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Biomaterials for vascular tissue engineering

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Abstract

Cardiovascular disease is the leading cause of mortality in the USA. The limited availability of healthy autologous vessels for bypass grafting procedures has led to the fabrication of prosthetic vascular conduits. While synthetic polymers have been extensively studied as substitutes in vascular engineering, they fall short of meeting the biological challenges at the blood–material interface. Various tissue engineering strategies have emerged to address these flaws and increase long-term patency of vascular grafts. Vascular cell seeding of scaffolds and the design of bioactive polymers for *in situ* arterial regeneration have yielded promising results. This article describes the advances made in biomaterials design to generate suitable materials that not only match the mechanical properties of native vasculature, but also promote cell growth, facilitate extracellular matrix production and inhibit thrombogenicity.

Keywords

biomaterials; biopolymers; degradable polymer scaffolds; endothelialization; *in situ* vascular regeneration; tissue engineering; vascular grafts

Coronary and peripheral vascular bypass graft procedures are performed in approximately 600,000 patients annually in the USA, most commonly with the saphenous vein or the internal mammary artery [201]. Although the use of autogenous vascular substitutes has had a major impact on advancing the field of reconstructive arterial surgery, these tissue sources may be inadequate or unavailable. Moreover, their harvest adds time, cost and the potential for additional morbidity to the surgical procedure [1–3]. Currently, expanded polytetrafluoroethylene (ePTFE), polyethylene terephthalate (Dacron®) and polyurethane are used to fabricate synthetic vascular grafts [4]. However, owing to thrombus formation and compliance mismatch, none of these materials have proved suitable for generating grafts less than 6 mm in diameter that would be required to replace the saphenous vein, internal mammary or radial artery as a vascular substitute [5–8].

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The functional importance of normal physiologic responses of the vascular wall in controlling thrombosis and inflammation has guided attempts to closely mimic the native arterial wall in the design of a new generation of vascular prostheses. These features include the structural components collagen and elastin, which are responsible for the tensile strength and viscoelasticity of the blood vessel, and create a fatigue-resistant tissue with long-term durability [9]. Furthermore, the endothelial lining in the native vasculature not only serves as a protective, thromboresistant barrier between blood and the surrounding tissue, but also controls vessel tone, platelet activation and leukocyte adhesion. Other elements that define an ideal biomaterial necessary to the design of a vascular graft are bio-compatibility, infection resistance, suturability and off-the-shelf availability.

The first tissue-engineered blood vessel substitute was created by Weinberg and Bell in 1986 [10]. They generated cultures of bovine endothelial cells, smooth muscle cells (SMCs) and fibroblasts in layers of collagen gel supported by a Dacron mesh. Although physiologic pressures were sustained for only 3–6 weeks, they did demonstrate the feasibility of a tissue-engineered graft with human cells. Since then, strategies to create a suitable material for a vascular graft have focused on three areas of research: coatings and surface chemical modifications of synthetic materials, biodegradable scaffolds and biopolymers. Each group can be further organized into tissue-engineering strategies for *in situ* vascular regeneration, in which the body's natural healing response is modulated by material design and fabrication, or strategies for *ex vivo* formation of a blood vessel substitute, whereby *in vitro* culture of human cells on polymer substrates before implantation defines their mechanical and biological properties.

