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Designer blood vessels and therapeutic revascularization

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Inadequate vascular perfusion leads to fatal heart attacks, chronic ulcers, and other serious clinical conditions. The body's capacity to restore vascular perfusion through angiogenesis and arteriogenesis is often impaired by pre-existing disease, and availability of native replacements for nonfunctional arteries is limited in many patients. Thus, recreating blood vessels of various calibres through novel engineering technologies has emerged as a radical option among therapeutic strategies for revascularization. Ranging from artificial, recycled or reassembled natural conduits to sophisticated microdevices, we refer to these as 'designer blood vessels'. Our common efforts to continuously improve vascular replacement design have provided many clues about our own blood vessels, but nature's ability to create nonthrombogenic, immunocompatible, strong, yet biologically responsive blood vessels remains unparalleled. Just as art reproductions never equal the original masterpiece, designer blood vessels may never attain nature's perfection. Nevertheless, they will provide a valuable option as long as they come close enough and are available to many.
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Abbreviations: BAM, bioartificial muscle; μCP, microcontact printing; EC, endothelial cell; ECM, extracellular matrix; bFGF, basic fibroblast growth factor; HIF-1α, hypoxia-inducible factor 1α; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PDMS, polydimethylsiloxane; PEO, polyethylene oxide; PGA, polyglycolic acid; PLA, polylactic acid; ePTFE, expanded polytetrafluoroethylene; SAM, self-assembled monolayer; SIS, small intestinal submucosa; SMC, smooth muscle cell; TE, tissue engineered; TEBV, tissue-engineered blood vessel; VEGF, vascular endothelial growth factor

Introduction

The capacity to restore vascular perfusion and thus oxygenation and delivery of nutrients to ischaemic or otherwise damaged organs is essential for repair and regeneration. Ischaemic episodes of cardiac and peripheral tissues trigger the formation of new capillaries from existing networks, a process known as angiogenesis. The level of perfusion supplied by the neomicrovasculature, however, is not enough to restore organ function, and the development of larger collateral vessels may occur *via* a process known as arteriogenesis. The building of these collateral vessels appears to be initiated by inflammatory cells, such as circulating monocytes (Schaper & Scholz, 2003), and besides endothelial cells (ECs), requires the recruitment of additional cell types including pericytes and vascular smooth muscle cells (SMCs). While angiogenesis tends to be initiated by ischaemia, arteriogenesis can occur under nonhypoxic conditions, and is regulated by a number of interdependent growth factors, cytokines, and mechanical stresses (Carmeliet, 1999).

In adults, however, the natural process of reforming the vascular structures necessary for proper tissue revascularization is usually not efficient enough to save compromised tissues, especially in the presence of existing vascular disease (Hill *et al*., 2003). Thus, while the identity and timing of the complex interactions among the numerous biological factors that regulate arteriogenesis and angiogenesis continue to be resolved, the great clinical need to restore vascular conduits has driven the medical and bioengineering communities towards the fabrication of blood vessel substitutes, which we dubbed 'designer vessels' for the purpose of this review.

Large-diameter vessels

Although most complications tend to occur in smaller diameter vessels even the largest arteries are susceptible to cardiovascular disease (Shah, 1996). Arterial walls, when weakened by defective formation or unchecked degradation of their extracellular matrix (ECM) scaffold, balloon outward excessively and threaten a patient's life. In the case of thoracic and abdominal aortic aneurysms, conditions frequently associated with advanced atherosclerotic disease in older patients, the arterial ballooning is typically treated through endovascular stenting or open surgical repair (Figure 1). The clinical success of intraluminally delivered endovascular stents fabricated of artificial materials, such as those comprised of stainless steel or titanium alloy framework housed in a polyester fabric sheath, depend highly on the accurate diagnosis of the aneurysm morphology. While postoperative complications, including endoleakage, stent migration, and

