TECHVIEW: MEDICAL TECHNOLOGY

Replacement Arteries Made to Order

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Blood vessels are the life-giving conduits that connect our tissues and our organs to the heart and to each other. Diseases of the blood vessels, principally of the small- and medium-caliber muscular arteries, account for the majority of deaths in the United States annually (*1*). The high incidence of atherosclerosis, in which formation of fatty plaques leads to clogging of the arteries, and related diseases means that diseased muscular arteries are surgically replaced more frequently than any other tissue in the body. In cardiac or peripheral bypass surgery, diseased arteries are usually replaced with autologous veins (that is, healthy veins from the same patient) or, less frequently, with autologous arteries. However, many patients who are in need of bypass surgery do not possess sufficient veins to act as replacements for their diseased arteries. These individuals then face palliative medical therapy and often suffer myocardial infarctions (heart attacks) or endure limb amputations as the blood flow becomes more and more constricted. Such medical realities have spurred many investigators to attempt to develop biological replacements for small-caliber arteries. If replacements were available, surgery would be possible for the many thousands of patients with atherosclerosis who currently cannot undergo bypass surgery. Although tissue engineering of blood vessels has been underway for more than two decades (*2*, *3*), the last 18 months have brought several important advances to this exciting field (*4*–*6*).

Normal muscular arteries have a trilamellar structure (Fig. 1), and each of the three layers confers specific functional properties on the blood vessels (*7*). The inner endothelial layer is a single cell layer that prevents spontaneous blood clotting in the vessel and regulates vascular smooth muscle cell tone. The intermediate or medial layer is composed of smooth muscle cells and extracellular matrix components such as collagen, elastin, and proteoglycans. The medial layer contributes the bulk of the mechanical strength to the vessel as well as its native ability to contract or relax in response to external stimuli. The outer adventitial layer, composed primarily of fibroblasts and extracellular matrix, harbors the microscopic blood supply of the artery as well as its nerve supply. Mimicry of some or all of the properties of the three layers of a healthy artery has been the strategy of all arterial tissue engineering approaches.

Huynh and colleagues at Organogenesis and Duke University recently reported a fascinating approach to arterial replacement (*4*). They prepared tubes of submucosal collagen from porcine small intestine and coated them with bovine fibrillar collagen. The inner surface of each vascular graft was treated with heparin-benzalkonium chloride. This heparin complex inhibited the coagulation cascade and helped to prevent clotting in the graft. The layers of collagen were chemically cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride to give the grafts mechanical strength. These nonliving vascular grafts were prepared with a minimum of chemical cross-linking to preserve their biocompatibility and to enable cellular infiltration in vivo. Eighteen of these grafts were implanted in rabbits and monitored for periods of 4, 8, or 13 weeks. During these time periods, all of the grafts remained open and no clots were observed. This impressive result was accompanied by the infiltration of the graft with smooth muscle cells and endothelial cells from the rabbit recipient. The implanted grafts incorporated host cells to such an extent that they exhibited small contractile responses to pharmacologic agents after they were removed from the rabbits. Although these results are certainly encouraging, the response of the human cardiovascular system to vascular grafts that are made from animal collagens remains unknown. Whether these grafts will stimulate an inflammatory response in humans and whether they will develop the host endothelial layer that may have contributed to their patency in animals, are unanswered questions.

Other researchers have reported the use of fibrillar intestinal collagen in vascular graft models (*8*, *9*). Intestinal collagenous layers have been treated with formaldehyde to improve their stiffness, and then have been used to "buttress" native vein grafts, thereby improving compliance matching between the graft and the adjacent native artery (*10*). A matching of compliance, or stiffness, between the graft and the native artery has been shown to decrease the incidence of scarring and vessel blockage at the suture line. Another intriguing report describes a model for partial liver replacement in which submucosal collagen layers from rat intestine are loaded with liver cells and grafted into the intestinal circulation of rat recipients (*9*). In contrast to using fibrillar collagen for the engineering of arteries, several investigators have prepared tubular gels made from denatured bovine collagen. Weinberg and Bell (*2*) first described this approach in their landmark paper in 1986, wherein they cultured vascular cells in preformed collagen gels to form a three-layered structure analogous to a native vessel. Ziegler and Nerem (*11*) have pursued this approach further and have made an extensive study of the interactions between cultured vascular cells and the collagen gel matrix. Collagen gel techniques have been well studied and continue to hold promise, but a graft with mechanical strength that is comparable to native vessels has not yet been obtained with these approaches.