Synthetic nondegradable polymers

ePTFE, Dacron & polyurethanes

Synthetic materials have been employed in vascular graft design for a variety of reasons, mainly due to the ease and flexibility of tailoring their mechanical properties. One such example is ePTFE, a porous polymer with an electronegative luminal surface that is not degradable. However, only 45% of standard ePTFE grafts are patent as femoropopliteal bypass grafts at 5 years, while autologous vein grafts display a 60–80% patency [11,12]. In standard ePTFE grafts, the fibril length or intermodal distance measures approximately 30 μm and neither transanastomotic nor transmural endothelialization occurs to any significant extent. Experimental ePTFE variants with a larger fibril length of 60 μm have been produced, which in animal models have facilitated luminal endothelialization [13]. Nonetheless, these observations have not been replicated in clinical studies. Currently, Dacron is most commonly used for aortic replacement and to a lesser extent as a conduit for femoropopliteal bypass surgery. Characteristically, knitted grafts incorporate a velour finish, which orients the loops of yarn upward, perpendicular to the fabric surface, thereby increasing available surface area and enhancing the anchorage of fibrin and cells to promote tissue integration. The preference for a velour finish has been primarily motivated by improved handling characteristics, with few data demonstrating that internal, external or double velour grafts exhibit greater patency rates [14]. Dacron grafts are often crimped longitudinally to increase flexibility, elasticity and kink resistance. However, these properties are lost soon after implantation, as a consequence of tissue ingrowth. Despite some evidence that suggests that platelet deposition and complement activation [15–18] are lower on ePTFE than Dacron prostheses, the patency rates of Dacron and ePTFE grafts are similar [19]. Polyurethane is a copolymer that consists of three different monomer types: a diisocyanate hard domain, a chain extender and a diol soft domain. At physiological temperatures, the soft domains provide flexibility while the hard domains impart strength. The most common medical-grade polyurethanes are based on soft domains made from polyester, polyether or polycarbonate. Various components have been added to the graft design to improve synthetic graft function and yield biohybrid conduits. For example,

Nakagawa *et al*. developed a poly(ether-urethane) graft reinforced with knitted polyester fibers for hemodialysis, which was found to be more durable than ePTFE [20]. Further development has yielded a poly(carbonate-urea)urethane vascular graft that exhibits a compliance profile similar to human arteries [21].

Polymer functionalization

The poor patency rates of synthetic polymers have motivated further strategies to functionalize the luminal surface of grafts and direct tissue regeneration. Coatings, chemical and protein modifications, and endothelial cell seeding on otherwise inert materials have been employed to improve endothelialization and inhibit inflammation. As a result, carbon deposition, photo-discharge and plasma discharge technologies have been utilized to deposit reactive groups onto polymer surfaces to interact with cell-specific peptides and influence protein adsorption to the surface [22]. For example, Nishibe and colleagues found that in a dog carotid implant model, fibronectin bonding improved graft healing in high-porosity ePTFE grafts [23]. Recent studies have documented that cell adhesion peptide sequences, such as the P15 peptide found in type I collagen, increase endothelial cell adhesion to ePTFE *in vivo* via integrin-specific binding [24]. Endothelial cell attachment can be significantly improved on surfaces coupled with another potent adhesion peptide, RGD, when compared with fibronectin-coated grafts [25, 26]. To this end, Zilla and colleagues were able to improve cell retention on shear stressed grafts by precoating them with RGD-crosslinked fibrin [27]. In addition, delivery of growth factors from polymer surfaces has also facilitated the rate of *in situ* endothelialization [28, 29]. For example, ePTFE grafts impregnated with fibrin glue containing FGF-1 and heparin has promoted transmural endothelialization and SMC proliferation in a dog model [30–32].

Several investigators have endeavored to endothelialize the luminal surfaces of synthetic vascular grafts to mimic the biologic responsiveness of the native vasculature [27,32–37], as seen in Table 1 & 2. The success of cell transplantation is limited because of difficulties in cell sourcing and attachment, and retention during pulsatile flow conditions [38]. Strategies that promote *in situ* regeneration of a functional endothelial lining have also met with difficulties owing to chronic inflammatory and prothrombotic responses to the synthetic polymeric materials [39]. Endothelial cells growing onto prosthetic graft surfaces that display a procoagulant phenotype can, in principle, promote rather than retard thrombosis [40]. Furthermore, activated endothelial cells may increase growth factor production and secretion that encourages SMC proliferation. Indeed, subintimal SMC proliferation occurs predominantly in areas that have an overlying endothelium [41]. This response can be seen with ePTFE grafts coated with anti-CD34 antibodies and implanted in pigs [42]. While the antibodies are able to capture endothelial progenitor cells and increase endothelial cell coverage, intimal hyperplasia at the distal anastomosis is significantly increased at 4 weeks.

The high rates of thrombus formation on vascular substitutes have led researchers to focus on modulating adverse inflammatory responses. One such example is the creation of nitric oxide-producing polyurethanes, in which the nitric oxide donor diazeniumdiolate is covalently bound to a polyurethane backbone [43]. Nitric oxide is produced by endothelial cells and functions to regulate vascular tone, prevent platelet aggregation and inhibit smooth muscle hyperplasia. Consequently, *in vitro* studies investigating the release of nitric oxide from modified polyurethane films have determined that the material does reduce platelet adhesion and vascular SMC growth, while stimulating endothelial cell growth [44]. Furthermore, the elastomeric copolymer, poly(1,8-octanediol citrate), with mechanical and degradation properties suitable for vascular tissue engineering, has exhibited decreased platelet adhesion and clotting relative to ePTFE [45]. *In vitro* studies evaluating the biocompatibility of these materials have demonstrated the potential for further application as vascular graft coatings, but require more robust *in vivo* test beds in order to determine their success.