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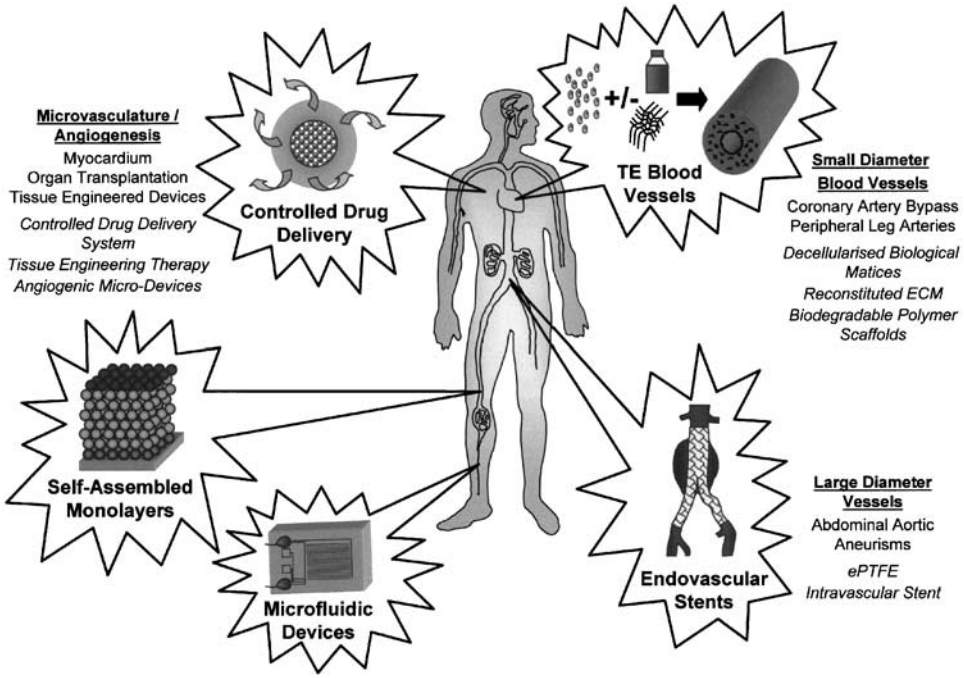

Figure 1 Designer blood vessels engineered to supplant large-diameter, small-diameter, and microvessels for therapeutic revascularization of various tissues.

rupture are reported to occur in 10–25% of patients (Thurnher & Grabenwoger, 2002), success rates following stenting interventions are high. Thus, while some potential alternatives to endovascular stenting, such as tissue-engineered (TE) tubes of autologous cells and biodegradable polymers (Shum-Tim *et al*., 1999), continue to be investigated pre-clinically, there is little incentive to create more nature-like sophisticated, large-diameter arterial substitutes.

Small-diameter arterial grafts

Coronary, carotid, and femoral arteries are examples of small-diameter vessels (3–5 mm i.d.) that tend to develop atherosclerotic lesions, stenosis, and other complications necessitating surgical intervention. Autologous native vessels, that is, saphenous veins and mammary arteries, are the most currently used option for obtaining small-diameter arterial replacements. Major advantages associated with this solution include 'ready-to-use' conduits and immune acceptance. The availability of suitable native replacements, however, is limited when multiple conduits are required, especially in patients with widespread pre-existing vascular disease.

Development of small-diameter tissue-engineered blood vessel (TEBV) represents both a major challenge and a major opportunity for the medical device industry (Figure 1). In order to function successfully, TEBVs must be nonthrombogenic, nonimmunogenic, and suturable, and must possess both adequate mechanical strengths and appropriate functional and healing responses. Tight manufacturing regulatory controls and an economical storage plan are also required for an off-the-shelf, mass-produced product line. While different solutions have been proposed to address each of these specific challenges, development of a fully functional TEBV with high long-term patency rates has yet to be accomplished.

The simplest solution of using synthetic conduits comprised of Dacron fabrics or expanded polytetrafluoroethylene (ePTFE), materials that perform adequately in high-flow, large-diameter environments, has been used to replace small arteries as well. However, in this capacity, the materials proved to be undesirable due to thrombogenecity and lack biological functional responses necessary for adaptation and functional responses. Attempts to improve the patency of the ePTFE through heparin coatings (Ritter *et al*., 1998), fibrin glue (Gosselin *et al*., 1996), or EC seeding (Herring *et al*., 1994) yielded promising – though sometimes mixed – results. Thus, efforts aimed at reproducing vascular function through the recreation of specific anatomical and physiological arterial properties led to the intense interest in TE strategies. By combining living cells with matrix scaffold structures, TE approaches strive to re-establish both the mechanical and the essential biological functions of native organs.

