CHAPTER 159

Engineering of Blood Vessels

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Introduction

Are human blood vessels replaceable? If so, what types of materials—synthetic or biological, living or nonliving—are best suited for these replacements? The field of vascular tissue engineering has emerged to answer these questions, with the goal of providing constructs that can repair or replace damaged vessels in vivo. This review summarizes the key concepts, requirements, and designs for the synthesis of artificial blood vessels.

In many vascular pathologies, the simplest replacement for a diseased vessel is an autologous transplant. For instance, in atherosclerosis, a narrowed coronary artery may be readily replaced by vessels of similar diameters, such as the internal mammary artery, radial artery, or saphenous vein; "bypass" surgeries of this sort are now routine, with half a million procedures performed annually. Healthy autologous vessels are not always available for transplantation, however, especially in patients whose vessels have already been harvested for previous operations. The clinical promise of tissue engineering lies in its potential to produce implantable vessels—either as single vessels or as entire networks—for the treatment of vascular obstruction or disease.

Whether an engineered vessel is suitable as a replacement depends on several factors: (1) *Strength:* Is the vessel sufficiently robust to withstand cyclic mechanical stresses generated by a pulsatile flow of blood through it? (2) *Biochemistry:* Does the vessel provide an antithrombotic, anti-inflammatory surface? Does the vessel respond appropriately to vasoactive compounds? (3) *Adaptability:* Does the vessel change its structure when faced with chronic changes in pressures or flows? Do the mechanical properties of the vessel adjust to approximate those of adjacent vessels? (4) *Organization:* Is the vessel histologically similar to a native one? The relative importance of these factors dep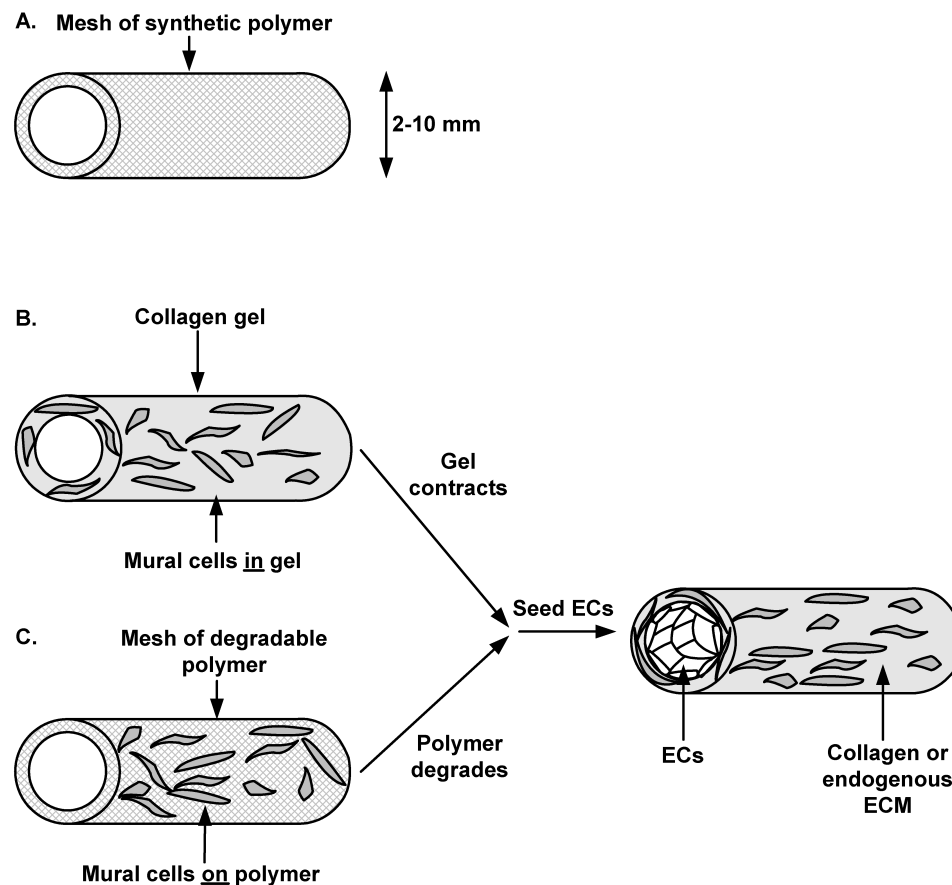ends on the specific application in mind. Thus, it is not necessary to synthesize vessels that are histologically indistinguishable from native ones (although this goal is desirable) before using these vessels in the clinical setting.