Degradable scaffolds

The use of biodegradable polymers as scaffolds on which layers of cells are grown is an alternate tissue-engineering approach for the development of a functional vascular graft. The scaffold degrades and is replaced and remodeled by the extracellular matrix (ECM) secreted by the cells. Polyglycolic acid (PGA) is commonly used in tissue-engineering applications as it degrades through hydrolysis of its ester bonds, and glycolic acid, in turn, is metabolized and eliminated as water and carbon dioxide. PGA loses its strength *in vivo* within 4 weeks and is completely absorbed by 6 months. Biodegradation rates can be controlled by copolymerization with other polymers, such as poly-L-lactic acid (PLLA), polyhydroxyalkanoate, polycaprolactone-copolylactic acid and polyethylene glycol [46–48].

Several investigators have explored the potential of PGA composite scaffolds in fabricating vascular conduits *in situ* and *ex vivo*. For example, partially resorbable Dacron grafts have facilitated infiltration and proliferation of vascular cells and promotion of capillary growth [49]. The regenerative potential of these conduits has led to further PGA and Dacron fiber blends with the purpose of optimizing compositional ratios for *in vivo* healing responses and graft strength maintenance [50,51]. As *in situ* regeneration via polymer degradation limits exact control over the remodeling process, other groups have demonstrated the ability to construct functional grafts *ex vivo*. Mooney and colleagues have seeded cells onto a PLLA/polylactide-coglycolide (PLGA) copolymer-coated PGA mesh [47,52]. Similarly, Vacanti and colleagues used PLGA to generate capillary networks for artificial microvasculature applications [53]. Furthermore, Niklason and colleagues have developed a pulsatile bioreactor to remodel PGA scaffolds seeded with bovine smooth muscle and endothelial cells [54]. After a 10-week culture period, the resulting tissue-engineered vessel displayed a burst pressure of up to 2300 mm Hg. After 5 weeks, the PGA scaffold had degraded to 15% of its initial mass. Consequently, mechanical stability was dependent on SMC production of collagen and culture medium supplements that promoted collagen crosslinking. Although the lumen of the graft did not present a confluent endothelium lining, vessels did display contractile responses to serotonin, prostaglandin and endothelin-1, and implants remained patent for 1 month in a swine model. Attempts to translate this approach to human cells have led to poor mechanical properties due to the limited proliferative capacity of human SMCs, especially when harvested from elderly patients. In addition, the notable absence of elastic fibers could limit fatigue resistance and predispose the vessels to subsequent aneurysmal degeneration.

Polyhydroxyalkanoates, linear polyesters that are produced by bacterial fermentation of sugar or lipids, have also been employed in graft design, as they can be modified to display a wide range of degradation rates and mechanical properties. Shum-Tim *et al.* engineered an aortic graft consisting of a polymer scaffold of PGA and polyhydroxyoctanoate (PHO) seeded with bovine carotid artery cells [55]. The inner layer of the construct was made of nonwoven mesh of PGA fibers, while the outer layers were composed of nonporous PHO. The PGA scaffold promoted cellular growth and ECM production, while the slower degradation rate of PHO provided mechanical support as this remodeling occurred. Significantly, the graft did not require extensive *in vitro* conditioning. The construct was implanted directly in the abdominal aorta of lambs with 100% patency noted at 5 months. Histological analysis revealed that the remodeled graft contained uniform collagen and elastin fibers that had aligned in the direction of blood flow. The mechanical stress–strain curve of the engineered construct approached that of the native vessel, although some permanent deformation was observed 6 months after implantation, indicating either insufficient or noncrosslinked elastin. Fu and colleagues investigated the effects of ascorbic acid and basic FGF, which stimulated cells on a PGA-poly (4-hydroxybutyrate) construct to proliferate and generate large quantities of collagen, thereby accelerating the improvement in mechanical integrity [56].

