MINIREVIEW

Blood Vessel Replacement: 50 years of Development and Tissue Engineering Paradigms in Vascular Surgery

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Summary

The gold standard material in bypass surgery of blood vessels remains the patient's own artery or vein. However, this material may be unavailable, or may suffer vein graft disease. Currently available vascular prostheses, namely polyethylene terephthalate (PET, Dacron) and expanded polytetrafluoroethylene (ePTFE), perform well as large-caliber replacements, but their long-term patency is discouraging in small-caliber applications (<6 mm), such as in coronary, crural or microvessel surgery. This failure is mainly a result of an unfavorable healing process with surface thrombogenicity, due to lack of endothelial cells and anastomotic intimal hyperplasia caused by hemodynamic disturbances. An ideal small-diameter vascular graft has become a major focus of research. Novel biomaterials have been manufactured, and tissue-biomaterial interactions have been optimized. Tissue engineering technology has proven that the concept of partially or totally living blood vessels is feasible. The purpose of this review is to outline the vascular graft materials that are currently being implanted, taking into account cell-biomaterial physiology, tissue engineering approaches and the collective achievements of the authors.

Key words
Small-caliber vascular grafts • Synthetic polymers • Biomaterials • Tissue engineering • Stem cells • Dynamic bioreactor • Shear stress

Introduction

Atherosclerosis accounts for almost one half of all deaths in Europe (Stehouwer et al. 2009). Although advanced pharmacological and minimally-invasive techniques offer a growing therapy option (Met et al. 2008), a surgical bypass of blood vessels on the heart or on a lower extremity remains the procedure of choice in a number of patients (Fig. 1) (Guyton 2006, Norgren et al. 2007). This approach is also more cost-effective, and in particular preserves the quality of the patient’s life better than primary amputation of a limb (Cheshire et al. 1992). A synthetic tube or a vascular prosthesis has to be implanted when the patient’s own artery or vein is not available. After more than half a century of development work, the results achieved with currently available materials are not optimal in terms of healing and tissue regeneration.

Long-term patency rates of prosthetic grafts are satisfactory in large-caliber arteries (>8 mm), where thrombogenicity (i.e. disposition to blood coagulation) may be overcome by massive blood flow, and the 5-year patency of aorto-iliac substitutes is 90 % (Brewster 1997). There is little difference between the results for prosthetic and autogenous (i.e. patient’s own) material in medium-caliber replacements (6-8 mm), e.g. in carotid or common femoral arteries (Ricotta 2005). However, in small-caliber vessels (<6 mm), such as coronary arteries (heart), infragenual blood vessels (below the inguinal ligament), and particularly in low-flow infragenicular
arteries (below the knee joint), the outcomes of vascular prostheses are rather disappointing. Five-year primary patency of prosthetic (ePTFE) above-the-knee femoro-popliteal bypass grafts is as low as 39%, whereas autovenous bypasses (i.e. performed with own vein) have a rate of 74% (Klinkert et al. 2004). A summary of currently-used vascular replacements is shown in Table 1.

Currently available vascular grafts fail due to the thrombogenicity of the artificial surface and intimal hyperplasia (IH), which is located at distal anastomosis (Fig. 2) of prosthetic grafts (i.e. the site of the junction to the artery). Unlike animal models, most of the prosthetic blood-contacting surfaces remain uncovered by tissue in humans (Berger et al. 1972). The etiology of IH, developing usually 2-24 months post implantation, is multifactorial and includes a compliance mismatch between a relatively rigid prosthesis and the more elastic native artery (Sarkar et al. 2006), graft/artery diameter mismatch, lack of endothelial cells (EC), surgical trauma and flow disturbances resulting in adaptive changes in the sub-endothelial tissue, characterized by proliferation and migration of vascular smooth muscle cells (VSMC) from media to intima, and synthesis of extracellular matrix (ECM) proteins (Haruguchi and Teraoka 2003).

To overcome these fundamental inconveniences, novel biomaterials research (Shin and Mikos 2003) and particularly tissue engineering modalities are increasingly being adopted (Isenberg et al. 2006).

Promising and clinically proven outcomes have been achieved with biohybrid and tissue-engineered vascular grafts.

An ideal vascular graft would possess the following characteristics: mechanical strength and compliance to withstand long-term hemodynamic stresses; non-toxicity; non-immunogenicity; biocompatibility; “off-the-shelf” availability in various sizes for emergency care; operative suture ability and simplicity of surgical handling; resistance to in vivo thrombosis; ability to withstand infection; complete incorporation into the host tissue with satisfactory graft healing and ability to grow when placed in children (Kakisis et al. 2005), and, last but not least, reasonable manufacturing costs. The task is so demanding and the potential rewards are so great that research in the field of small-caliber vascular substitutes has been compared with the search for the Holy Grail (Conte 1998).

The aim of this review is to outline the characteristics and drawbacks of currently-used vascular substitutes, with special emphasis on the physiological cellular response to materials and on vascular tissue engineering approaches, based on the collective experience of the authors.