Natural options for arterial scaffolds

The extracellular scaffold, providing structural and mechanical properties, anchorage for cells, and storage for soluble factors, is essential for any natural tissue, and is thus a major component for any successful tissue replacement. Scaffold materials tested for TEBVs have ranged from biodegradable polymer meshes to native intact scaffolds. Additional inclusion of exogenous cells, ECs, fibroblasts, and SMCs, from a variety of tissue sources and/or absorbed active viruses capable of luring and infecting the surrounding host cells continues to be tested in an effort to repopulate the scaffolds and improve functionality. While the constructs based mostly on the use of natural components display the characteristics of native arteries more effectively, the mechanical properties of such constructs remain inferior to what is expected for an arterial conduit.

Among the tested options, the use of nature's own designed scaffolds is likely to yield the first generation of small-diameter, designer blood vessels. After the removal of the original cellular component through sequential protease treatments and detergent extractions, decellularized conduits of natural matrix structures from tissues ranging from cadaveral arteries to umbilical veins to small intestinal submucosa (SIS) xenografts have been tested for their potential to replace vascular matrix and encourage the ingrowth of host cells postimplantation.

Implantation of decellularized arteries, consisting predominantly of collagen, elastin, and associated glycosaminoglycans, into dogs was reported to yield 94% 6-year patency (Wilson *et al*., 1995). Already investigated in clinical applications including urinary tract repair and meniscal replacement, the porcine SIS appears to be a promising decellularized matrix material. In addition to the typical ECM components, this scaffold was found to contain a number of growth factors that enhance neovascularization and infiltration of host cells upon implantation.

Used as an experimental vascular graft biomaterial in dogs since 1989 (Badylak *et al*., 1989), grafts are reportedly completely endothelialized at 28 days postimplantation, histologically similar to native arteries at 90 days postimplantation, and present no evidence of intimal hyperplasia, infection, or aneurysmal dilation at 5-year follow-up investigations (Lantz *et al*., 1993). Scientists from Organogenesis Inc. (Huynh *et al*., 1999) made improvements upon the decellularized porcine SIS by crosslinking it with ethyl carbodiimide to enhance its mechanical performance, and by coating with a heparin-benzalkonium chloride complex to reduce thrombogenecity. When these TEBVs were implanted into rabbits as infrarenal replacements, the grafts were rapidly populated by infiltrating host cells. Additionally, they reported little to no immune response.

The success of using decellularized natural matrix scaffolds is attributed to the advantage of already having the proper composition and architecture. The high conservation of matrix molecule, specifically collagen, sequences among the different species is thought to engender immunoprotection. Despite these, a major remaining challenge is the notorious susceptibility of naturally derived materials to degradation through enzymatic digestion following implantation. This has led to the use of crosslinking treatments, such as glutaraldehyde, ethyl carbodiimide, dehydrothermal treatment, and ultraviolet radiation, in an effort to stabilize the tissues (Berglund *et al*., 2003). Although glutaraldehyde, one of the more popular crosslinkers that forms bonds between amine moieties such as the ε-amine of the lysine residues, improves the mechanical performance and stability of bioprosthetic valves and pericardial tissues (Ionescu *et al*., 1974; Sung *et al*., 1996), it is also linked to their calcification (Schmidt & Baier, 2000) and leaching of cytotoxic monomers (Gendler *et al*., 1984). Furthermore, such harsh chemical treatments can alter cell–matrix interactions and destroy the efficacy of associated growth factors. Recent strides have been made to minimize calcification (Rao & Shanthi, 1999), but the potential for residual toxicity may continue to be a concern.

The other option of using natural individual ECM to reconstitute scaffolds introduces some additional degrees of flexibility to the design process. Thus, several types of solubilized matrix proteins can be combined with cells from a variety of sources *ex vivo*, also making it possible to incorporate genetic engineering methodologies and to direct cell-mediated remodelling prior to implantation. Thus, thorough controlled manipulation of the chemical and physical environments during the developmental phase can also be achieved.