Yet another versatile polymer, polycaprolactone (PCL), slowly degrades by hydrolysis of ester linkages, with elimination of the resultant fragments by macrophages and giant cells. Shin'oka *et al.* reported the use of PCL-based scaffolds to engineer venous blood vessels [57,58]. The PCL–polylactic acid copolymer was reinforced with woven PGA and seeded with autologous smooth muscle and endothelial cells harvested from a peripheral vein. After 10 days, the construct was implanted as a pulmonary bypass graft into a 4-year-old child [59]. Subsequent studies with autologous bone marrow cells on the constructs have reported greater than 95% patency at a mean follow-up of 16 months [60]. Further evaluation of endothelial cell function and mechanical properties of vascular grafts constructed with autologous bone marrow cells was conducted with a canine inferior vena cava model [61]. Interestingly, the biochemical properties and wall thickness of cell-seeded scaffolds were similar to those of the vena cava 6 months after implantation.

Biodegradable polymer systems provide the opportunity for spatial and temporal release of various growth factors to promote vascular wall regeneration. For example, VEGF release from PLGA scaffolds has been shown to promote angiogenesis *in situ* [62]. Likewise, FGF-2 release from poly(ester urethane) urea (PEUU) scaffolds amalgamates the favorable mechanical properties of polyurethanes with the bioactivity of an angiogenic protein [63]. While degradation via hydrolysis serves as a powerful tool in vascular tissue engineering to control the release of bioactive molecules from the polymer matrix, incorporation of proteolytic sites into the material can further optimize presentation of these molecules to the surrounding environment via cell-mediated degradation.

Although tissue-engineered vascular grafts based on biodegradable scaffolds have yielded promising results, some drawbacks exist. Challenges of cell sourcing are compounded by long culture periods that range between 2 and 6 months, and the proliferative capacity of cells isolated from elderly patients is limited. The mechanical strength of the materials may be comparable to that of native vessels, but compliance mismatch limits long-term patency. Table 3 summarizes other notable work with degradable scaffolds.

Biopolymers

An alternative strategy to synthetic and degradable scaffold-based vascular grafts is the manipulation of proteins that constitute the architecture of native ECM. The generation of protein polymers that mimic native structural proteins and adopt the characteristics of the arterial wall offers a unique approach to develop a vascular graft. Ultimately, the success of this approach is dependent on appropriate cell migration, adhesion and proliferation, as well as ECM production, on the biomimetic surfaces.

One such protein is type I collagen, a major ECM component in the blood vessel [64]. Collagen fibers function to limit high strain deformation, thereby preventing critical rupture of the vascular wall [65,66]. Collagen gels and fibers reconstituted from purified collagen are ideal in artificial blood vessel development due to their low inflammatory and antigenic responses [67]. Furthermore, integrin-binding sequences in collagen allow for cell adhesion during fibrillogenesis. As mentioned previously, Weinberg and Bell first reported the use of collagen gels as substrates for cells in vascular tissue engineering. Since then, Habermehl and colleagues have developed a process to obtain large quantitites of collagen from rat tail tendons to scale-up production [68].

Variables such as fiber orientation, crosslinking conditions and cell seeding techniques have been explored to improve the mechanical integrity of collagen-based constructs. A wide range of crosslinking agents can enhance covalent links between the collagen fibers, the most efficient of which is glutaraldehyde [69]. The cytotoxicity of this chemical, however, has led to the development of alternative crosslinking mechanisms, such as the enzymatic reactions of lysyl

oxidase and transglutaminase, as well as photocrosslinking [70–72]. Various groups have investigated fiber orientation and SMC alignment as a means to increase mechanical properties in the circumferential direction of a tubular construct [73–75]. Preconditioning treatments involve applying mechanical strain or shear stress to the construct and compaction of SMC-containing collagen gels around a mandrel in order to increase mechanical strength [76–78].

The shortcomings of a stiff collagen-based scaffold have motivated researchers to explore the potential of more elastic fibrin gels in vascular tissue engineering [79]. Fibrin is formed when fibrinogen polymerizes into a fibrillar mesh with the addition of thrombin. An advantage of this biopolymer is the ability to produce it with the patient's own blood, thereby preventing an inflammatory response upon implantation [80]. Fibrin also binds to critical proteins that direct cell fate, such as fibronectin and VEGF [81]. *In vivo* degradation can be controlled with the proteinase inhibitor aprotonin and crosslinking agents, although there are concerns that the concentrated presence of these natural proteins may interfere with local coagulation cascades [82].

