endothelium and had a histological appearance similar to native vasculature. On long-term follow-up, there was no evidence of graft failure, suggesting that in contrast to prosthetic grafts, in which infection is particularly problematic, this graft material may be infection-resistant. This favorable result was confirmed by Huynh et al who used an acellular submucosal graft enriched with bovine collagen. After implantation, remodeling of intestinal submucosal grafts resulted in mechanical properties similar to normal vasculature. When grafted into pigs with peritoneal contamination, intestinal submucosa showed significantly lower rates of graft infection and a trend to lower rates of pseudoaneurysm compared with standard PTFE prostheses, indicating that these grafts may confer advantages over synthetic prostheses when deployed in a contaminated field.

Other xenogeneic organs used as scaffolds include decell-

Growing Arteries Using Mandrels

Mandrels, in contrast to scaffolds, are molds that allow the formation of an artery and are removed before implantation. Tsukagoshi et al used the host’s inflammatory reaction to a foreign body (the mandrel) to create a vascular graft in vivo using autogenous fascia as a framework for a fibrocollagenous tube. Dorsal fascia was harvested from rabbits and wrapped around a silicone rod before subcutaneous implantation into the thigh. One month later, the implant was excised, the silicone mandrel was removed, and the tube was used as vascular graft; 73% patency was observed. Unfortunately, there was neointimal in-growth at the anastomotic ends of the graft, similar to the pattern seen with prosthetic grafts. This approach had already been tried in humans by Sparks in 1969, but with high rates of thrombosis and aneurysm. This may have been because of variable tissue quality in grafts generated from elderly patients in areas of poor blood flow.

Engineering Artificial Arteries In Vitro

Permanent Prosthetic Scaffolds

Weinberg and Bell created the first tissue-engineered vascular graft by culturing bovine fibroblasts and smooth muscle cells on a Dacron mesh before seeding with endothelial cells. The in vitro burst strength of this graft was 323 mm Hg. More recently, collagen and dermator sulfate have been embedded into prosthetic scaffolds before seeding with fibroblasts or smooth muscle cells and endothelial cells. Collectively, this approach has produced patency rates in the order of 80% at 23 to 26 weeks after grafting into animal common carotid arteries. Variations on this approach include the use of endothelial progenitor cells and mesothelial cells in place of endothelial cells. Endothelial progenitor cells make a particularly attractive cell source because they can be collected from the patient’s peripheral blood and do not require invasive harvesting methods. Other modifications of the permanent scaffold paradigm include culturing cells under fluid flow conditions. A possible limitation of this approach is restricted vascular remodeling imposed by the permanent synthetic scaffold.

Biodegradable Scaffolds

This approach is similar to that used for permanent prosthetic scaffolds, except that the scaffold degrades as the artery is formed and remodeled. Shum-Tim et al demonstrated that a polymer of polyglycolic acid and polyhydroxalkanoate seeded with cells from lamb carotid arteries and grafted into lamb aortae had 100% patency at 5 months. In a similar study, Niklason showed 100% patency 4 weeks after implantation of pigs with modified polyglycolic acid scaffolds that had been seeded with bovine smooth muscle cells and exposed to pulsatile flow conditions for 8 weeks. Contractile response of these grafts was only 5% of normal rabbit aorta, but they had burst pressures in excess of 2000 mm Hg. McKee et al hypothesized that the production of strong engineered arteries in this model was limited by the tendency of smooth muscle cells to lose their phenotype in culture and divide a finite number of times before undergoing senescence. They therefore induced ectopic expression of the human telomerase reverse transcriptase subunit in adult human smooth muscle to extend the cells’ lifespan. Another scaffold material investigated for vascular grafting is esterified hyaluronic acid. This material is used in skin and cartilage tissue engineering and has the theoretical advantage that it is spontaneously degraded by hyaluronidases into products that induce production of extracellular matrix and angiogenesis. This is potentially important because biodegradable scaffolds may not always degrade completely and may induce an inflammatory reaction. Unfortunately, the grafts based on esterified hyaluronic acid had a low axial strength and high stiffness when compared with normal porcine arteries.