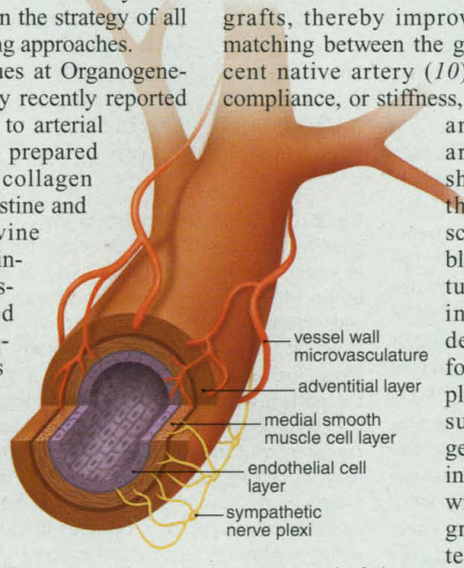

Fig. 1. Normal arteries are composed of three component layers: the inner endothelial layer, the intermediate medial layer, and the outer adventitial layer. The endothelial cell layer is composed of a monolayer of confluent cells that prevents thrombosis and regulates vascular smooth muscle cell tone. The medial layer is composed of alternating microlayers of smooth muscle cells and extracellular matrix components arranged around the circumference, which confer much of the mechanical integrity to the artery. The adventitial layer is a loosely organized connective tissue that contains fibroblasts and matrix proteins, and is the source of the microvasculature and sympathetic innervation that sustains the muscular artery wall.

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Exogenous extracellular matrices such as submucosal collagen and denatured collagen gels have been used to support the growth of vascular cells in vivo and in vitro, respectively. Taking another approach, L'Heureux and colleagues last year reported a unique strategy for arterial replacement that incorporates cultured cells without any type of exogenous extracellular matrix (*6*, *12*). These investigators cultured smooth muscle cells from umbilical vein and skin fibroblasts into flat sheets. The cellular sheets were then rolled around a mandrel in sequential layers to construct a tubular vessel. After a suitable amount of time in culture, the inner lumen of the tubular construct was seeded with endothelial cells that grew to form a third, inner layer. In this way, a multilayered artificial vessel was created in vitro that mimicked the three-layered structure of a normal artery. These artificial vessels could withstand impressive pressure stress, displaying rupture strengths of greater than 2000 mm Hg. Such rupture strengths are comparable to those of native human coronary arteries. This important result was the first demonstration of the feasibility of creating a mechanically robust vascular structure solely from cultured cells and their secreted extracellular matrix proteins. When grafted into a dog transplant model, the vessels displayed a 50% thrombosis rate after one week of implantation. Nevertheless, this work raises the possibility that functional vessels could be produced in culture from cellular components alone.

