INVITED REVIEW

Biomaterials in the development and future of vascular grafts

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Recent developments in the field of tissue engineering have re-invigorated the quest for more suitable biomaterials that are applicable to novel cardiovascular devices, including small-diameter vascular grafts. This review covers both commercially available and relevant newly developed experimental materials, including elastic polymers (polyurethane), the biodegradable and bioresorbable materials, and the naturally occurring materials, focusing on their potential applications in the development of future vascular substitutes. (J Vasc Surg 2003;37:472-80.)

The search for vascular substitute materials has thus far been a half-century endeavor.1 The initial failure of materials such as metal, glass, ivory, silk, and nylon brought 2 important criteria into focus: thrombogenicity and durability. Research was thus directed at inert materials that minimally interact with blood and tissue. Polyethylene terephthalate (PET, Dacron) and expanded polytetrafluoroethylene (ePTFE) are the products of this research and are currently the standard biomaterials of prosthetic vascular grafts. Examined by means of decades of use, both Dacron and ePTFE grafts have been shown to perform well at diameters >6 mm, but neither material has been suitable for small-diameter (<4 mm) applications. Thus, finding a solution for small-diameter bypass grafting has become a major focus of attention. The mid- to long-term failure of existing synthetic grafts is essentially caused by unfavorable healing processes, namely incomplete endothelialization and myointimal hyperplasia (IH). Seeking completely non-reactive substances is likely unrealistic. Optimizing tissue-biomaterial interactions to elicit desirable results is thus a major emphasis of research. Various modifications have been applied to Dacron and ePTFE grafts to improve their function. Elastic polymers can constitute a temporary scaffold through which tissue ingrowth in vivo eventually replaces the prostheses and leave a complete biological vascular conduit. The emergence of tissue-engineering technology has made the development of a novel biologically viable vascular substitute feasible, and it may prove to be the ultimate solution for small-diameter vascular grafting. The purpose of this review is to highlight currently used and experimental biomaterials and their potential applications in the development of future vascular grafts, focusing on those used for conventional open vascular reconstructions.

CURRENT MATERIALS

As aforementioned, the 2 standard polymers used for vascular grafts in clinical practice are Dacron and ePTFE. Both PET and PTFE molecules are highly crystalline and hydrophobic, the 2 properties that prevent the polymers from hydrolysis. The hydrophobicity of the polymer has important implications in predicting surface interactions with blood and tissue.

Dacron. PET was first introduced in 1939. DuPont further developed it and patented its widely known Dacron fiber in 1950.2 Vascular grafts made from Dacron were first implanted by Julian in 1957 and DeBakey in 1958.1 Various modifications have been applied to Dacron and ePTFE grafts to improve their function. Elastic polymers can constitute a temporary scaffold through which tissue ingrowth in vivo eventually replaces the prostheses and leave a complete biological vascular conduit. The emergence of tissue-engineering technology has made the development of a novel biologically viable vascular substitute feasible, and it may prove to be the ultimate solution for small-diameter vascular grafting. The purpose of this review is to highlight currently used and experimental biomaterials and their potential applications in the development of future vascular grafts, focusing on those used for conventional open vascular reconstructions.

Dacron. PET was first introduced in 1939. DuPont further developed it and patented its widely known Dacron fiber in 1950.2 Vascular grafts made from Dacron were first implanted by Julian in 1957 and DeBakey in 1958.1 Clinically available Dacron grafts are fabricated in either woven or knitted forms. The multifilament Dacron threads in woven grafts are fabricated in an over-and-under pattern, which results in very limited porosity and minimal creep of the finished graft. Knitted grafts are made with a textile technique in which the Dacron threads are looped to create greater porosity and radial distensibility. The velour technique that extends the loops of yarn on the surfaces of the fabrics has been used in an attempt to increase tissue incorporation. A crimping technique is used to increase the flexibility, distensibility, and kink-resistance of textile grafts. Prosthetic rings or coils are applied to the external surface of the grafts as external support to resist kinking and possible mechanical compression.

The high porosity of the knitted graft necessitates preclotting as a means of preventing transmural blood extrav-
asation. Gelatin (Vascutek, Renfrewshire, Scotland), collagen (Boston Scientific, Oakland, NJ), and albumin (Bard Cardiovascular, Billerica, Mass) are used to seal knitted Dacron graft pores. The gelatin and collagen in the Vascutek and Boston Scientific grafts are cross-linked by low concentrations of formaldehyde, a method that results in a weak linkage that allows the gelatin or collagen to be degraded in the body in <2 weeks. Bard uses glutaraldehyde to cross-link albumin, and the albumin is absorbed in 2 months.