Interestingly, SMCs embedded in fibrin gels produce more collagen and ECM than cells that are entrapped in collagen gels [83]. One such example is the fibrin-based vascular graft developed by Swartz and colleagues, who incorporated ovine SMCs and endothelial cells into the gel [84]. The grafts were implanted in the jugular veins of lambs, and remained patent for 15 weeks. Upon histologic examination, the constructs were found to contain both collagen and elastin, with the mechanical integrity comparable to that of native coronary arteries. Furthermore, Tranquillo and colleagues demonstrated that the enmeshed SMCs directed compaction and alignment of both the fibrin fibers and the cell-synthesized collagen fibers in a circumferential orientation around a nonadhesive mandrel [85].

The elasticity afforded by fibrin-based grafts is a critical factor in vascular tissue-engineering design. Researchers have also explored the potential of incorporating scaffolds with more extensible proteins such as elastin, a key structural element in native vasculature. Crosslinked elastic fibers form concentric rings around the medial layer of arteries, providing elasticity to the vascular graft by stretching under a stress and recoiling back to the original dimensions as the load is released [86–89]. In addition, elastin regulates vascular SMC activity by inhibiting SMC proliferation. Unlike collagen, the stable crosslinked fiber network of native elastin makes isolation and purification techniques difficult. Therefore, different strategies have emerged to incorporate elastin into tubular constructs. Whereas some investigators have attempted to promote elastogenesis in vascular grafts indirectly with SMC culture techniques [90–92], others have developed protocols to process insoluble and soluble elastin [93]. One such example includes a freeze-drying protocol for collagen and elastin to produce a porous scaffold [94].

More recently, the development of recombinant genetic and protein engineering has enabled the synthesis of bioinspired protein polymers that not only mimic structural proteins but also direct cellular fate by emulating the ECM *in vivo* [95–98]. Specifically, polymers with pentapeptide repeat motifs similar to VPGVG exhibit elastic behavior with features that are consistent with native elastin, including a mobile backbone and the presence of β turns [99–102]. The biosynthetic machinery of microorganisms can be exploited to produce significant quantities of these recombinant protein polymers that have been designed from primary amino acid sequences and self-assemble into a distinct 3D folded structure [100,103]. These elastin-mimetic biopolymers, in turn, can be cast as hydrogels or electrospun into nanofibrous scaffolds [104–108].

Nanocomposites

Recent developments in the field of nanotechnology have facilitated vascular tissue-engineering efforts in mimicking the nanostructure of native vasculature, thereby directing mechanical and biologic performance of the bulk material. One such application is electropinning of synthetic polymers and naturally occurring materials into nanofibers [109–112]. The advantages of this strategy include the ability to form scaffolds with high porosity as well as high surface area-to-volume ratio, thus simulating the dimensions and structure of native collagen and elastin fibrils [113,114]. In particular, He and colleagues have demonstrated the utility of electrospinning with the generation of a nanofibrous scaffold composed of collagen-blended degradable poly(L-lactic acid)-co-poly(ε-caprolactone) [115]. Results indicated that the blended nanofibers supported endothelial cell attachment and spreading, and preserved the endothelial cell phenotype.

Enhancement of base material properties with the addition of fillers has resulted in various nanocomposites. In general, these materials have demonstrated a reduction in thrombogenicity while improving mechanical properties. For example, Kannan and colleagues have generated a polymer based on poly(carbonate-urea)urethane and polyhedral oligomeric silsesquioxane nanoparticles, and have reported the nanocomposite's heparin-like behavior at the blood–material interface [116,117]. Furthermore, the polymer displayed a greater degree of compliance match to natural arteries compared with ePTFE and Dacron. Other groups have utilized the strength and flexibility of carbon nanotubes as fillers to enhance base polymer properties [118,119]. These efforts have indicated that although the composite polymers decrease thrombogenicity on their surfaces, toxicity of carbon nanotubes remains a concern [120,121].