Engineered Arteries

Synthetic, Large-Diameter Arteries (Greater Than 5 mm)

Modern efforts to synthesize artificial blood vessels began with the seminal work of the French surgeon Alexis Carrel in the early 1900s. In experiments with transplantation in small animals, Carrel demonstrated that many synthetic materials—rubber, glass, metals—could be used to form large-diameter arteries that were stable for several months in vivo. Successful incorporation of these foreign substances into the vascular tree required precise surgical techniques that Carrel pioneered; these techniques minimized trauma of adjacent vascular segments during suture and attempted to preserve laminar flow of blood downstream from the synthetic graft. With the development of advanced polymers in the 1930s and 1940s and the subsequent refinement of techniques to mold, weave, or extrude these polymers into three-dimensional structures, it became possible to create grafts whose mechanical properties resembled those of native vessels (in contrast to the rigid tubes used by Carrel). These flexible grafts reduced radial mismatches between grafted and host vessels during expansion and contraction in vivo. Large-diameter vascular grafts are now commercially available for clinical use in a variety of different chemistries, including polytetrafluoroethylene (Teflon), polyester (Dacron), polyurethane, and polyacrylate (Figure 1A).

The primary limitation of these grafts is their poor long-term patency (20 to 70 percent remain open in 2 years, depending on anatomical location) due to gradual intimal

A. Mesh of synthetic polymer

2-10 mm

B.   **Collagen gel**

Gel contracts

Mural cells _in_ gel

Seed ECs

C.   Mesh of degradable polymer

Polymer degrades

ECs

Collagen or endogenous ECM

Mural cells _on_ polymer

Figure 1   Processes for engineering arteries. **(A)** Commercially available large-diameter grafts consist of a woven polymer tube. **(B)** Collagen-based small-diameter grafts consist of a contracted sheath of collagen and mural cells that is lined by a monolayer of ECs. **(C)** Small-diameter grafts made from degradable polymers consist of tubes of mural cells and endogenously produced ECM, with a lining of ECs. Collagen-based and degradable polymer-based approaches may also be used to synthesize large-diameter arteries.

growth and occlusion. Several strategies to address this issue have been proposed; a common theme is the use of an endothelial lining to prevent the adherence of blood cells, adsorption of plasma proteins, and migration of mesenchymal cells to the surface of the synthetic polymers. Initial attempts to implement this idea relied on injection of a suspension of autologous endothelial cells (ECs) into the lumen of a graft during or before implantation of the vessel. Seeded ECs often detached from the graft, however, upon exposure to hemodynamic stresses mediated by the flow of blood. Recent approaches to enhance the adhesion of ECs to a graft have focused on derivatization of its surface with adhesive peptides (either in the form of adsorbed extracellular matrix proteins, or as small adhesive motifs such as Arg-Gly-Asp). Clinical trials of these EC-lined synthetic vessels in humans have demonstrated that the presence of an internal lining of ECs reduces the extent of intimal formation in implanted grafts.

A complementary strategy to enhance patency relies on the stimulation of ECs to migrate over the surface of a graft from junctions between host vessels and the graft, and thereby to form a complete monolayer. Postsurgical injec-

tion of chemotactic and chemokinetic agents such as vascular endothelial growth factor (VEGF) and acidic and basic fibroblast growth factors (aFGF and bFGF) appears to promote migration of ECs over some synthetic materials in animal models. It remains to be seen how much the incorporation of ECs—whether injected into lumens of grafts or attracted from host vessels—can improve the patency rates of synthetic large-diameter vessels in humans, and whether there are inherent limitations to the long-term performance of synthetic materials as blood vessels in vivo.