Dacron has a good stability and can persist for more than 10 years after implantation without significant deterioration. However, knitted Dacron grafts have been prone to dilate when implanted into the arterial environment, more because of fabrication technique than the polymer itself. Direct etiological association between graft dilation and the later clinical complications has been rare. Other than this, there are no clinical differences grafts in complications and graft patency between woven and knitted grafts in their use as aortoiliac bypass grafts. Five-year patency rates are 93% for aortic bifurcation grafts, but only 43% for above-knee femoropopliteal bypass grafts, and even lower for below-knee grafts.

Blood and tissue reactions to implanted grafts start immediately after the restoration of circulation. The first step is a dynamic protein adsorption/desorption to synthetic material surfaces, known as the Vroman effect, followed by platelet adhesion, inflammatory cell infiltration, and endothelial cell (EC) and smooth muscle cell (SMC) migration. A coagulum containing fibrin, platelets, and blood cells builds up during the first few hours to days and stabilizes in a period of 6 to 18 months, forming a compact layer. The histological characteristics observed within Dacron grafts is a compact fibrin layer on the blood-contacting surface and densely packed foreign body giant cells between the outer layer of the graft wall and surrounding connective tissue capsule. The fibrin layer within the midgraft remains acellular, regardless of whether the grafts are woven or knitted. An external velour surface permits more extensive and perigraft tissue and leaves acellular cellular coverage can be found at the midgraft region years after human implants. In the outer wrap-reinforced graft, the wrap limits the infiltration of the cells from perigraft tissue and leaves acellular fibrin matrix inside the graft wall. The densely fabricated wrap is manufactured on the outer surface of some of the Gore-tex grafts as a reinforcement to the graft wall. This wrap was beneficial in reducing post-implantation dilation. However, with the newer manufacturing technologies currently used, the wrap is felt by many investigators to be unnecessary.

Several modifications to the basic graft have been proposed for improving its function. One is to increase the graft permeability on the basis of the notion that the rate of tissue ingrowth is associated with graft porosity (in limited porosity ranges) and that transmural capillary ingrowth can provide the cell source for the surface endothelialization. In a baboon model, enhanced tissue ingrowth with complete endothelialization occurred in ePTFE grafts with a 60-μm or 90-μm intermodal distance, but the 90-μm intermodal graft demonstrated focal areas of neointimal desquamation at late periods. Increased tissue ingrowth and EC coverage and higher patency rates of the high-porosity ePTFE have also been reported in canine models. However, a human trial with high-porosity ePTFE failed to show any untreated ePTFE graft of 56%, 46%, and 42%, respectively. The significance of heparin bound to synthetic grafts will be further discussed in this review.

**Expanded polytetrafluoroethylene.** PTFE was patented by DuPont in 1937 as Teflon. Because of its particular relatively inert characteristics, it was considered to be an ideal electrical insulator. Its medical use began with its application in artificial heart valves in the early 1960s. In 1969, Gore patented expanded ePTFE (Gore-tex), which is the material used in vascular grafts. The expanded polymer is manufactured by means of a heating, stretching, and extruding process that produces a microporous material more supportive of firm tissue adhesion.

The PTFE molecule is biostable, and the graft made from it does not undergo biological deterioration within the body. The surface of the graft is electronegative, which minimizes its reaction with blood components. ePTFE grafts are manufactured by means of stretching a melt-extruded solid polymer tube, which then cracks into a non-woven porous tube. The characteristic structure of ePTFE is a node-fibril structure in which solid nodes connect through fine fibrils, with an average intermodal distance of 30 μm for a standard graft.

Like Dacron grafts, ePTFE grafts perform well as aortic substitutes, with a 5-year primary patency rate of 91% to 95%. When used for femoropopliteal bypass grafting, the 3- and 5-year patency rates are only 61% and 45%, respectively, whereas the autogenous vein grafts have 5- and 10-year cumulative patency rates of 77% and 50%, respectively.