Alternative tissue sources

Decellularized allogeneic or xenogenic tubular tissues that contain an intact and structurally organized ECM have been investigated as vascular conduits, which include human umbilical vein and bovine and porcine carotid arteries. Although a readily available supply of artificial arteries is attractive, drawbacks include the inability to tailor matrix content and architecture, progressive biodegradation and the risk of viral transmission from animal tissue. Decellularization removes most cellular antigenic components in allogeneic and xenogeneic tissue. A combination of physical agitation, chemical surfactant removal and enzymatic digestion disrupts cells and removes protein, lipids and nucleotide remnants [122–125]. Following decellularization, chemical crosslinking is used to enhance mechanical strength and reduce immunogenicity [126,127]. The addition of an external support such as a Dacron mesh is also common to provide mechanical strength and prevent late dilation. Efforts to improve the durability and healing response of decellularized scaffolds have included coating with heparin and FGF, as well as seeding with endothelial cells, bone marrow-derived cells, and adipose-derived stem cells [128–136].

Alternative tubular tissue sources have been utilized as vascular substitutes as well. For example, decellularized small intestinal submucosa is composed of collagen, fibronectin, proteoglycans, growth factors, glycosaminoglycans and glycoproteins [137]. Consequently, implantation of the small intestinal submucosa construct as a vascular graft leads to neovascularization, host cell migration and adhesion, and matrix remodeling [138–141]. The development of a tissue-engineered vascular conduit from yet another avascular tissue source has been documented by Campbell and colleagues [142]. The intraperitoneal graft model employs the peritoneal cavity as an *in situ* bioreactor for the creation of a tubular construct seeded with layers of host cells. The investigators observed that foreign objects implanted into the peritoneal cavity became encapsulated by a fibrous capsule containing myofibroblasts and a surrounding layer of mesothelial cells [143]. They then inserted silastic tubing into the

peritoneal cavities of dogs, rabbits and rats. After 2–3 weeks, the tubing was removed, and the cell-encapsulated construct was grafted into the carotid artery (rabbit), abdominal aorta (rat) and femoral artery (dog) of the animal in which it was grown [144,145]. Remodeling of the autologous grafts included differentiation of myofibroblasts to smooth muscle-like cells, increased wall thickness, elastin and collagen production, and circumferential alignment of cells and matrix proteins [146]. The constructs displayed endothelium-dependent relaxation when stimulated with acetylcholine and were patent in rabbits for at least 16 months and in dogs for 6.5 months.

Recently, sheet-based tissue engineering and bioreactor conditions have enabled the expansion of *in vitro* culture of cells into a cohesive cell sheet comprised of various cell types and endogenously expressed ECM. Thermoresponsive polymers, such as poly *N*-isopropylacrylamide and methyl cellulose, have served as coatings on culture flasks in order to facilitate the removal of cultured cells and underlying ECM as a uniform sheet. These sheets have been further processed into blood vessels by layering and wrapping them around a mandrel for incubation [147–149]. While this maturation period can be as extensive as 10 weeks, the resulting graft does not require exogenous biomaterials for mechanical support. L'Heureux *et al.* have demonstrated the utility of assembling arterial bypass grafts exclusively from a patient's own cells by implanting the substitutes into primate models [150]. *In vivo* results indicated that the grafts were antithrombogenic and mechanically stable for 8 months, with histology and microscopy displaying complete tissue integration, regeneration of a vascular media, as well as elastogenesis and a collagen fiber network.

Conclusion & future perspective

The development of a synthetic arterial substitute represents a major milestone of 20th century medicine, yielding technology that has saved the lives of millions of patients. Nonetheless, a durable small-caliber (diameter: <6 mm) conduit remains elusive, and patency rates for infrainguinal revascularization through the use of a prosthetic graft have changed little over the past 30 years. The challenges of creating the ideal tissue-engineered vascular substitute are numerous, but significant progress has been made towards understanding the importance of both the mechanical and biologic requirements of biomaterials for this application. Investigators continue to strive for the generation of multifunctional materials with optimized release and presentation of bioactive molecules in order to guide *in situ* vascular regeneration. For example, the challenges of sufficiently balancing polymer degradation rates with ECM production and cellular infiltration has resulted in polymers designed with cell-binding sequences, enzymatic cleavage sites, and tethering of chemoattractant molecules [151,152]. This ‘bottom-up’ approach to materials design enables researchers to finely modulate the nanostructure of a material in order to influence its bulk properties. The success of these efforts will depend on the generation of composite scaffolds that mimic the complexity of native vascular matrix in order to improve elasticity and compliance of the native blood vessel while inhibiting adverse responses at the blood–material interface. *In vitro*, *in vivo* and computational models are also providing new insights into the complex interplay of cellular, biochemical and biomechanical processes that lead to graft failure. However, a better understanding of vascular progenitor cell biology is required to harness the potential of progenitor cells in endothelialization of arterial grafts. Through continued collaboration among vascular surgeons, biologists, material scientists and biomedical engineers, existing barriers in the creation of an arterial substitute will undoubtedly be broken.