Biological, Small-Diameter Arteries (Less Than 5 mm)

In contrast to the clinically successful application of synthetic vessels for replacement of large arteries, the use of synthetic vessels as small-diameter arteries has not yet proven to be feasible. The intimal growth that slowly leads to possible occlusion in large-diameter grafts rapidly induces failure of small-diameter ones: Occlusion of small, synthetic vessels by thrombus occurs readily, and the mismatch in mechanical compliances between synthetic

polymers and native tissues often results in intimal formation. These deficiencies have led to the idea that engineered vessels should ultimately be made of living, biologically active materials, which may provide signals to reduce the occurrence of thrombosis and intimal hyperplasia, and which may resemble native arteries in mechanical properties.

COLLAGEN-BASED ARTERIES

Some groups have attempted to synthesize purely biological vessels from gels, particularly collagen, with ECs and mural cells cultured on and in the gel, respectively (Figure 1B). In 1986, Weinberg and Bell demonstrated this design by gelling collagen, in which smooth muscle cells (SMCs) were suspended, around a tubular mesh [1]. Subsequent gelling of collagen and adventitial fibroblasts around the muscular layer and injection of ECs into the lumen of the tube generated a construct that segregated ECs, SMCs, and fibroblasts into compartments that grossly resembled the intima, media, and adventitia of arteries. The endothelial layer served as a selective barrier to large proteins and produced measurable amounts of prostacyclin, a vasodilator and potent inhibitor of platelet aggregation in vivo.

Engineered vessels made from gelled collagen primarily suffer from a lack of sufficient mechanical strength, due to abnormal cellular organization and low densities of collagen and cells. Although contraction of collagen by SMCs and fibroblasts helps initially to increase the burst strengths of engineered arteries, these strengths are still much lower than those of native vessels. Moreover, a gradual decrease in strength often follows the initial increase, because the collagen used in these vessels has a low density of cross-links, and thus is particularly sensitive to digestion by collagenases expressed by SMCs and fibroblasts. Improvements on the model of Weinberg and Bell have therefore focused on inactivation of collagenases (by transfection, or by addition of protease inhibitors), and on increasing the strength of the vessel (by cross-linking collagen with aldehydes or radiation). Although promising, improvements based on transfection or addition of small molecules run counter to the idea that engineered vessels should be composed solely of cells and proteins found in vivo.

To address these concerns, L'Heureux and others developed a process in 1998 to engineer vessels with high burst strength solely from human vascular cells and the extracellular matrix (ECM) produced by them [2]. This process coaxed mural cells to produce thick sheets of ECM, by supplementing culture media with antioxidants and metal salts that promoted cross-linking of deposited collagen. Winding of these sheets of cells and matrix around a cylindrical mandrel, removal of the mandrel, and seeding of ECs into the lumen of the resulting tube generated a construct with burst strengths greater than that of human saphenous vein. The superior performance of these vessels, compared to that of vessels synthesized from gelled collagen, may result from their higher cellular densities and greater numbers of endogenous cross-links in the ECM.

ARTERIES MADE FROM DEGRADABLE POLYMERS

A second strategy to form biological vessels uses biodegradable polymers, as proposed by Langer and Vacanti in 1993 (Figure 1C) [3]. Here, the principle is to use a degradable polymer as a mechanical support for vascular cells. While the polymer slowly degrades, the seeded cells will produce ECM to replace the lost volume of the polymer; if carefully controlled, the rates of degradation and synthesis balance to eventually yield a construct composed solely of cells and endogenous ECM. Appropriate selection and placement of hydrolyzable bonds in these polymers enable the tuning of degradation times on the order of days to years. Because these scaffolds may be molded or woven into a variety of shapes, it may be possible to use degradable polymers to produce noncylindrical arteries (in contrast to the method of L'Heureux, which appears limited to cylindrical geometries).

In 1999, Niklason and others demonstrated this strategy by seeding SMCs onto a degradable tubular mesh in a bioreactor [4]. As in the work of L'Heureux, the culture media was supplemented to enhance the secretion of collagen by SMCs and the cross-linking of ECM. Upon degradation of the mesh, seeding of ECs into the lumen of the tubes formed a biological vessel. This construct contracted when exposed to the vasoconstrictor $PGF_{2\alpha}$, displayed a histologically appropriate organization, and remained patent 1 month after implantation in small animals. Efforts to extend this work to the synthesis of human vessels are currently focused on methods to obtain sufficient populations of autologous human vascular cells.