The initial host response to ePTFE grafts is similar to that of Dacron grafts. A fibrin coagulum or amorphous platelet-rich material develops in a time sequence that is similar in both materials. Lack of luminal surface cellular coverage can be found at the midgraft region years after human implants. In the outer wrap-reinforced graft, the wrap limits the infiltration of the cells from perigraft tissue and leaves acellular fibrin matrix inside the graft wall. The densely fabricated wrap is manufactured on the outer surface of some of the Gore-tex grafts as a reinforcement to the graft wall. This wrap was beneficial in reducing post-implantation dilation. However, with the newer manufacturing technologies currently used, the wrap is felt by many investigators to be unnecessary.

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advantage in platelet deposition as compared with standard 30-μm internodal distance ePTFE grafts.\textsuperscript{29} Another modification has focused on the luminal surface of the graft. Carbon coating is used to increase the surface electronegativity so as to diminish thrombus formation. Early studies demonstrated decreased platelet deposition on carbon-coated grafts, but the overall patency rates were not improved when compared with those of uncoated grafts.\textsuperscript{28,30} A prospective multicenter clinical study, consisting of 81 carbon-impregnated ePTFE and 79 standard ePTFE grafts for below-knee popliteal and distal bypass grafting, showed no difference in patency rates between the 2 groups as long as 2 years after implantation.\textsuperscript{31} However, a recent report on a multicenter trial in Europe involving 128 carbon-coated ePTFE and 126 standard ePTFE grafts for infrarenal bypass grafting demonstrated the significantly greater 1- and 2-year patency rates of the carbon-coated versus the standard grafts by means of life-table analysis.\textsuperscript{32}

The attachment of anticoagulant or antithrombotic agents to the graft has also been explored. The most investigated is heparin binding. Heparin-bound ePTFE grafts demonstrated reduced thrombogenicity and improved patency rates at 8 weeks compared with the standard graft in the rat infrarenal aortic position.\textsuperscript{33} Whether the anticoagulation works through continuous release of heparin from the material that establishes an effective concentration at the interface between blood and the graft surface or through non-consumptive mechanisms of active function of the heparin immobilized on the material surface is unclear. A major concern with the administration of heparin on the graft surface is the duration of heparin function. Premature release or disturbance of functional heparin or the presence of a physical barrier because of adherent blood components implies a theoretical inefficacy of the approach. In a recent report by Fisher et al from Gore & Associates,\textsuperscript{34} heparin was covalently linked to a pretreated bioactive surface of ePTFE grafts, which were then implanted into canine aortoiliac arteries. The surface heparin activity measured by means of antithrombin III uptake per unit area was 24.7 ± 7.9 pmol/cm\textsuperscript{2} at 2 weeks and remained at 15.3 ± 3.7 pmol/cm\textsuperscript{2} at 12 weeks. Although it seems promising, the actual benefit of this treatment needs to be proved in longer-term in vivo studies.

Various bioactive substances have been integrated onto synthetic grafts by means of a number of delivery methods to modulate the graft healing process. One example is fibrin glue (FG) delivery of growth factors onto the ePTFE grafts. The growth factors can be slowly released from the FG, retaining their bioactivities in vivo. ePTFE grafts impregnated with FG containing fibroblast growth factor (FGF)-1 and heparin that were implanted into canine bypass graft models elicited greater endothelialization and tissue incorporation than untreated or FG/heparin (no FGF) treated grafts.\textsuperscript{35,36} Many growth factors, such as FGF-2, platelet-derived growth factor, and vascular endothelial growth factor, have been tested by using various delivery systems.\textsuperscript{14} Although most of the earlier studies were done on synthetic grafts, the concept may also apply to scaffolds, which will be discussed.

More experimental modifications have been reviewed elsewhere.\textsuperscript{14} Despite the difference in both chemical and physical properties between ePTFE and Dacron grafts, the patency rates are comparable at all positions.\textsuperscript{10,11,37,38} Little or only marginal clinical improvement has been achieved from various modifications of the basic grafts.

**Elastic polymer—polyurethane.** Both ePTFE and Dacron grafts are relatively non-compliant. The compliance mismatch has been thought to contribute to the development of IH at the anastomotic regions.\textsuperscript{39} Elastic polymers have been introduced to create radially compliant vascular grafts.