Mandrels as Molds

In this approach, the nascent vascular construct molds into the shape of a mandrel, which is removed before grafting. For
example, Hirai and Matsudau prepared an artificial tunica media by pouring bovine aortic smooth muscle cells and type I collagen into a glass mold and incubating in a 37°C oven.64 However, the resultant tissue was fragile, with a burst pressure of <100 mm Hg. A follow-up study in which the collagenous tubes were seeded with jugular endothelial cells and reinforced by wrapping with a Dacron mesh resulted in 64% patency 24 weeks after implantation into canine vena cava.65 Interestingly, these tubes remodeled with time; smooth muscle cells acquired a contractile phenotype, elastic lamellae were formed, and the specific gravity of tissues increased. Wrapping the exterior of the collagenous tube with segmented polyester or high-pore-density polyurethane improved the compliance of the resultant conduit and provided a burst pressure of 240 mm Hg66 and patency rate of 100% at 6 months.67 However, the use of reinforcing materials may impair vascular remodeling of grafts and therefore partially explains observed losses of compliance in the months after implantation.68 To improve the strength of grafts based on collagen gels without resorting to prostheses, Burgland et al constructed acellular sleeves of cross-linked collagen to act as a scaffold. Collagen and neonatal human dermal fibroblasts were added to this biological scaffold before seeding with human coronary endothelial cells. Brittleness was an issue with these glutaraldehyde cross-linked constructs and the authors noted that better results may be achieved with other methods of cross-linking.69

L'Heureux used a PTFE mandrel to engineer a vascular graft by seeding consecutive layers of human cells over a period of 12 weeks. Cultured vascular smooth muscle cells were placed on the mandrel to form the media of the engineered construct, then fibroblasts were wrapped around the media to form the adventitia before the mandrel was removed and endothelia seeded onto the exposed luminal surface. The resulting vessel had the ultrastructural and functional features of a normal artery and a burst pressure of 2000 mm Hg. However, it was not as compliant as native vasculature, and methods to make the vascular constructs more compliant tended to reduce strength.70 A nonendothelialized version of this graft was implanted into dogs and showed 50% patency at 1 week.71

In all these studies in which the cells are expanded in culture before tissue engineering an artery, numerous variables must be controlled72 because maturation conditions influence the properties of the end product.62,73–75 For example, addition of ascorbic acid and retinoic acid to culture medium enhances collagen production, and hence mechanical properties, of the vascular construct,76 whereas physical stretching of the graft, such as by pulsatile flow through the lumen, influences cell proliferation and differentiation.77–81

A New Approach

The Intraperitoneal Graft Model

The rationale for this approach stems from observations that implantation of foreign bodies in the peritoneal cavity initiates an inflammatory response and culminates in the production of a fibrous capsule that contains layers of myofibroblasts covered by a single layer of mesothelial cells.82 Importantly, mesothelial cells possess anticoagulant activi-

A, “Device” within which the tissue capsule has grown over 2 to 3 weeks in the peritoneal cavity of the dog. Cells floating in the peritoneal fluid have entered the “device” through holes in the sheath and formed a tissue capsule around an inner tube, whose diameter can be varied. The outer surface of the sheath had been coated with surfactant to prevent adhesions. The “device” was inserted (distal end first) through a 2- to 3-cm incision in the skin of the abdomen and a smaller incision in the underlying peritoneal wall and the flange (arrow) positioned on the outer surface of the peritoneal wall. Purse-string sutures sealed the peritoneal incision and 3 loose sutures held the flange in place, and then the skin incision closed. For harvest, an incision was made in the skin, the flange sutures were cut, and the “device” was slid out, followed by closure of the incisions. B, The tissue capsule and inner tube are removed from the outer sheath by cutting around a tab at its distal end and sliding them out. C, The tissue capsule can then be slid off the inner tube.

ty.32,83 Hence, eversion of this capsule forms a fibromuscular tube with a nonthrombogenic luminal lining.