**Historical background**

The history of vascular surgery tracks progress made in suturing and replacing damaged vessels. In
ancient times, vascular interventions were limited to compressing and cauterizing injured vessels to control bleeding. Ambrose Paré developed a vessel ligature in the 15th century, but surgical repair of vessels did not begin until 1759, when Hallowell and Lambert repaired a brachial artery injury with a suture. The first vascular anastomosis (connection of blood vessels) was performed by Nicholas Eck as a porto-caval shunt in dogs in 1877 (Starzl 2003). Carrel and Guthrie optimized the vascular anastomosis transplantation technique and even tissue culture for organ replacement in the early 1900s, and Alexis Carrel received the Nobel Prize for Physiology or Medicine in 1912.

Goyannes first used an autogenous popliteal vein graft for popliteal aneurysm repair in 1906. A femoro-popliteal bypass with a reversed saphenous vein graft was first performed by Kunlin in 1948, initiating a very successful era for this type of graft that has lasted until the present day (Lopez and Ginzberg 2008). At the same time, the first fresh arterial allografts (foreign tissue of the same species) began to be used in human vascular reconstructive surgery (Gross et al. 1948). An artificial vascular prosthesis was first implemented as an aortic replacement with a Vinyon “N” tube in a dog experiment (Voorhees et al. 1952). The same material was implanted in humans to replace an aneurysm (dilation) of the abdominal aorta (Blakemore and Voorhees 1954), leading to rapid progress in vascular surgery and prostheses research and use (Sauvage 1986).

### Arterial replacements

#### Biological vascular grafts

The gold standard for vascular replacement remains the autologous native vessel, which possesses the most physiological properties. In coronary artery bypass grafting (CABG), the internal mammary artery and the radial artery are superior to a greater saphenous vein graft (SVG), which is also often used (Beghi et al. 2002). In the case of lower limb or peripheral bypass surgery, the material of choice is also greater saphenous vein (vena saphena magna). It can be cleansed of valves and anastomosed in situ, so that the vasa vasorum remain intact (Kachlik et al. 2007) and the blood flow direction is reversed. In this type of graft, preservation of desired endothelial properties can be expected; however, several studies have failed to confirm superiority to the more widespread ex vivo reversed setting of the SVG (Lawson et al. 1999). It should be noted that, despite good clinical performance, SVG is also liable to atherosclerosis and intimal hyperplasia occurring throughout the length (Jarjeant and Rabinovitch 2002). In addition, almost 30-40% of patients lack an appropriate saphenous vein (Faries et al. 2000) due to previous phlebitis, vessel removal, varicosities, hypoplasia or anatomical unsuitability. Additionally, any surgical vessel harvest is associated with indispensable donor site morbidity (Swenne et al. 2006).

Alternatives include the use of other native vascular materials only in carefully selected indications.
and sometimes with limited clinical performance: arteries, such as the right gastroepiploic artery for coronary application (Sasaki 2008), and veins, such as the lesser saphenous vein (vena saphena parva) (Chang et al. 1992), arm veins (Calligaro et al. 1997) for coronary and peripheral bypasses, or deep leg veins (Ali et al. 2009) for infected aortic graft replacement, visceral revascularization or even primary lower limb bypass.

Although the use of fresh (cold-stored) or cryopreserved homografts (i.e. human allografts from cadaver donors) was abandoned in the early 1960s because of difficulties in preserving them, late graft deterioration, aneurysm formation and the anticipated availability of synthetic prostheses, they have been reintroduced for managing aortic prosthetic graft infection (Kieffer et al. 2004), lower extremity primary revascularization (Dardik et al. 2002, Fahner et al. 2006, Matia et al. 2007) and simultaneous or sequential revascularization surgery in solid organ transplant-recipients (Matia et al. 2008). Although allografts are not routinely used (usually in limb-threatening situations, in redo surgery or in an infected field), some studies suggest improved patency of these grafts, e.g. in the case of preserved externally-supported human umbilical vein (harvested from newborns) when compared to ePTFE (Johnson and Lee 2000).

The application of heterografts (xenografts, i.e. tissue from different species) (Schmidt and Baier 2000) involves mainly studies on alternative hemodialysis vascular access. Although bovine carotid artery heterografts did not show superiority to PTFE (Hurt et al. 1983), and decellularized bovine ureteric grafts have been implanted with ambiguous results (Chemla and Morsy 2009), glutaraldehyde-crosslinked bovine mesenteric vein provided a considerable reduction in infection, thrombosis and reintervention rate (Katzman et al. 2005). Importantly, decellularized natural tissue of allogenic (human) or xenogenic (animal) origin serves as a scaffold for cell seeding within the scope of tissue engineering of vascular grafts (Dahl et al. 2003). A summary of biological vascular conduit materials is presented in Table 2.