Polyurethanes (PUs) were originally developed commercially in Germany in the 1930s as surface coatings, foams, and adhesives.\textsuperscript{40} Segmented PUs are copolymers comprising 3 different monomers, a hard domain derived from a diisocyanate, a chain extender, and a soft domain, most commonly polyol. The soft domain is mainly responsible for flexibility, whereas the hard domain imparts strength. The selection of the 3 monomers can produce materials with different mechanical characteristics, which makes PU an attractive biomaterial. Lyca is the trade name of a segmented polyether PU that was commercialized in 1962 by DuPont.

As a biomaterial, PU was first used in manufacturing implantable roller pumps and left ventricular assist devices and as a coating for early artificial hearts.\textsuperscript{41} The superior elastic and compliant mechanical properties and acceptable biocompatibility of PU make it an appealing material for vascular grafts. Developing PU-based small-diameter vascular grafts has attracted great interest from industry.

The first generation of PU vascular grafts was developed with polyester PUs, which resulted in devices such as Vascugraft by B. Braun Melsungen AG (Melsungen, Germany). Although the initial report demonstrated good biocompatibility,\textsuperscript{42} the graft underwent surface chemical modification and deterioration in vivo.\textsuperscript{43,44} A clinical trial with Vascugraft for below-knee bypass grafting was aborted after 8 of 15 grafts had occluded in the first year.\textsuperscript{44} It has been reported that PUs with polyester polyols as soft segments are hydrolytically unstable.\textsuperscript{45}

Polyether-based PUs, such as in the Pulse-Tec (Newtec Vascular Products of North Wales, UK) vascular access graft, were then used. Polyetherurethane was relatively insensitive to hydrolysis but susceptible to oxidative degradation.\textsuperscript{45} The Pulse-Tec graft suffered from in vivo biodegradation and died in the product pipeline. Vectra (Thoratec Laboratories Corporation, Pleasanton, Calif) is another vascular access graft made with polyetherurethane. The graft is manufactured with an average pore size of 15 μm and a non-porous layer under the luminal surface, which makes it impervious to liquids.\textsuperscript{46} In a multicenter trial involving 142 patients receiving either Vectra or ePTFE vascular access grafts with a follow-up time as long as 12 months, no difference was found in the patency or compli-
cation rates of the 2 grafts, but the Vectra grafts allowed earlier access. However, it was noted that the PU graft elongated with time after implantation, and the incidence of pseudointimal formation near the anastomosis was higher than that in the ePTFE grafts. The Vectra graft received Food and Drug Administration (FDA) clearance in 2000. A small-diameter coronary bypass graft created by the company with the same material is currently undergoing clinical trial.

A new generation of PU grafts uses polycarbonate-based PUs that eliminate most ether linkages and are more resistant to biodegradation. A non-woven polyesterurethane graft, fabricated with a spray-phase-inversion technique, showed no significant degradation for as long as 6 months in rat aorta. The graft demonstrated faster endothelialization, early stabilization of neointimal proliferation, and a thinner neointima compared with ePTFE grafts. The Corevita graft (Corvita, Miami, Fla), comprising a porous polyurethane inner tube filled with a glutaraldehyde cross-linked gelatin-heparin matrix reinforced on the outside with knitted Dacron mesh, displayed no signs of aneurysm at 1 year after implantation in canine femoral arteries. When compared with ePTFE grafts, the PU graft overall showed no appreciable difference in neointimal formation in the canine aortic model. The graft made of poly(carbonate-ureaurethane (Chronoflex, CardioTech International, Woburn, Mass) is expected to have better stability than the early release graft in vivo. At 2 years, the grafts were implanted in aortoiliac arteries in 4 dogs for 36 months. No evidence of polymer degradation or graft deterioration was found. Histologically, there was IH in midgraft regions and around anastomoses, cellular infiltration and collagen deposition inside the wall, and a fibrous capsule on the outer surface of the graft with, reportedly, no foreign body reaction. The graft is currently undergoing clinical trial. Carboxylated PU treatment of the graft can create a surface with reactive carboxylic acid groups to which hirudin has been covalently bound. The antithrombin activity of immobilized hirudin may be expected to improve the graft performance.

Tissue reactions to PU grafts are discrepant in the literature because factors such as different compositions of polymers, graft fabrication, porosity, and surface modifications all affect the results. No conclusion can be made at this point as to whether PU grafts may be functionally superior to ePTFE or Dacron grafts until more data become available.