MATURATION OF ENGINEERED ARTERIES

Whether synthesized from collagen or degradable scaffolds, engineered vessels rarely possess sufficient mechanical strength as is to withstand in vivo stresses. Many groups have examined the use of shear and transmural stresses transduced by lumenal flow to enhance the strength of these constructs. Shear stress acts on the endothelial lining to induce the release of growth factors that may promote the proliferation of SMCs, while transmural stretch (especially as a result of pulsatile flow) increases the synthesis of ECM by mural cells and encourages the circumferential alignment of SMCs. The strengths of matured, engineered vessels made of human cells can exceed 2,000 mmHg; by comparison, the burst strength of a human saphenous vein is approximately 1,500 mmHg, whereas that of immature constructs is on the order of 200 mmHg. To date, useful schedules of maturation have arisen mostly by trial and error. As the effects of mechanical stresses on vascular cells become better studied, rational design of processes to control the mechanical properties of engineered arteries will become possible.

Engineered Microvascular Networks

Whereas artificial arteries are used to treat dysfunction of single vessels, engineered microvessels are envisioned

for the treatment of ischemia mediated by the degeneration or absence of entire microvascular networks. As with small-diameter arteries, engineered microvessels will require the synthesis of completely biological tissues to remain patent in vivo. In contrast to the manual preparation of constructs used for macroscale arteries, it is neither practical nor necessary to assemble microvessels by hand into an interconnected network. Two complementary strategies have been proposed for vascularization of a tissue (Figure 2): the addition of angiogenic growth factors to attract capillaries from a host into the desired tissue, and the organization of ECs in an implanted tissue into networks that anastomose with microvascular beds from the host.

Microvascular Networks Induced by Growth Factors

Many growth factors, such as VEGF, aFGF, and bFGF, induce angiogenesis in vivo; presentation of these factors in a concentration gradient results in directional growth of capillary sprouts toward the regions of high concentration.

To take advantage of this natural biological process for the vascularization of tissues, many groups have tried to infuse an ischemic tissue or transplant with angiogenic factors (Figure 2A). The factors may be slowly released from micropellets of degradable polymers impregnated with the desired proteins, or locally produced by transfected cells (whether exogenously added or generated in situ from the host). Initial results have indicated that strategies for vascularization that rely on release of a single growth factor, such as VEGF, often do not yield fully functional microvascular beds. Instead, the vessels that form are usually highly permeable, poorly invested by mural cells, and prone to regression over time.