Adding cells to the scaffold – more options and additional challenges

In 1986, Weinberg & Bell (1986) described the fabrication of a tubular vascular graft containing an adventitia analogue comprised of fibroblasts and Type I reconstituted collagen, a media analogue comprised of SMCs and reconstituted collagen, and an EC monolayer. While the resulting TEBV displayed many of the morphological characteristics of native arteries, its mechanical properties were very weak, and Dacron support sleeves were necessary to withstand the haemodynamic pressures after implantation. These challenges, however, were considered to be relatively straightforward, and success was anticipated to follow promptly.

Over the following 20 years, a number of research laboratories have adapted this artery model to investigate cellular behaviour and TE construct functionality. One of the major hypotheses investigated has been that a more faithful recreation of the natural organization of the medial layer will enhance the function of TEBV. Indeed, obtaining a circumferential alignment of SMCs, by casting a tubular construct about a central mandrel (L'Heureux *et al*., 1993), significantly increased the circumferential stiffness modulus as compared to that of freely compacted constructs. Additional attempts to control the organization of reconstituted TEBVs investigated the effect of collagen fibre prealignment (Barocas *et al*., 1998), achieved by allowing fibrillogenesis of the collagen solution to occur in the presence of a magnetic field. The formed collagen fibres became preferentially aligned in an orientation perpendicular to the field, but the initial improvements associated with this strategy decreased with time in culture, as cells seeded in these collagen matrices ultimately prevailed in the process of remodelling.

Mechanical stimulation has also been employed to induce construct remodelling, and thus improve the mechanical performance of cell-seeded ECM gels. Cyclic stretching of tubular SMC-seeded collagen constructs was found to enhance the switch of SMCs from a synthetic to a contractile phenotype (Kanda *et al*., 1993). More importantly, cyclic strain (10% at 1 Hz frequency) resulted in increased stiffness moduli and ultimate stress parameters of collagen-based TEBVs seeded with rat SMCs, and to a lesser extent of constructs containing human aortic SMCs or human dermal fibroblasts (Seliktar *et al*., 2000). The mechanical stimulation was also found to increase the expression and activation of matrix metalloproteinase (MMP)-2 (Seliktar *et al*., 2001), an enzyme known to degrade short collagens and previously associated with the remodelling of native arteries (Galis & Khatri, 2002). Interestingly, when the dynamic conditioning was applied in the presence of nonspecific inhibitors of MMP activity, with the intent to preserve construct integrity, the opposite result was obtained. Nonspecific inhibition of MMP activity ablated the enhancing effects of mechanical conditioning upon the organization and mechanical properties of the TEBVs (Seliktar *et al*., 2001). These observations, obtained using SMC-seeded

collagen constructs, led to the important realization that biologically active factors such as MMPs, known to contribute to the dissolution of matrix, are also important for the assembly of a collagen matrix. Besides its implication for the remodelling of native tissues, the deeper understanding of this new complex interrelation between cells and ECM may offer new opportunities for the design of TE substitutes.

A strategy aimed at enhancing collagen assembly entails nonenzymatic glycation through culturing cell-seeded constructs in growth media containing elevated levels of ribose and/or glucose (Girton *et al*., 2000). The covalent crosslinking of the collagen fibres through glycation reactions led to increased construct strengths and stiffness moduli over time. This strategy, still being investigated, seems to provide a method for accelerated matrix crosslinking without the viability loss observed with glutaraldehyde treatment.

More recently, research groups have started investigating the use of alternative ECM components when forming cell-seeded gels. Fibrin, one of the most popular substitutes, was selected based on its natural ability to provide a provisional matrix for tissue ingrowth during wound healing, and is being investigated in small-diameter vascular substitutes, as well as for bioartificial heart valves (Ye *et al*., 2000; Jockenhoevel *et al*., 2001).