There have also been attempts at using PU with other biodegradable materials in the manufacture of biodegradable vascular grafts, which will be discussed. One major concern about PU grafts is the potential carcinogenic effect of its degradation products. In 1991, the FDA terminated the use of PU foam as a surface-coating material for breast implants, after it had been marketed for >20 years. A statement issued by the FDA suggested that the implanted foam might degrade and form 2,4-toluene diamine, which has been shown to cause liver cancer in laboratory animals.

The extent to which the initial compliance may affect the long-term function of the graft remains controversial. It has long been realized that fibrous tissue formation within and surrounding an implanted graft would compromise graft compliance. In a follow-up study of 8 patients with iliofemoral artery woven Dacron grafts, the average graft diameter variation during the cardiac cycle was 6% at 1 month after implantation, and it decreased to 1% after 1 year. The mechanical behavior of vascular grafts in vivo is governed not only by the properties of the implanted graft, but also by the nature and the amount of tissue incorporation.

PRE-CLINICAL INVESTIGATIONAL BIOMATERIALS FOR VASCULAR GRAFTS AND TISSUE-ENGINEERING SCAFFOLDS

Bioresorbable polymers

Tissue-biomaterial interactions, which ultimately often result in graft failure, are inevitable as long as the prosthesis remains implanted. Biodegradable polymers possess the advantage of leaving behind no prosthetic materials to keep stimulating persistent foreign-body reactions. Biodegradable polymers, however, undergo fragmentation with exposure to biological environments, which results in smaller degradation products that may or may not remain present either at the implantation site or in distant locations, such as the lymphatic system. Theoretically, it is possible to tissue-engineer a “neoartery,” assuming there is adequate load bearing to resist dilatation and include cellular components with desirable physiologic characteristics.

The 2 most investigated biodegradable polymers are polyglycolic acid (PGA) and polylactic acid (PLA). PGA is highly crystalline and is hydrophilic. It was used to make the first synthetic absorbable suture. The suture loses its mechanical strength 2 to 4 weeks after implantation because of in vivo hydrolytic degradation of the polymer. PLA is more hydrophobic than PGA because of the presence of an extra methyl group in the lactide molecule, which limits the water uptake and results in a lower hydrolysis rate. Lactic acid is a chiral molecule that, therefore, exists in 2 stereoisomeric forms, D-PLA and L-PLA. L-PLA is semicrystalline with high mechanical strength, and its hydrolytic product is naturally occurring L-lactic acid. Thus it is more frequently used in scaffold design.

The copolymers of glycolic acid and lactic acid marketed under the trade name Vicryl and polyglaclrin 910 (PG910) are widely used in the medical field as absorbable sutures, orthopedic devices, and drug-delivery systems. Biodegradation, tissue regeneration, and mechanical strength. A fully biodegradable vascular graft made from Vicryl sheets was investigated in 1979. These early grafts were prone to aneurysmal dilation and rupture. Grafts composed of woven PGA have been evaluated in a rabbit model in our laboratory. An inner capsule composed of a confluent layer of ECs and smooth muscle-like
myofibroblasts amid dense collagen fibers was formed 4 weeks after implantation. Macrophage infiltration and phagocytosis were in parallel with the resorption of the PGA, which no longer could be identified within 3 months. In this initial experiment, 10% of the PGA grafts showed aneurysmal dilation, with no difference between 1 to 3 months and 3 to 12 months, which suggests that the critical time for the development of aneurysms is during prosthetic resorption, before the ingrowth of tissue with adequate strength to resist hemodynamic pressures.

Polydioxanone (PDS), a material used clinically in bone pins and suture clips, is a more slowly resorbed compound. Grafts made of PDS showed similar endothelialization of the regenerated luminal surface after implantation, and PDS remained present for as long as 6 months. The explanted specimens of these PDS grafts were able to withstand mean static bursting pressures of 6000 mm Hg and 2000 mm Hg without fatigue.65

A critical feature of bioresorbable grafts is that they must regenerate a tissue complex of sufficient strength before loss of prosthetic integrity to minimize the possibility of aneurysmal dilation as a requirement for clinical efficacy.