Because signals provided by mural cells play a crucial role in the maturation of vascular beds in vivo, current efforts have focused on the release of a combination of growth factors to promote angiogenesis and to recruit mural cells. In 2001, Mooney and others demonstrated that implantation of composites that sequentially release an angiogenic factor (VEGF) and a factor chemotactic and
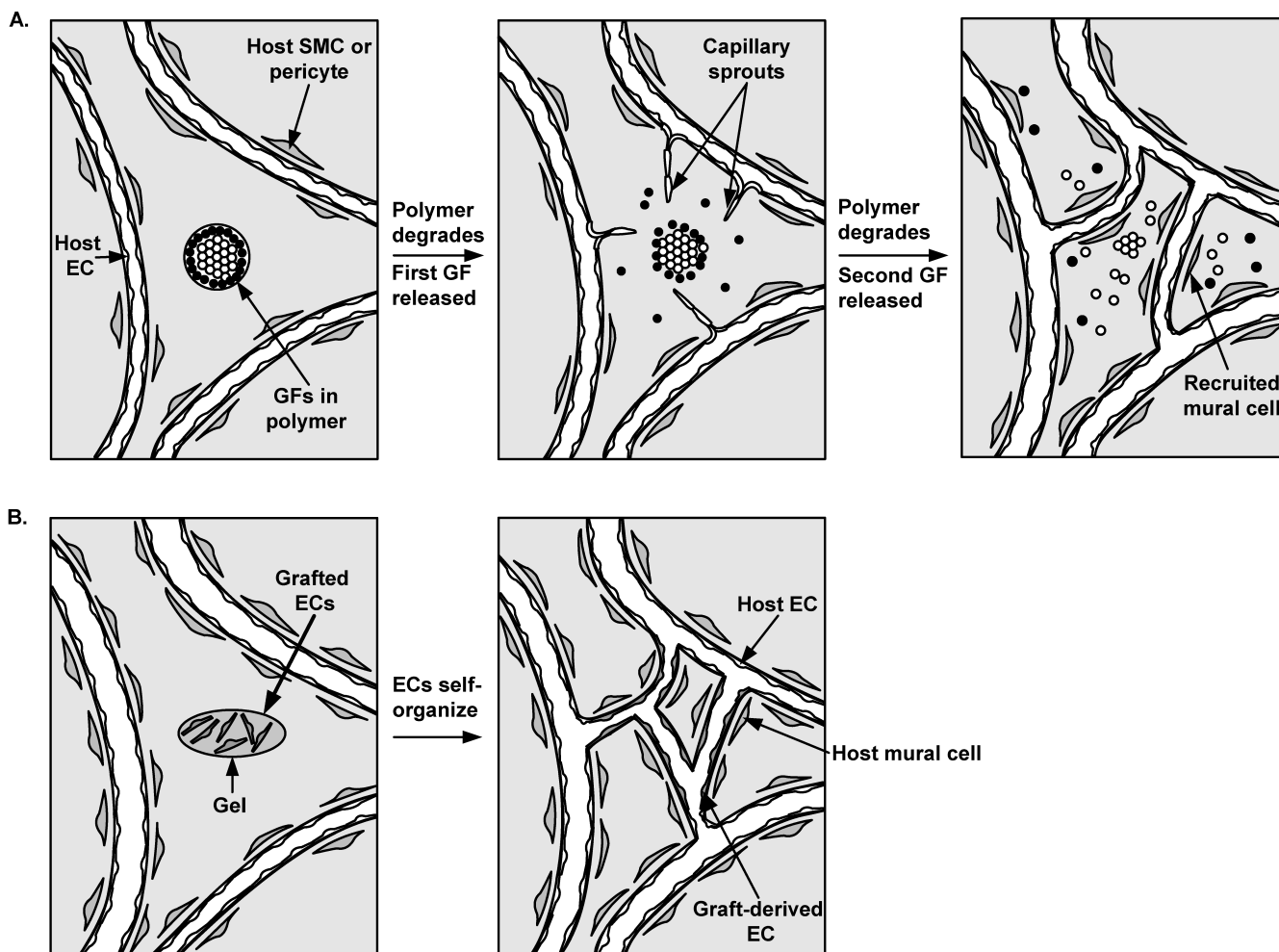

Figure 2   Processes for engineering microvascular networks. **(A)** Slow release of growth factors (GFs) from degradable pellets promotes the invasion of implanted tissue by microvessels derived from the host. **(B)** Self-organization of injected ECs (labeled red) into networks, and anastomosis of those networks with host microvasculature, generate a viable network at the site of injection.

mitogenic for mural precursors (platelet-derived growth factor; PDGF) induced the sprouting and maturation of capillary networks from the host into the grafted area [5]. These composites consisted of layers of a degradable polymer, one of which degraded more quickly than the other and hence allowed a gradual, two-stage release of growth factors. The final microvascular networks induced by these composites were well-invested by mural cells, exhibited appropriate permeability, and regressed less than vessels induced by a single growth factor. The idea of using the release of two or more factors to promote angiogenesis and mural recruitment has expanded to encompass many permutations (VEGF/PDGF, bFGF/PDGF, VEGF/bFGF/PDGF, and so on), each of which may display different potencies for inducing vascularization in different organs. Exhaustive searches for an optimal combination of growth factors and an effective schedule of release will undoubtedly yield improvements in the densities and stabilities of engineered vascular networks.