In 1999, this knowledge was applied to the creation of an artificial artery (Figure). By implanting silastic tubing into the peritoneal cavities of rabbits and rats, free-floating avascular tissue tubes formed over 2 weeks, with few intestinal adhesions.84 When compared with native aortae by Western analysis and immunohistochemistry, the tissue tubes were shown to express similar levels of α-smooth muscle actin and desmin, less smooth muscle myosin heavy chain, but higher levels of β-actin and vimentin; collagen levels were similar to native aortae but elastin expression was low. Moreover, 3 months after autologous grafting into rat aortae, the volume fraction of myofilaments of the graft cells resembled that of the native artery, suggesting that the myofibroblasts had differentiated into smooth muscle-like cells. This remodeling effect was shown to be caused by mechanical factors (wall stretching), most likely pulsatile blood flow.75 Also, over the 4 months that the graft was implanted in the rat aorta, the construct remodeled such that it resembled the native vessel with regard to wall thickness, cell number, elastic lamellae in the media, and vasa vasorum in the adventitia. Functionally, the graft was responsive to vasoactive agents and displayed endothelium-dependent vasorelaxation in response to acetylcholine (∼10% to 20% response of normal aorta) by 6 weeks.
There was a patency rate of 68% in the absence of any heparin or spasmylecty over a period of up to 4 months. The presence of elastin in this engineered vessel after grafting is particularly encouraging, because the lack of elastin in engineered grafts is believed to cause late dilatation of engineered conduits in high-pressure circuits such as the aorta and thus may contribute to aneurysm formation. Further, elastin is believed to be important for graft survival and its degradation may contribute to adverse remodeling.

Lengths of tissue tubes have also been successfully grafted by end-to-end anastomoses into the carotid artery of the rabbit (1.9-mm diameter; 3-cm-long) and femoral artery of the dog (3.5-mm diameter; 7-cm-long), with 80% to 90% patency for at least 16 months in rabbits (MacGinley et al, unpublished data) and 6.5 months in dogs. In dogs, the burst strength of tissue tubes was identical to that of the native femoral and carotid arteries (>2500 mm Hg), with similar suture holding strengths of 10 to 11.5 N.

This model generates an artery over a relatively short period of 2 to 3 weeks and does not require any in vitro manipulation. It has not yet been grown in humans. However, an implantable “device” within which the tissue capsule grows, reducing the risk of adhesions, has recently been developed and tested in the dog peritoneal cavity before clinical trial (Figure A through C). Although it does require 2 surgical interventions, implanting the “device” into the peritoneal cavity is a simple process and graft retrieval from this natural bioreactor can occur simultaneously with bypass grafting. Also, by using different tubular molds, the engineered artery can be grown to any desired diameter (1.5 to 7 mm) and length (up to 25 cm, to date).

Can Bone Marrow Cells Produce Tissue Engineered Arteries?

Although foreign body reactions may be the bane of grafts using biodegradable polymers that have not fully degraded, they are the cornerstone of the intraperitoneal model. The silastic tube implanted in the peritoneal cavity serves as a foreign body that induces an inflammatory reaction in the host. This inflammation is then harnessed to create the graft from the host’s own cells, thus avoiding the problems associated with allograft rejection.

But what is the origin of these cells? To investigate this question, foreign bodies (silastic tubing or boiled blood clots) were inserted into the peritoneal cavity of mice. After 3 days after implantation, rounded CD45+ cells were attached to the surface; ultrastructurally, these cells resembled macrophages. The capsule matured over the 2-week implantation period such that it became a fibrous structure containing multiple layers of cells with ultrastructural and immunohistochemical features of myofibroblasts. Very few cells now stained with antibodies to CD45. To determine whether differentiation of bone marrow-derived cells had occurred, female mice were irradiated to destroy their bone marrow and then were transfused with bone marrow cells from congenic male mice. After 4 weeks, foreign bodies were implanted into the peritoneal cavity of these female mice and the resulting tissue capsule removed 14 days later. In situ hybridization showed that most of the smooth muscle-like cells of the mature capsule contained the Y-chromosome, thus indicating their donor bone marrow origin.