Grafts constructed from ≥2 bioresorbable polymers with different resorption rates or from bioresorbable polymers with a non-resorbable material component have been designed to dictate mechanical strength considerations. The woven grafts composed of yarns of 74% PG910 and 26% PDS demonstrated a 100% 1-year patency rate, with no aneurysms in the rabbit aorta model.66 The PG910 was totally resorbed within 2 months, and the PDS was totally resorbed within 6 months. The regenerated arteries withstood 800 mm Hg of pulsatile systolic pressure ex vivo without bursting. Partially resorbable grafts containing 69% PG910 and 31% polypropylene or 70% PDS and 30% polypropylene were implanted into canine aortoiliac arteries. The overall patency rate was 90% for PG910/polypropylene and 86% for PDS/polypropylene for as long as 1 year, with no aneurysms.67 PG910 was totally resorbed within 2 months, and PDS was totally resorbed within 4 months. Both grafts elicited tissue ingrowth, which remained histologically stable from 4 months through 1 year. Polypropylene was chosen as the non-resorbable component because Dacron was found to inhibit the arterial regeneration stimulated by the resorbable component.67

Grafts prepared from a mixture of 5% PLA and 95% PU were evaluated in rat aorta.68,69 The grafts formed neointima in 6 weeks and neomedias with elastic laminae in 12 weeks after implantation, but aneurysmal dilatation developed in 3 of 8 grafts, and another 2 dilated after 1 year. PU/PLA lattices started to disintegrate on day 12 and completely fragmented within 1 year. The authors suggested that a relatively compliant scaffold was necessary to induce circumferential orientation of SMCs. As aforementioned, the initial graft compliance can be compromised by tissue ingrowth in vivo. A graft comprised of a polyetherurethane scaffold and sealed with polyethylene glycol (PEG)/PLA copolymer exhibited good compliance, and the compliance increased with the degradation of the PEG/PLA components in vitro. Yet, when implanted in vivo, the compliance reduced 20% after 12 weeks.70

**Scaffolds for in vitro tissue engineering.** After initial attempts at directly implanting bioresorbable grafts that are totally dependent on tissue ingrowth in vivo, cell seeding onto bioresorbable scaffolds has been exploited to initiate functional tissue regeneration. SMC seeding onto PU/PLA scaffolds was found to enhance neomedia generation and optimal media cell orientation.68 A Harvard-Massachusetts Institute of Technology group led by Langer and Vacanti constructed a tubular scaffold with woven polyglycolic acid as an outer layer and non-woven PGA as an inner layer.71 Autologous cells with mixed population from arterial explants were seeded onto the scaffolds. After 7 days of in vitro culture, the constructed vessels were implanted into ovine pulmonary arteries. All 7 were patent for as long as 12 weeks. The polymer scaffold was replaced by cells and extracellular matrix (ECM) with time. However, the vessels demonstrated an increase in diameter. They then designed a more durable PGA/polyhydroxyalkanoate (PHA) scaffold.72 The inner layer was made of non-woven PGA designed to degrade in 6 to 8 weeks, and the outer layer was made of nonporous PHA. PHAs are naturally occurring polyesters produced by several microorganisms.62 They can be degraded by hydrolysis, but have a degradation time of years. The PHA homopolymers are highly crystalline, relatively hydrophobic, and usually extremely brittle. Copolymers such as hydroxybutyrate with hydroxyvaleric acid are less crystalline and more flexible. The PHA used in this study was a copolymer of polyhydroxyoctanoate (PHO) and hydroxyhexanoic acid and has a high tensile set of 35% after 100% elongation. The constructed scaffolds had good tensile strength, flexibility, and handling. Using this scaffold, the authors showed that all the tissue-engineered vessels were patent, with no aneurysms for as long as 150 days after implantation into ovine abdominal aorta. The PGA layers were completely replaced by tissue within 3 to 4 months. Development of endothelium and of a media, containing collagen with the presence of elastin fibers, was evident.72

Organized tissue can only be generated in appropriate mechanical conditions. Culturing SMC-seeded PGA scaffolds with pulsatile flow for 8 weeks results in organization of SMCs into multilayer structures with orientated collagen fibrils between cells. The vessel structure displayed a contractile response to vasoconstrictors, although the magnitude was only 15% to 20% of that of the native artery.73 The ECM accumulates after exposure to in vivo hemodynamic environments. The content of elastin and proteoglycans was demonstrated by means of biochemical analysis to peak at 8 and 16 weeks after implantation, respectively, after exceeding their native artery levels and then decreased, approaching that of native artery. Nevertheless, collagen content continuously increased to approximately 5 times that of the native artery within 24 weeks, without decline.74 ECM deposition is necessary for the establishment of graft strength, but excessive matrix formation indicates unfavor-
able tissue remodeling. Much still needs to be learned to control this balance.