**Synthetic vascular grafts**

For more than 50 years, two polymers have been used for synthetic vascular prostheses: 1) polyethylene terephthalate (PET), Terylene or Dacron, and 2) polytetrafluoroethylene (PTFE), Teflon or Gore-Tex. Both of these molecules are highly crystalline and hydrophobic. Prosthetic rings or coils can be applied to the external surface of both materials to resist kinking and compression in anatomically required positions (long bypasses, grafts crossing the joint or body midline).

PET/Dacron (polyethylene terephthalate, \[-O-C=O-C_2H_4O-C=O-CH_2-CH_2-\]) was introduced in England in 1939, and was further developed and patented as Dacron by DuPont in 1950. It is a thermoplastic polymer resin of the polyester family and is used in synthetic fibers of round cross-section. These fibers are bundled into multifilament yarns, which can be woven (over-and-under pattern) or knitted (looped fashion) into textile vascular graft fabrics and tubes. A crimping technique (an undulating surface) is sometimes used to increase distensibility and kink-resistance. The porosity of a textile Dacron graft is defined by water permeability, which is greater for knitted Dacron. Knitted Dacron is impregnated with albumin, collagen or gelatin to make it more impervious, to decrease the porosity/permeability, and to avoid the need for blood preclotting prior to implantation. Depending on the cross-linking agent (formaldehyde or glutaraldehyde), the albumin impregnation is degraded 2 to 8 weeks after implantation (Marois et al. 1996). Although collagen impregnation increased platelet deposition and delayed the healing process in a dog experiment (Guidoin et al. 1996), a prospective randomized trial with aorto-iliac prostheses indicated that collagen impregnation does not stimulate the coagulation cascade more than conventional Dacron (De Mol Van Otterloo et al. 1991). There are no differences in clinical graft patency between woven and knitted Dacron, when used as an aorto-iliac bypass graft (Quarmby et al. 1998). Dacron is reported to dilate over time, but direct association with graft complications and failure has been rare (Blumenberg et al. 1991).

Host reactions to the vascular prosthesis start immediately after restoration of blood circulation. The tissue-prosthesis and blood-prosthesis interfaces are complex microenvironments, and the physico-chemical properties of the surface of the prosthesis, such as charge, energy, wettability and roughness, may be responsible for the graft patency. The first step is the plasma protein adsorption/desorption process typical for any blood/material interface (Vroman and Adams 1969), followed by platelet deposition, white blood cell and erythrocyte adhesion, and eventually endothelial and smooth muscle cell migration. Fibrin deposits, which contain platelets and blood cells, build up during the first few hours to days after implantation. They are stabilized
over a period of up to 18 months and form an inner
compact fibrin layer. Fibrin also fills the interstices
within the graft wall. Generally, this fibrin
platelet pseudointima remains acellular; however, after 5 months,
capillaries and fibroblasts can grow into the tight
interstitial spaces even in humans (Stewart et al. 1975)
and reach the inner fibrin layer. Only a few sparse small
islands of endothelialization appeared in areas remote
from the anastomosis region on woven excised Dacron
grafts (Wu et al. 1995). External fibrin matrix
surrounding the graft is gradually invaded by
macrophages, and granulation tissue and foreign-body
giant cells (FBGC) are usually seen under the external
surrounding connective tissue capsule (Rahlf et al. 1986).

In the case of knitted Dacron, endothelial islands can
occasionally be observed on human explants during redo
surgery or autopsy 1-11 years after implantation (Shi et al. 1997).
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surrounding connective tissue capsule (Rahlf et al. 1986).

PTFE (polytetrafluoroethylene, [-CF₂-CF₂-]) was
patented by DuPont as Teflon in 1937, and ePTFE was
patented by Gore as Gore-Tex in 1969. ePTFE is an
expanded polymer which is manufactured by a heating,
stretching, and extruding process resulting in a non-
textile porous tube composed of irregular-shaped solid
membranes (“nodes”). The molecule is relatively
biostable, i.e. less prone to deterioration in biological
environments than PET (Guidoin et al. 1993a,b), and the
graft surface is electronegative, which minimizes its
reaction with blood components. It is characterized by a
node-fibril structure, and its average porosity is described
by the internodal distance (IND), which is usually 30 to
90 µm. However, the actual available ingrowth spaces
between fibrils are much smaller than IND.