Executive summary

Synthetic polymers

- Nondegradable materials

- Expanded polytetrafluoroethylene, Dacron® and polyurethane are currently used as synthetic vascular grafts.
- Polyurethane is better able to match the compliance of native vasculature, but the patency rates of grafts composed of synthetic, nondegradable materials is relatively poor.
- Functionalization of the polymer surfaces via chemical modification and coatings enables improved endothelialization and thromboresistance of the materials.

- Degradable scaffolds
  - Biodegradable polymers act as scaffolds upon which cells and the surrounding environment can modulate vascular remodeling.
  - Degradable polymers, including polyglycolic acid, polyhydroxyalkanoates, polycaprolactone and polyethylene glycol, have been utilized in generating cell-seeded scaffolds for vascular substitutes.
  - Degradable scaffolds have been further functionalized with proteolytic sites for the controlled release of bioactive molecules from the polymer matrix and optimized presentation of these factors to the surrounding environment via cell-mediated degradation.
  - While degradable polymers have enabled improved extracellular matrix (ECM) production and vascular cell infiltration into the graft site, compliance mismatch, prolonged cell culture periods and the challenges of cell sourcing remain significant obstacles in utilizing biodegradable materials in the clinical setting.

Biopolymers

- The generation of protein polymers that mimic native structural proteins and adopt the characteristics of the arterial wall offers a unique approach to develop a vascular graft.
- Collagen and fibrin gels and fibers are able to bind to critical proteins that direct cell fate, and are therefore ideal in the formation of artificial blood vessels. While aligned, crosslinked collagen fibers contribute to the mechanical integrity of the graft, the more elastic fibrin mimics the role of elastin in native vasculature.
- ECM production and mechanical integrity can be further modulated by smooth muscle cell seeding, culture techniques and preconditioning treatments.
- The biosynthetic machinery of microorganisms can be exploited to produce significant quantities of recombinant protein polymers that have been designed from primary amino acid sequences and self-assemble into a distinct 3D folded structure. The generation of elastin-mimetic protein polymers is one such example of a vascular tissue-engineering application.
- Decellularized allogeneic or xenogenic tubular tissues that contain an intact and structurally organized ECM have been investigated as vascular conduits, which include human umbilical vein and bovine and porcine carotid arteries. These grafts have met with some success, but drawbacks include the inability to tailor matrix content and architecture, progressive biodegradation and the risk of viral transmission from animal tissue.

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Table 1

Synthetic polymers: *in vivo* vascular regeneration

Synthetic polymer	Coating composition	Cell type seeded	Preconditioning scheme	Culture time	EC function	*In vivo* system	Explant histology	Patency	Ref.
ePTFE	Fibrin glue with FGF-1 and heparin	–	–	–	–	Canine thoracoabdominal aortic position	EC confluence, minimal intimal hyperplasia, increased capillarization, increased collagen content	At least 140 days	[30]
ePTFE	P15 peptide	–	–	–	*In vitro*, 100% HUVEC confluence on treated polymer at day 8	Arteriovenous grafts in sheep	Decreased thickness of neointimal hyperplasia and increased endothelialization	At least 28 days	[24]
ePTFE	Growth factor -reduced Matrigel™-containing VEGF	–	–	–	High expression of bFGF and a low expression of TGF-β in VEGF-treated EC	Rat abdominal aorta	Increased EC rate, increased myointimal hyperplasia and SMC density	At least 30 days	[29]
Microporous polyurethane	FGF-2, heparin, gelatin	–	–	–	EC proliferation	Rat aorta	Increased endothelialization	At least 4 weeks	[153]
ePTFE	Anti-CD34 antibodies	–	–	–	–	Implanted grafts between carotid artery and internal jugular vein	Rapid endothelialization, increased intimal hyperplasia	At least 28 days	[42]
High-porosity ePTFE	Fibronectin bonding	–	–	–	–	Pig and dog carotid implant model	Increased tissue ingrowth, including EC layer, including thrombus-free area, complete organization of neointima	80% at 6 weeks	[23]

bFGF: Basic FGF; EC: Endothelial cell; ePTFE: Expanded polytetrafluoroethylene; HUVEC: Human umbilical vein endothelial cell; SMC: Smooth muscle cell.