Microvascular Networks Formed from Grafted Endothelial Cells

It has been known since the early 1980s that cultures of ECs embedded in a collagen gel may organize to form short "cords" comprised of several cells linked in a chain. Some of these cords have open lumens and interconnect to form rudimentary networks. The application of these self-organized structures to microvascular engineering lies in their potential to anastomose with preexisting microvascular networks. In theory, implantation of exogenous ECs into an ischemic tissue may allow the organization of these ECs into
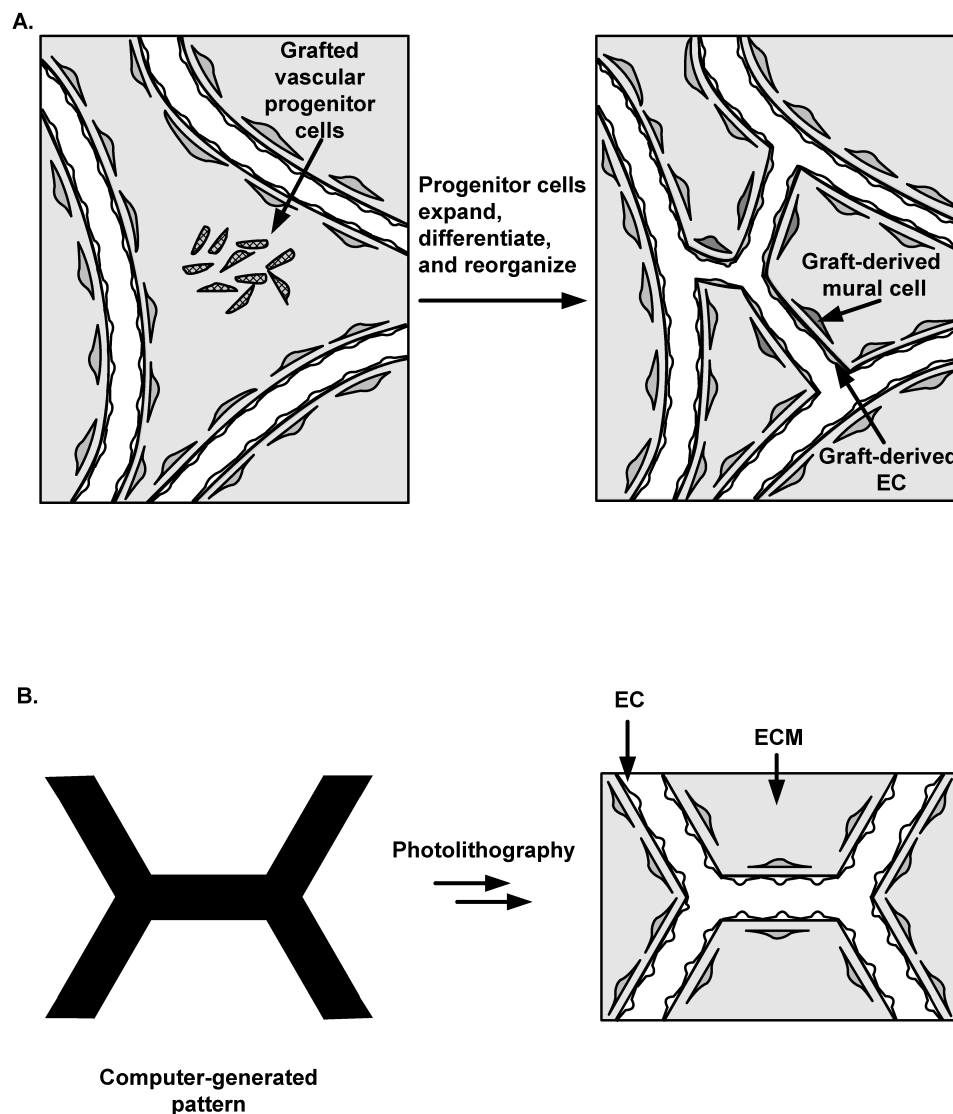
Figure 3   New strategies in vascular tissue engineering. **(A)** Vascular progenitor cells may differentiate in situ to form both ECs and mural cells, which subsequently organize into networks. Graft-derived cells are labeled red. **(B)** Lithographically formed patterns may guide the placement of vascular cells and enable the engineering of microvessels that have predefined shapes.

networks that connect to host vessels (Figure 2B). Because cellular self-organization occurs in parallel throughout the volume of the grafted tissue, this approach appears particularly well suited for the vascularization of thick tissues.