Optimal IND of 60 µm (high porosity) was
experimentally proposed for tissue ingrowth and
endothelialization of 4mm ePTFE grafts in a baboon
model (Golden et al. 1990). Though a human trial with
high-porosity ePTFE showed capillary ingrowth, it did
not extend more than half the distance from the outside,
and did not produce an endothelial lining (Kohler et al. 1992). Human host responses to standard low-porosity

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**Table 2: Biological vascular grafts in clinical use.**

<table>
<thead>
<tr>
<th>Biological vascular grafts</th>
<th>Autografts</th>
<th>Allografts (homografts)</th>
<th>Xenografts (heterografts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>Venous</td>
<td>Arterial</td>
<td>Venous</td>
</tr>
<tr>
<td>Advantages</td>
<td>Closest approximation, less diameter mismatch, internal mammary artery anatomically nearby, excellent function</td>
<td>Durable and versatile, good results, infection resistance, relative availability</td>
<td>Off the shelf availability, better resistance to infection, transplant-recipient patients</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Availability, vasospasm (radial artery), donor site morbidity</td>
<td>Availability, harvest injury, vein graft disease</td>
<td>Antigenicity, graft deterioration, early occlusions, chronic rejection, intake of drugs, infection risk</td>
</tr>
<tr>
<td>Healing</td>
<td>Intimal thickening, myointimal hyperplasia (radial artery)</td>
<td>Endothelial desquamation, vein dilation, wall thickening, arterialization, re-endothelialization</td>
<td>Endothelial denudation, immune response, fibrotization</td>
</tr>
<tr>
<td>First use</td>
<td>Jaboulay and Briau 1896</td>
<td>Goyannes 1906</td>
<td>Gross et al. 1948</td>
</tr>
<tr>
<td>Review e.g.</td>
<td>Nezic et al. 2006</td>
<td>Cooper et al. 1996</td>
<td>Fahner et al. 2006</td>
</tr>
</tbody>
</table>
ePTFE (IND ≤ 30 μm) material are similar to the responses to Dacron grafts: a thin fibrin coagulum or amorphous platelet-rich material develops over time, and a lack of luminal surface endothelial cellular coverage is found after human implants. Collagenous external encapsulation develops within 1 to 6 months, penetrating the material structure with minimal cellular infiltration (Guidoin et al. 1993a). Systematic evaluation and meta-analysis of randomized controlled trials comparing Dacron and ePTFE showed no evidence of an advantage of one material over the other (Roll et al. 2008).

Polyurethanes (PU) comprise a large family of elastic polymers containing a urethane [-NH-(CO)-O-] group. Polyurethanes were originally developed in Germany in the 1930s. They were commercialized by DuPont in 1962, and have been available for biomedical applications since the 1960s (Boretos and Pierce 1967). Generally, they are copolymers consisting of three different monomers: crystalline (hard) and amorphous (soft) segments, the former accounting for rigidity and the latter for flexibility, which can be varied by the manufacturer. The third monomer serves as a chain extender (Zdrahala 1996). The disadvantage of the first generation polyester-based PU was hydrolytic biodegradation, which resulted in abortion of one of the clinical trials (Zhang et al. 1997). The next generation of polyether-based PU, which is hydrolysis-resistant but more susceptible to oxidation, underwent successful clinical assessment as hemo dialysis access graft (Glickman et al. 2001), and received Food and Drug Administration (FDA) approval in 2000.

The latest generation of polycarbonate-based PU is hydrolytically and oxidatively stable, and promoted faster luminal endothelialization and less neointimal formation as small-calibre vascular prostheses in a rat experiment compared to ePTFE (Jeschke et al. 1999). After 36-month implantation in aorto-iliac position in dogs, a histological analysis of poly(carbonate-urea) PU grafts showed well-developed neointima only in distal anastomosis, only minor hydrolysis of the amorphous segments and, in addition, the grafts retained their compliance with time – a feature not observed in Dacron or ePTFE. These promising results led to a phase I clinical trial (Seifalian et al. 2003).

According to their microstructure, polyurethanes can be divided into fibrillar or foamy. Both structures tend to lack communicating spaces for potential capillary ingrowth. Upon implantation, the blood surface of the fibrillar PU is covered by a fibrin layer that is thinner than on knitted Dacron, and the outer surface is encapsulated by scar formation containing FBGC. In microporous foamy PU with 15 μm pore size, relatively little capillary ingrowth can be accomplished, whereas with increasing pore size up to 157 μm, the undesired inflammatory FBGC reaction could be diminished and capillary sprouting was allowed from the outside in a Chacma baboon model. However, before its completion, transmural ingrowth slowed down when reaching the dense inner fibrin layer that was “squeezed” into the pores from the inside (Zilla et al. 2007).

Although PU grafts possess many interesting features, e.g. the presence of EC under poor hemodynamic conditions, excellent healing, good surgical handling and low suture bleeding (Tiwari et al. 2002), further investigations are required before more recommendations can be made, because there is a lack of evidence for their use in human peripheral bypass surgery (Dereume et al. 1993). An outline of synthetic vascular prostheses is summarized in Table 3.