The PGA polymer does not possess cell-anchoring sites. Surface modifications have been investigated to facilitate cell attachment, spatial cell distribution, or both. Treatment with 1N NaOH transforms ester groups on the surface of PGA fibers to carboxylic acid and hydroxyl groups. The resultant hydrolyzed surface increased its adsorption of serum proteins and doubled seeded SMC attachment density.75 Incorporation of the RGD sequence to the polymer surface can direct receptor-mediated cell adhesion.76 Patel et al77 synthesized a biotinylated PLA-PEG (polyethylene glycol) copolymer. Biotinylated-RGD peptide was immobilized on the polymer surface by avidin. With patterning technology, the authors were able to achieve a controlled directed cell distribution. ECs adhered and spread only on the RGD-functionalized lines, separated by no cell zones in between. This technique and the concept of controlling specific cell distribution represent new possibilities in the tissue engineering field.

It has been noticed that SMCs in proximity to residual PGA fragments display an undifferentiated phenotype that is evidenced by a high mitotic rate and low expression on contractile proteins.58 Degradation of polymer can produce acidic products and create a low pH microenvironment, which stimulates chronic inflammation and induces fibrocollagenous tissue formation that impairs the compliance of the graft and eventually may cause graft failure.

**Synthetic protein-based polymer.** Synthetic protein-based polymers, such as elastic protein-based polymer, represent a new class of biomaterial. They are produced by means of recombinant DNA technology and are biocompatible and biodegradable. A model polymer is poly(GVGVP), the core sequence of which is a highly conserved repeating sequence in elastin. Poly(GVGVP) cross-linked with γ-irradiation exhibited an elastic modulus that was similar to the femoral artery.78 Degradation of the polymer can be achieved by the incorporation of carboxyamides containing amino acids such as asparagine and glutamine into the chain. The carboxyamides hydrolyze to form carboxylates, resulting in polymer breakdown. Depending on the preceding and following amino acids, the degradation can occur at times ranging from days to years. Consequently, chemical clocks can be introduced to control the polymer degradation rate. Cell attachment can be achieved by means of incorporating RGD sequences into the polymer. It has been shown that ECs among other cell types can attach to polymer poly[40(GVGVP), [GRGDSP]], spread, and grow to confluence.79 Both remarkable elasticity and controllable degradation make elastin-based polymers potentially desirable materials for scaffolds in blood vessel tissue engineering.

**Naturally occurring materials**

The advantage of synthetic materials is that their microstructure, strength, and speed of degradation can be controlled during production; natural materials, however, facilitate cell repopulation and tissue remodeling.

**Type I collagen.** Type I collagen is a major component of most connective tissues and is present throughout the arterial wall. Its native state is resistant to most proteases but is readily degraded by a wide variety of proteases once denatured.80 Increasing intermolecular cross-links among its 3 consisting peptide subunits can increase the tensile strength of collagen fibers and make it less susceptible to degradation. It has long been recognized that collagen, with its integrin-binding domains, facilitates cell attachment and that a collagen matrix can support tissue growth. Because of its unique biological and physical properties, collagen has been extensively used in tissue-engineering applications.

The first complete tissue-engineered biological blood conduit was constructed in 1986, with collagen gel as the scaffold.81 Cultured bovine SMC and fibroblasts were separately embedded in collagen gel and assembled to form media and adventitia. ECs were seeded to the luminal surface to form a monolayer of endothelium. Although the graft obtained 92% EC coverage on the inner surface and longitudinal SMC organization, it failed to show the requisite mechanical strength, even when reinforced with Dacron meshes. L’Heureux et al in 1993 modified this model by using human umbilical vein ECs and SMCs and human skin fibroblasts.82 They encountered the same mechanical limitation. To improve the mechanical strength of the collagen constructs, several approaches have been used. Appropriate culture media and mechanical conditioning of the constructed conduit stimulate its histologic organization and improve its mechanical strength.83-85 Fabricating collagen/elastin fibers into scaffolds with techniques such as electrospinning instead of by using a collagen hydrogel represents a new approach that may eventually be able to provide enhanced scaffold strength.86