Table 2

***Ex vivo* engineering of synthetic polymers**

Synthetic polymer	Coating composition	Cell type seeded	Preconditioning scheme	Culture time	EC function	*In vivo* system	Explant histology	Patency	Ref.
ePTFE	Fibrin glue	Human autologous ECs, mostly from the cephalic vein	Controlled seeding rotation device (6 rph, 37°C, 3 h)	7–11 days	–	Infrainguinal position above and below the knee in humans	–	62.8% after 7 years	[27]
ePTFE	Fibronectin	Autologous ECs were seeded on top of SMCs	Cell seeding occurred via controlled rotation within graft at 1 rpm for 2 h	1 day	EC retention greater when seeded on top of SMCs	Infrarenal aorta in rabbit model	Decreased neointimal thickening	At least 100 days	[154]
Dacron®	Cells mixed with venous blood	Canine bone marrow-derived $CD34^+$ cells	Four-step seeding/preclotting technique	–	92% surface endothelialization	Canine descending thoracic aorta	Enhanced EC coverage and increased microvessel infiltration	At least 4 weeks	[155]
Chrono-flex-polyurethane	None	Autologous canine jugular vein ECs	ECs were electrostatically seeded onto grafts for 16 min	–	Mature, confluent endothelium	Canine femoral artery	Enhanced neointimal development and minimal thrombus formation	At least 6 weeks	[156]
ePTFE	Fibronectin	Endothelial progenitor cells (EC-like cells grown from mononuclear blood cells)	EPC seeding and maintenance in organ culture under nonflow conditions	48 h	60% endotheliaization	Rabbit carotid model	–	28 days	[157]

EC: Endothelial cell; EPC: Endothelial progenitor cell; ePTFE: Expanded polytetrafluoroethylene; SMC: Smooth muscle cell.

Table 3

Biodegradable scaffolds

Polymer assembly	Cell type seeded	Preconditioning scheme	Culture time	*In vivo* system	Explant histology	Patency	Ref.
Polyglycolide fibers reinforced with a Dacron® outer sleeve	–	–	–	Rabbit aorta	Endothelial- and smooth muscle-like cell infiltration	At least 9 months	[49]
Poly(ether urethane urea) (Lycra) scaffold impregnated with PEG/PLA	–	–	–	Canine carotid arteries	Improvement of transmural tissue ingrowth and vascularization; thin, uniform intima and EC lining	At least 3 months	[158]
PLGA fiber mesh compounded with collagen microsponge was frozen and lyophilized prior to glutaraldehyde crosslinking	–	–	–	Pulmonary trunks of beagle dogs	No intimal thickening; EC monolayer and parallel alignment of SMCs; reconstructed vessel wall with elastin and collagen fibers	At least 6 months	[159]
Electrospun PCL	–	–	–	Rat abdominal aorta	Confluent endothelium at 12 weeks; neocapillary formation; thin neointima	At least 24 weeks	[160]
Electrospun PLLA membrane consisting of nanofibers	Bone marrow MSCs	MSCs seeded onto membranes, then rolled into 0.7 mm mandrel	3 days	Rat common carotid artery	Development of elastic lamina layer; no significant intimal hyperplasia; organized vascular cell infiltration	At least 60 days	[161]
Nonwoven PGA mesh coated with poly 4-hydroxy-butyrate	Ovine vascular myofibroblasts and ECs	Sequential seeding and culture of myofibroblasts and ECs	21 days	Main pulmonary artery in lambs	Stiffer tissue properties compared with native artery due to increased collagen content and no elastin production; no thrombus formation or calcification; appropriate EC and SMC layers were present	At least 100 weeks	[162]

EC: Endothelial cell; MSC: Mesenchymal stem cell; PCL: Polycaprolactone; PEG: Polyethylene glycol; PGA: Polyglycolic acid; PLA: Polylactic acid; PLGA: Polylactide-coglycolide; PLLA: Poly-L-lactic acid