Vascular grafts with modified lumen

Several attempts have been adopted to suppress the thrombogenicity of synthetic material by affixing chemicals or anticoagulants to the graft lumen: early studies showed decreased platelet deposition on carbon-coated ePTFE grafts, but the overall patency rates were not improved (Kapfer et al. 2006). A heparin-bonded Dacron graft exhibited slightly better patency than an untreated ePTFE graft (Devine et al. 2004), and heparin-immobilized ePTFE provided better thromboresistance in humans (Bosiers et al. 2006). Applying a polyethylene glycol (PEG)-hirudin/iloprost coating to 4 mm ePTFE prostheses reduced IH and led to superior patency in a pig experiment (Heise et al. 2006). Heparin coating significantly reduced aortic graft thrombosis in rats both for ePTFE and for PU (Walpoth et al. 1998). A dipyridamole (anti-platelet drug) coating positively influenced the patency rate of a 5 mm PU vascular prosthesis in animal experiments (Aldenhoff et al. 2001). Fibroblast growth factor-1 and heparin affixation enhanced ePTFE graft capillarization and surface endothelialization without significant IH in a canine aorta model (Gray et al. 1994). Antithrombotic agents are of course routinely administered to patients with vascular disease, with an obvious influence on graft patency and overall cardiovascular mortality (Watson et al. 1999).

Interestingly, an effort has been made to increase the resistance of synthetic vascular grafts to infectious
agents. Antibiotics such as rifampicin bonded to Dacron did not reduce the incidence of vascular graft infection (Earnshaw et al. 2000); however, a silver-coated collagen-impregnated Dacron prosthesis offers an alternative approach in the treatment of vascular graft infection (Mirzaie et al. 2007).

Composite and vein-interposition vascular grafts
Attempts at improving disadvantageous anastomotic hemodynamics include alternative surgical techniques and interposition of an autologous vein segment.

Despite the theoretical and experimental advantage of end-to-end (straight) rather than end-to-side (Y-shaped) anastomosis between the graft and the artery (O’Brien et al. 2007), clinical trials did not prove superiority, and moreover an increased limb amputation rate was revealed in the end-to-end setting, probably due to exclusion of collateral vessels (Schouten et al. 2005). Suturing materials and techniques may also decrease the anastomotic compliance difference, e.g. in the case of interrupted sutures (more than one single suture). However, running sutures are most widely used, because they are quicker and achieve better hemostasis (Tiwari et al. 2003).

Own venous tissue is placed in the form of a vein patch or cuff between an ePTFE graft and the artery to reduce the anastomotic compliance mismatch and to improve the prosthesis patency. Procedures implemented during the 1980s and 1990s include the Miller cuff, the Linton patch, the Taylor patch and the St. Mary boot (Moawad and Gagne 2003). When the patient’s own vein graft is of insufficient length, feasible options that increase the patency rates may be: a composite bypass—prosthesis and vein are spliced together with an additional anastomosis (Bastounis et al. 1999), a sequential bypass, which is formed as a distal venous extension graft to the preceding proximal prosthetic bypass (Mahmood et al. 2002), and a bridge graft, which connects two patent distal arteries with a short vein segment (Deutsch et al. 2001). Although adjunctive arterio-venous fistula placement increases the blood flow through femoro-distal (i.e. below knee) bypasses, thus theoretically preventing thrombosis, several trials failed to show evidence of

### Table 3. Synthetic vascular grafts in clinical use.

<table>
<thead>
<tr>
<th>Synthetic vascular grafts</th>
<th>PET (Dacron, Terylen)</th>
<th>ePTFE (Teflon, Gore-Tex)</th>
<th>Polyurethane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woven</td>
<td>Woven</td>
<td>Knitted</td>
<td>Fibrillar</td>
</tr>
<tr>
<td>Knitted</td>
<td>Low-porosity (&lt;30 μm IND)</td>
<td>High-porosity (&gt;45 μm IND)</td>
<td>Foamy</td>
</tr>
</tbody>
</table>

#### Advantages
- PET: Better stability, lower permeability and less bleeding
- ePTFE: Greater porosity, tissue ingrowth and radial distensibility
- Polyurethane: Biotability, no dilation over time

#### Disadvantages
- PET: Reduced compliance and tissue incorporation, low porosity, fraying at edges, infection risk
- ePTFE: Dilation over time, infection risk
- Polyurethane: Stitch bleeding, limited incorporation, infection risk, perigraft seroma formation

#### Healing
- PET: Inner fibrinous capsule, outer collagenous capsule, scarce endothelial islands
- ePTFE: Fibrin luminal coverage, very sporadic endothelium, transanastomotic endothelialization in animals
- Polyurethane: Luminal fibrin and platelet carpet, connective tissue capsule with foreign body giant cells, no transmural tissue ingrowth

#### First use
- Ku et al. 1957
- Norton and Eiseman 1975
- Boretos and Pierce 1967

#### Review e.g.
- Xue and Greisler 2003
- Nishibe et al. 2004
- Tiwari et al. 2002

IND (internodal distance).
benefit (Laurila et al. 2006).