**Decellularized biological scaffolds.** To obtain a physiologic matrix scaffold resembling that of the native artery, decellularized native vessel was introduced.87-90 The cells with their surface antigens are removed by means of detergent and enzymatic extraction methods, leaving a well-preserved acellular matrix that provides a scaffold for autologous cell ingrowth and allows favorable tissue remodeling. Allogenic scaffolds have achieved minimal immunoreactivity and good durability for as long as 6 years, without aneurysmal degradation.87 Initially, patency was reported in 15 of 16 implants at 3 days to 6 years in canine femoral and carotid arteries,87 but a later study showed 5 of 9 implants failed from acute occlusion in coronary bypass grafts, and only limited cellular repopulation occurred in a follow-up period of 6 months.88 Detergent was eliminated from the process because of a concern that its remnant may be cytotoxic. The difficulties with the supply of human materials impose a significant limitation on allogenic scaffolds. In this respect, xenogeneic materials have an advantage. However, unlike allogenic grafts in which the ECMs bear little antigenicity, interspecies matrix immunogenicity exists for xenografts.89 Xenogeneic acellular scaffolds elicit significant chronic immunoresponsive inflammation, which is sufficient to destroy elastin structures.90,91
necessary to either remove or mask the antigens from structural proteins for xenogeneic sources to be used in vascular tissue engineering.

Autologous EC seeding was expected to address both the thrombogenicity of exposed collagen and the remaining antigencity of the matrix, but the actual results have been disappointing. When decellularized allogeneic porcine carotid matrix seeded with autologous ECs was tested in carotid arteries, 46% of the 2-cm-long grafts failed within 1 week. The remaining grafts had a patency rate of 71% at 4 months. The patent grafts had well-preserved collagen and elastin structures, EC coverage, myofibroblast ingrowth, and some degree of inflammatory reaction at explantation.

Scaffolds constructed from decellularized porcine intestine with cross-linked type I bovine collagen deposited on the luminal surface have also been tested. The constructs reportedly provided the necessary mechanical and hemodynamic properties at implantation, and the scaffold was cellularized and remodeled within 90 days in rabbit carotid artery bypass graft model studies. Implanted as canine femoral artery interposition grafts, the constructs demonstrated myofibroblast repopulation through the scaffold and incomplete endothelial coverage. Eight of 9 grafts were patent for as long as 9 weeks, but with significant anastomotic IH. The diameter reductions were 7% at midgraft and 56% and 42% at proximal and distal anastomoses, respectively. No aneurysmal dilation was documented, but non-infectious serosal cavities around the grafts developed in all dogs at various points. No prediction can currently be made on the prospect of this approach.

ENDOVASCULAR GRAFTS

Endovascular grafting to date has used variations of the same class of biomaterials that are currently used for open graft placement. However, a substantial effort is underway to develop novel chemistries, biomechanics, and surface modifications for these devices, work that is beyond the scope of this review. The biomaterials discussed in this review are mainly focused on conventional vascular grafts. Most of the principles are applicable to endovascular grafts. However, certain distinctive aspects should be considered for endovascular graft development. For instance, endovascular grafts require an extremely thin-wall design, which enables them to be compressed to fit into a delivery sheath or catheter. The porosity requirement for endovascular grafts may not necessarily be the same as for conventional grafts. The wall structure of the grafts may be affected by deployment procedures. In addition, endovascular grafts are surrounded by blood clots and atherosclerotic lesions, which may result in tissue reactions that are different from that of the conventional grafts.

CONCLUSIONS
ePTFE and Dacron are the standard materials for large-diameter vascular grafts, but no ideal alternative to autologous vein grafts is currently available for small-diameter applications. We are on the verge of integrating our understanding of biological reactions to vascular grafts with the principles of tissue engineering and innovations of technology to develop a new generation of vascular substitutes. A living vascular graft with predictable and desirable biological functions will likely be constructed by culturing blood vessel cells on biological/synthetic scaffolds in bioractors with optimal hemodynamic and biomechanical conditions and supplemented with spatially and temporally controlled 3-dimensional delivery of bioactive agents, the use of genetic engineering techniques, or both. This may provide the ultimate solution for the current dismal long-term patency rates of small-caliber synthetic grafts.

REFERENCES