The biology of native arteries is strongly influenced by the pulsatile hemodynamic forces to which they are exposed. Increased blood pressure is known to cause vessel wall hypertrophy, as well as many changes in vascular cell physiology (*13*). As early as 1974, vascular grafts were created in animals by placing a pulsating balloon pump subcutaneously (*3*). The pulsating tubular balloon stimulated the formation of an encapsulating fibrous sheath, which then functioned adequately as an arterial graft in the same animal. Work in my laboratory has explored the possibility of culturing arterial grafts in vitro under physiologically pulsatile conditions (*5*, *14*). We have cultured autologous vascular smooth muscle and endothelial cells on highly porous, degradable polymer scaffolds. Scaffolds with growing vascular cells are subjected to pulsatile radial distensions during culture that mimic the human cardiovascular system. A perfusion system containing a bellows pump provides a pulsatile flow of sterile fluid through the lumens of the vessels, which distends the developing arteries with each pulse. After about 8 weeks, the polymer scaffold degrades substantially and is replaced by a dense smooth muscle cell medial layer and an inner endothelial lining. Engineered vessels cultured under pulsatile conditions possess many of the physiologic and mechanical characteristics of native arteries, including burst pressures of more than 2000 mm Hg. With the use of these techniques, we implanted the first autologous tissue–engineered arteries in miniature swine and found the implants to be functional for up to 4 weeks. Although these preliminary results are encouraging, much more work needs to be done before we fully understand the biological effects of various culture conditions and cell-polymer interactions on the cultured vascular cells, both in vitro and in vivo.

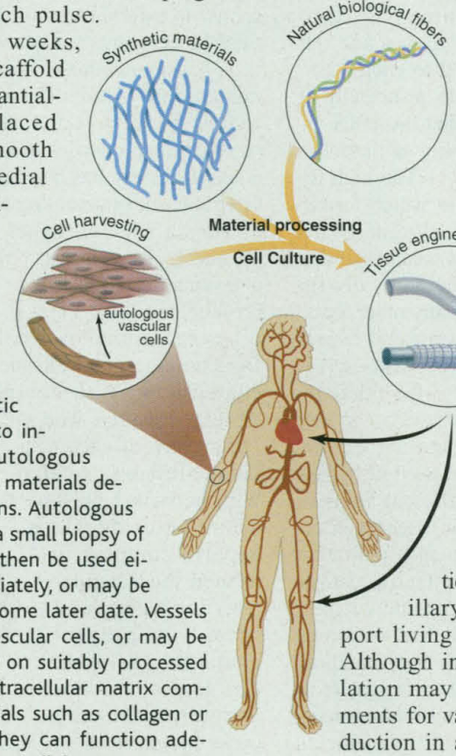

Fig. 2. Different components of engineered arteries. The most authentic engineered blood vessels are likely to incorporate some combination of autologous cells, synthetic materials, and natural materials derived either from animals or humans. Autologous vascular cells can be harvested from a small biopsy of the patient's tissue. These cells may then be used either to produce blood vessels immediately, or may be cryopreserved for vessel growth at some later date. Vessels may be cultured exclusively from vascular cells, or may be produced from cells that are grown on suitably processed polymer scaffolding or exogenous extracellular matrix components. Alternatively, natural materials such as collagen or elastin may be processed so that they can function adequately as vascular grafts, without any cellular components. Regardless of which technique emerges as superior, the clinical goal will be to produce implantable arteries that cause a minimum of inflammatory, infectious, and thrombotic complications. These arteries will be used primarily in restoring circulation to the heart and the extremities of patients with vascular disease.

What will the replacement arteries of the future look like? Although the final template is not clear, reports in the last 2 years have shown several exciting possibilities (Fig. 2). Nonliving tubular grafts have been produced from natural biological materials that can become functioning neo-arteries after implantation in vivo. Living vascular grafts that possess remarkable mechanical properties have been cultured in vitro from animal vascular cells and from non-autologous neonatal human cells. Although seeding living vascular grafts with xenogeneic cells (cells from another species that have been rendered immunologically neutral) is a long-term possibility, it is likely that any cell-based vascular grafts in the near future will incorporate autologous vascular cells. The eventual structure of living vascular grafts may incorporate cells cultured alone or may include cells combined with synthetic or natural materials. Lastly, there remain the challenging hurdles of creating a functional nerve supply and capillary network in vitro to support living vascular tissues (Fig. 1). Although innervation and microcirculation may not be essential requirements for vascular conduits, their production in any cultured tissue would constitute a giant leap forward for the field of tissue engineering. Functional engineered arteries may one day revolutionize the treatment of vascular disease. They will also be a crucial enabling technology for the development of other engineered tissues, all of which will require a blood supply to sustain them after transplantation into humans.

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