Vein interposition techniques are generally useful in the case of distal anastomosis located below the knee, but are unimportant in the above-the-knee position (Mamode and Scott 2000). The underlying mechanism consists in reducing the compliance mismatch and suppressing IH (Cabrera Fischer et al. 2005). Research derived from this evidence led to the evolution of specially formed ePTFE grafts, capable of better harnessing the hemodynamic forces by an enlarged anastomotic hood. Interestingly, clinical studies have shown patency rates of this pre-cuffed ePTFE (Panneton et al. 2004) and carbon-lined ePTFE Distaflo graft (Fisher et al. 2003) that are comparable to interposition vein cuffs.

Biosynthetic/biohybrid vascular grafts (biografts)

The idea of introducing viable biological components into an artificial material-based vascular graft was established to produce more biocompatible vascular substitutes. A viable endothelial layer is the best antithrombogenic surface. There are three possible sources of graft lumen endothelialization in vivo post implantation: trans-anastomotic ingrowth from the native artery, transmural tissue and capillary ingrowth through the prosthesis wall, and a “fall-out” or blood-borne source from circulating progenitor cells. Unlike animals, humans seldom achieve spontaneous endothelialization more than 1-2 cm from an anastomosis, and transmural ingrowth is hampered by the structure of currently-used prostheses (Zilla et al. 2007).

The concept of seeding endothelial cells onto the graft lumen before implantation was experimentally implemented (Herring et al. 1978), and consequently this autologous EC “transplantation” managed to improve the patency of human Dacron prostheses (Herring et al. 1984) in a non-smoker population. Endothelial cells from a subcutaneous vein segment (e.g. saphenous, cephalic or jugular vein) are harvested and immediately seeded onto the graft lumen within the timeframe of one operation. The results of the first clinical trials performed during the 1980s were controversial and disappointing, mainly due to insufficient cell density (Bordenave et al. 1999). However, sophisticated EC extraction and retention techniques (Tiwari et al. 2001) and the search for more abundant cell sources, such as microvascular endothelium from fat tissue or an omentum biopsy, improved the outcome of this single-stage cell seeding method (Alobaid et al. 2005).

On the other hand, the double-stage approach involves a prolonged incubation and cell multiplication period (2-4 weeks) between cell harvesting and implantation (Bordenave et al. 2005). Nevertheless, in vitro flow pretreatment or shear stress preconditioning of the cell-material constructs before exposing them to arterial conditions enhances cell retention by inducing structural changes and adaptation (Rademacher et al. 2001). This means that an endothelial cell monolayer is physiologically exposed to certain mechanical forces, namely hydrostatic pressure resulting from blood pressure, shear stress resulting from tangential friction of blood flow against the vessel wall, and finally longitudinal and circumferential cyclic stretch resulting from repeated blood vessel distension due to the cardiac cycle (Lehoux et al. 2006). Chronic laminar versus turbulent shear stress seems to be of utmost importance for endothelial and vascular smooth muscle cell function (Chien 2007), regulating signal transduction in these cells (Daculsi et al. 2007), their alignment, molecule secretion, cytoskeleton reorganization, gene expression, cell migration, proliferation and survival. These processes ultimately influence thrombogenesis and atherogenesis (Yoshizumi et al. 2003), and similar cellular events and phenotypes (Rémy-Zolghadri et al. 2004, Fernandez et al. 2006) can be observed when cells are seeded on biomaterials (Fernandez et al. 2007).

Early results of clinical in vitro double-stage endothelialization were promising (Magometschnigg et al. 1992, Meinhart et al. 1997), and recently published overall 5-year and 10-year patency of endothelialized fibrin glue- precoated ePTFE femoro-popliteal bypass grafts of 69 % and 61 %, respectively (Deutsch et al. 2009) has already been correctly reported to close the gap between prosthetic and vein grafts (Meinhart et al. 1997). Interestingly, the feasibility of endothelium-seeded vascular prostheses was also confirmed in coronary bypass grafting, though it is not widely used (Laube et al. 2000).

To improve the above-mentioned clinical success, additional cell sources and cell retention technologies are being investigated. Vascular cells are anchorage-dependent, and integrin receptor-mediated adhesion occurs via ECM proteins that are adsorbed from the cell culture media in vitro or from blood in vivo (Bačáková et al. 2004). Thus, to regulate cell attachment, biomaterials are coated with ECM proteins such as collagen, laminin and fibronectin (Vara et al. 2005) or are modified by covalent bonding of short adhesive peptides.
which may be cell specific, such as the Arg-Gly-Asp (RGD) sequence (Bačáková et al. 2007). Fibrin, as a natural scaffold for cell migration and healing, plays a pivotal role in tissue engineering of vascular grafts (Filová et al. 2009). In addition, less specific physical surface modifications, non-ligand based techniques (Salacinski et al. 2001) and surface nanoarchitecture (de Mel et al. 2008) are being explored.