The same female mouse model with male marrow engraftment was used to show that cells of bone marrow origin are the major source of smooth muscle-like cells when the arterial media is severely damaged. This was contrary to accepted dogma that extensive smooth muscle cell migration and proliferation occurs at local sites to heal an injured artery. However, these findings were later confirmed by others using various models of vascular injury including restenosis after angioplasty, graft vasculopathy, and hyperlipidemia-induced atherosclerosis. Human studies in sex-mismatched transplant recipients have also shown that bone marrow cells contribute to graft vasculopathy in renal and cardiac allografts.

Similarly, Feigl et al found that a prosthesis made of athrombogenic polyurethane walls, but with a central portion of thrombogenic Dacron in contact with circulating blood, but not with the walls of the vascular conduit, stimulated remodeling of the Dacron surface that proceeded from activated mononuclear cells to myofibroblastic cells and endothelial formation.

This raises the intriguing possibility that the bone marrow-derived cells of the artificial artery formed in the peritoneal cavity may, in fact, be monocytic cells that have transdifferentiated into both smooth muscle and endothelial cells. Several lines of evidence give this hypothesis credence. For example, Cebotari et al showed that after implantation, decellularized vascular scaffolds became lined with cells coexpressing leukocyte (CD18) and endothelial (CD31) markers. Recently, endothelial progenitor cells were shown to express monocyte/macrophage markers such as CD14, Mac-1, and CD11c, whereas expression of progenitor-cell markers AC133 and c-kit was minimal. Importantly, these cells did not show significant proliferation but secreted angiogenic growth factors. Simper et al showed that with different culture media, human mononuclear cells, and purified CD34+ cells can give rise to smooth muscle cells expressing α-smooth muscle actin, myosin heavy chain, calponin, and the integrin α5β1, or to cells expressing typical endothelial cell markers CD31, von Willebrand factor, and vascular endothelial cadherin. Other studies have also suggested that endothelial progenitor cells are derived from monocytic cells, and that monocytes contribute to sites of angiogenesis. Monocytic cells localize at sites of arterial injury and invade the vessel wall after thermal vascular injury and coexpress macrophage and smooth muscle cell markers. The capacity for monocytes/macrophages to transdifferentiate to myofibroblasts has also been reported by several groups. However, whereas these studies are all supportive of monocyte transdifferentiation into cells of the blood vessel wall, the issue is not fully resolved because Sata et al showed that the cells responsible for neointimal hyperplasia in several models of vascular injury are c-kit+"sca-1"lin", raising the possibility that more primitive cells may be involved in this process.

Conclusion

Over the past 40 years, numerous approaches to make an artificial artery have been described. By using the body’s own
immune system, we have shown that tubular structures can be grown in the peritoneal cavity from cells of bone marrow origin over a 2-week period, and that they can function as autologous vascular grafts while undergoing extensive remodeling to resemble native vessels, complete with elastin. This raises the possibility that patients will be able to grow tissue-engineered arteries within their own body cavity according to need and to specified dimensions. This will eliminate the shortage of healthy vessels suitable for transplantation, and the use of artificial prostheses and scaffolds. We are now beginning to learn about the bone marrow origins of the cells of the graft, although hematopoietic cells are already well known to be involved in healing after vascular injury. With knowledge of the factors that control their differentiation along vascular pathways within the in vivo bioreactor, it may be possible to produce "off-the-shelf" tissue banks of vascular conduits that do not have the problems of senescence associated with adult vascular smooth muscle cells.

References

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