Initial attempts to engineer microvascular networks by implanting gels in which ECs were embedded did not succeed, primarily because the ECs did not survive long enough to anastomose with vessels from the host. Subsequent improvements have focused on the enhancement of cellular survival: For example, Schechner and others used ECs transfected with the antiapoptotic gene Bcl-2 to prolong survival of ECs [7], whereas Herron and others used immortalized telomerase-active ECs [7]. In both of these studies, grafts of transfected ECs survived for a longer period of time than those of untransfected cells did, and eventually organized into networks that anastomosed with host vasculature. Portions of the engineered networks were well invested by layers of mural cells and were stable for about 2 months.

As in the engineering of arteries, the use of transfected cells for microvascular engineering may compromise the biological activity of the formed tissue in unpredictable ways (e.g., whether telomerized ECs exhibit the same functional responses as normal ECs is debatable). Some groups have attempted to extend the viability of grafted ECs without the use of transfection by coinjecting mural precursors with ECs. In vitro, coculture of mural cells and ECs in collagen gels results in the formation of invested vascular networks; in 2004, Jain and others demonstrated that co-injection of mural cells and ECs in vivo results in networks that anastomose with host microvasculature and that are stable for longer than 1 year [8].

Future Directions

Vascular tissue engineering has evolved from the substitution of macroscale tubes for portions of the arterial tree, to the combined use of growth factors, woven polymers, purified populations of cells, and genetic manipulation to construct macro- and microvessels. It has resulted in the clinical application of large-diameter tubular grafts for repair of aortae and peripheral vessels, and in the development of small-diameter vessels that may someday be implanted in humans. Clinical trials are underway to test the effectiveness of angiogenic growth factors in inducing the formation of microvascular networks in ischemic tissues. Strategies based on the use of seeded cells for the engineering of arteries and microvessels appear promising and await the development of methods to culture sufficient numbers of autologous vascular cells before these therapies can be widely applied in humans.

Two recent developments may potentially revolutionize the engineering of blood vessels (Figure 3): First, in 1997, Isner and others discovered that human peripheral blood contains multipotent progenitor cells that can differentiate into cells that express EC- or SMC-specific markers [9].

This work has spawned the idea of using these progenitors to produce large numbers of autologous vascular cells in vitro for cell-based engineering, or perhaps, to allow injected progenitor cells to expand in vivo into invested networks. Second, in 2000, Vacanti demonstrated that photolithography, a technique widely used in the microelectronics industry to pattern metals, ceramics, and semiconductors, may be applied to construct polymeric structures that resemble microvascular networks in shape [10]. Lithographic tools may be able to provide micrometer-scale control over the placement of vascular cells in three dimensions, and thus enable the synthesis of microvessels of a selected topology. Further advances in these areas will expand the toolkit available to tissue engineers and should lead to the synthesis of custom-made vascular tissues for the treatment of human disease.

Glossary

Autologous: Derived from the same organism (e.g., an autologous transplant).

Biodegradable polymer: A polymer that degrades in physiological conditions into small molecules that are readily absorbed or secreted.

Patency: The ability of a vessel to support the flow of blood through it (usually in reference to large vessels).

Photolithography: A process that uses irradiation of light-sensitive materials through a selective mask to generate patterns with micrometer-scale resolution.

Tissue engineering: The synthesis of living structures that can repair or replace defective tissues.

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Yannas, I. V. (2000). Synthesis of organs: In vitro or in vivo? *Proc. Natl. Acad. Sci. USA* **97**, 9354–9356. *This review by Yannas outlines two complementary approaches for generation of functional microvascular networks in a tissue: recruitment of microvessels from a host, and anastomosis of graft-derived microvessels with those of the host.*

Further Reading

Nerem, R. M., and Seliktar, D. (2001). Vascular tissue engineering. *Annu. Rev. Biomed. Eng.* **3**, 225–243. *In this review, Nerem and Seliktar describe many important considerations in the construction and maturation of engineered arteries, with a particular emphasis on the use of lumenal flow to strengthen the walls of as-synthesized constructs.*

Capsule Biography

Dr. Tien, Mr. Golden, and Ms. Tang are members of the Department of Biomedical Engineering at Boston University. Their research focuses on the engineering of living tissues that have complex three-dimensional architectures. Their work is supported by the NIH and the Whitaker Foundation.