An alternative approach aimed at improving seeding efficiency is to use progenitor cells. Bone marrow cells infiltrated into the ePTFE vascular grafts and implanted in the aortic position of dogs retained complete endothelialization and patency after six months (Noishiki et al. 1996). Similarly, human endothelial progenitor cells (EPC) were isolated from peripheral blood (Asahara et al. 1997), and it has been shown in a canine model that a subset of CD34+ bone marrow cells can be mobilized to the circulation and can colonize the flow surfaces of Dacron vascular prostheses (Shi et al. 1998). Moreover, seeding of bone marrow-derived cells (BMC) accelerated early Dacron graft endothelialization without increasing thrombogenicity in a dog model (Bhattacharya et al. 2000). Taken together, due to their high proliferative capacity and differentiation potential, stem cells may represent the next era of cell-sourcing technology (Riha et al. 2005).

Genetically-modified cells have also been considered for the construction of vascular replacements. For example, genetically-modified endothelial cells over-expressing tissue plasminogen activator (t-PA) and urokinase-type PA, or bone marrow mesenchymal stem cells transduced to express endothelial nitric oxide synthase (eNOS), would promote cell repopulation of the graft and help to eliminate thrombotic events (Zarbiv et al. 2007). However, the use of genetically-modified cells inherently raises ethical questions. A conventional approach is directly to load the materials with anti-coagulant, anti-inflammatory and cell growth-regulating substances, such as heparin and heparin-like molecules, as mentioned above (Walpoth et al. 1998, Lee et al. 2002), hirudin (Heise et al. 2006), dipyridamole (Aldenhoff et al. 2001), growth factors, such as vascular endothelial growth factor (Ehrbar et al. 2005) or fibroblast growth factor (Gray et al. 1994, Sato et al. 2008), or antinflammatory and antiproliferative drugs paclitaxel (Lim et al. 2007), sirolimus (Ishii et al. 2008) and inhibitors of CDK2 kinase (Brooks et al. 1997). These drugs can be incorporated directly into the prosthesis wall or delivered through drug-eluting stents (Lee et al. 2008), catheters and perivascular collars (for review see Sriram and Patterson 2001). Artificial materials releasing NO are also being developed, consisting of synthetic polymers (e.g. polyurethane, PTFE) incorporated with NO donors, such as diazeniumdiolates and S-nitrosothiols (Varu et al. 2009).

Living vascular grafts, totally-engineered blood vessels (TEBV)

The concept of completely biological living grafts implies the ability to remodel, grow, self-repair and respond to the immediate environment. Similarly to the native artery, the graft would consist of a functional endothelial cell layer resting on metabolically active smooth muscle cells which are in a contractile (i.e. non-synthetic and anti-atherogenic) phenotype (Muto et al. 2007). The graft would also contain enough collagen and elastin proteins to display desirable viscoelastic properties, and would lack any synthetic foreign material that would initiate chronic inflammatory responses or be susceptible to infection. If a synthetic material is used, it should be non-immunogenic, non-thrombogenic and of appropriate compliance. In the ideal case, it should be degradable, providing a temporary scaffold for vascular tissue regeneration, gradually removed and replaced by the newly-forming tissue.

In the construction of tissue-engineered vascular grafts, three major components must be addressed: a scaffold to provide the initial graft shape and strength, adhesive matrix and living vascular cells (Baguneid et al. 2006). For the scaffold, four major approaches can be identified: permanent synthetic support, natural acellular tissues, a biodegradable scaffold, and non-scaffold technology (Campbell and Campbell 2007).

The first seminal attempt involved seeding bovine EC, VSMC and fibroblasts onto collagen gel tubes (Weinberg and Bell 1986). An external Dacron mesh reinforcement had to be added because of poor mechanical strength. An example of the use of a natural scaffold involves seeding human cells on decellularized porcine aorta (Bader et al. 2000) or small intestinal submucosa implanted as vascular grafts (Lantz et al. 1993). The most commonly used biodegradable polymer is polyglycolic acid (PGA). Under shear stress preconditioning, VSMC followed by EC were seeded onto this scaffold, and the resulting vascular grafts were implanted in pigs, with excellent results (Niklason et al. 1999). Seeding bone-marrow cells (BMC) onto a biodegradable scaffold enabled the establishment of a
totally-engineered autograft implanted into the inferior vena cava of a dog, and explant analysis showed that BMC were able to differentiate both in endothelial and smooth muscle cells (Matsumura et al. 2003).

The non-scaffold or “self-assembly” approach produced a graft composed exclusively of human cells by wrapping and culturing fibroblast and VSMC cellular sheets on a PTFE mandril, which was then removed and EC were seeded in the lumen. This was the first completely biological TEB V to display mechanical resistance comparable to that of human vessels (experimental burst strength up to 2000 mm Hg). A short-term experiment in a canine model demonstrated good surgical handling (L´Hereux et al. 1998). Another vascular graft was developed without a scaffold by inducing an inflammatory reaction. A silastic tube was placed into the peritoneal cavity of rats and rabbits and was in 2 weeks spontaneously covered by layers of myofibroblasts, collagen matrix, and a single layer of mesothelium. It was everted to resemble the blood vessel and grafted in carotid or aortic position, and remained patent after 4 months (Campbell et al. 1999).

A TEBV construct consisting of autologous bone marrow cells seeded onto a biodegradable scaffold was first clinically implanted to replace the pulmonary artery in a pediatric patient with a cyanotic defect (Shin’oka et al. 2001), and there was no graft-related complication in a group of 42 patients (Shin’oka et al. 2005). Another clinical success was achieved by TEBV produced by the cell-sheet multilayer method (L’Heureux et al. 2006) and implanted as a hemodialysis access graft in 10 patients (L’Heureux et al. 2007b). A recent paper reports primary 1-month and 6-month patency of 78 % and 60 %, respectively, meeting the approved criteria for a high-risk patient cohort (McAlister et al. 2009).

A summary of composite, biohybrid and totally-engineered vascular grafts is outlined in Table 4.

### Venous replacements

Unlike arterial reconstructions, venous reconstructions are limited to large-diameter central veins, such as the inferior (Schwarzbach et al. 2006) or superior vena cava and the ilico-femoral veins (Kalra et al. 2003). Autologous size- and length-matched veins such as superficial femoral vein, internal jugural vein, left renal vein and mainly the gold-standard composite saphenous spiral vein graft (Doty et al. 1999) produce the best results, e.g. for a bypass for an occlusion of the superior vena cava of iatrogenic origin (catheter-related thrombosis). Venous allogenous homografts, either fresh or cryopreserved, have been used in experimental and clinical venous reconstruction (Sitzmann et al. 1984). The patency of externally supported ePTFE is better than the patency of grafts fashioned from ePTFE alone. However, the use of synthetic prostheses may be limited by low-flow thrombogenicity, contaminated tissue beds, as in venous vascular trauma or tumor resection cases, e.g. in portal vein resection for pancreatic carcinoma (Leon et al. 2007). Venous reconstructions may be combined with an
adjuvant arterio-venous fistula to improve graft patency. Interestingly, the success rates of repeated percutaneous angioplasty approached those of operative reconstruction (Wisselink et al. 1993).

**Hemodialysis vascular access**

Creating a native arterio-venous fistula for chronic hemodialysis access is obviously superior to creating a prosthetic fistula. However, patients with depleted veins receive a prosthetic access graft, which has to provide sufficient blood flow and sustain repeated puncturing. It is difficult to identify the ideal access graft from a large number of biologic and prosthetic conduits. Randomized trials suggest that cuffed ePTFE grafts offer reasonable patency and that “early access” PU grafts can provide a safe conduit. Biologic/semibiologic grafts give better results than synthetic grafts, in some respects (Scott and Glickman 2007).

**Collective experience of the authors**

Static experiments performed in our laboratory enabled us to assess the cell colonization of various biomaterials without flow of culture medium. Clinically-used PET vascular prostheses (produced by VÚP, Brno, Czech Republic) were impregnated with biodegradable polyester-based copolymers (Pamula et al. 2008) as a background for further modification with multilayers of adhesive matrix proteins, such as collagen, fibronectin,
laminin and particularly fibrin, which can be derived in autologous form from the patient’s blood (Brynda et al. 2005, Filová et al. 2009). For the purposes of vascular tissue engineering, we explored the adhesion and growth of animal-derived endothelial and vascular smooth muscle cells (Chlupáč et al. 2008a) (Fig. 3). Research work carried out on some fibrin-based surfaces (Riedel et al. 2009) has led to a common patent (Brynda et al. 2008).

Dynamic experiments were conducted under defined medium flow to better mimic in vivo conditions. Dacron vascular prostheses were modified on the luminal surface with ECM protein assemblies (Chlupáč et al. 2006a), and an investigation was made of the adhesion and growth of human patient-derived EC (Chlupáč et al. 2006b). Their resistance to shear stress was also investigated (Chlupáč et al. 2006c). The gene expression profile of endothelium seeded on similar planar ECM analogues was also determined (Chlupáč et al. 2008b). Hybrid vascular graft constructs in vitro seeded with autologous EC and preconditioned under shear stress in a bioreactor (Provitro Co., Berlin Germany) are currently being investigated in a porcine model.

Conclusions

Open arterial surgery in the form of a lower limb bypass remains the standard treatment for extensive and multilevel atherosclerotic disease. Over the past 50 years, no synthetic alternative has been found to compare with the patency rates of gold-standard autologous conduits. However, appropriate native material is often unavailable and alternative vascular prostheses show poor clinical performance. To address this issue, luminal modifications of grafts, interposition of vein segments and particularly tissue engineering of small-sized blood vessels have been introduced. Biohybrid and tissue-engineered vascular grafts have been manufactured with the help of materials research, complex tissue culture and cell seeding technologies. The outcomes of experimental and clinical implants seem to be favorable. It is likely that cell therapy will become a frequent option in vascular and endovascular surgery in the coming decades.

Conflict of Interest

There is no conflict of interest.

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