Despite many of the advances described above, weak mechanical properties continue to restrict the majority of investigations to benchtop research. From a mass-production standpoint, a major challenge associated with TEBVs fabricated from cell-seeded reconstituted ECM is the expense and complications associated with *in vitro* culturing prior to implantation. The scaling up of natural components and culture environments used in academic experimental studies, expensive bioreactors, and storage requirements may render this approach cost prohibitive for mass production. While hybrid combinations of reconstituted matrices with biological or synthetic support structures may overcome deficiencies in the mechanical properties, in the end, the major utility associated with this approach may be as an *in vitro* test bed to develop new TE technologies, to address questions in basic vascular biology, and to screen potential pharmacological agents.

Letting the cells do the building

Another approach using solely biological components has been developed, in which monolayers of cultured SMCs and fibroblasts isolated from human umbilical veins and surgical biopsies (L'Heureux *et al*., 1998) are cultured for approximately 3–5 weeks in ascorbate-supplemented growth media. Secretion of significant amounts of endogenous matrices by these cells eventually allowed the careful lifting of the intact monolayers, which were subsequently wrapped around tubular mandrels to form multilayered constructs. Additional layers were periodically added during an additional 8 weeks in culture. Immunostaining and histological analysis revealed a highly structured, circumferentially oriented ECM containing collagens type I, III, and IV, laminin, fibronectin, and elastin. Grafts fabricated by this method were reported to exhibit exceptional mechanical strengths, with burst pressures of 2600 mmHg. After additional processing through seeding of human umbilical vein ECs on the inner lumen, this construct was tested *in vivo* into mongrel dogs. Although initially patent, these human-based TEBVs thrombosed and occluded 1 week following implantation. Presumably, such complications likely caused by immunological and foreign body responses might be avoided by constructing the graft entirely from autologous components with an intact EC lining.

Self-assembled biologically based TEBVs were also built *in vivo* (Campbell *et al*., 1999). The implantation of silastic tubing into the peritoneal cavity of rats or rabbits was shown to induce a foreign body response that created a capsule of macrophage-derived myofibroblasts. These self-assembled tissues were removed from the tubing, everted, and autologously grafted *via* end-to-end anastomoses into either the carotid arteries or abdominal aortas. Transplanted grafts were reported to remain patent in excess of 4 months.

The implementation of an autologous approach might also benefit from privileged regulatory status, as a precedent exists for an autologous cartilage repair strategy not subjected to approval by the Food and Drug Administration. In spite of their innovative design and promising initial functional data, the development of TEBVs from cell-secreted matrices faces considerable challenges. Issues including extended culturing requirements and highly labour-intensive fabrication processes must be addressed to provide an off-the-shelf product capable of being produced in clinically relevant quantities.

Biocompatible polymer scaffolds – stronger than nature?

Polymers designed to present a hemocompatible interface and potentially even resorb over time provide an alternative to enzymatically unstable natural materials and mechanically weak reconstituted protein matrices. Resorbable synthetic scaffolds have shown great promise. Polyglycolic acid (PGA), polylactic acid (PLA), and other biodegradable polyesters are among the best characterized implantable materials, and have been used to make sutures and other medical devices since the 1970s (Lichtenstein, 1970; Faulborn *et al*., 1975).

Recapitulating the lessons learned from the *in vitro* development of constructs based on natural components with the added strength of biodegradable polymers, bovine SMCs were seeded onto tubular PGA scaffolds, followed by pulsatile conditioning in the presence of supplements known to stimulate the production of matrix components (Niklason *et al*., 2001). Thus, as the polymer scaffolds resorbed, they were gradually replaced with endogenous ECM. After 8 weeks, the TEBVs were capable of nominal vasoactivity, possessed lumens suitable for EC seeding, and exhibited exceptional mechanical properties, with burst pressures exceeding 2100 mmHg. These TEBVs maintained patency for 2 weeks after implantation into miniature swine, but gradual deterioration of constructs eventually led to pathological responses and decreased blood flow (Niklason *et al*., 1999). One potential explanation was that the highly acidic localized regions created by the residual PGA degradation elicited strong inflammatory responses *in vivo*.

Build it and they will come?

When acellular constructs fabricated from PGA and varying quantities of Dacron and polydioxanone were implanted into rabbits, capsules of mesenchymal cells formed in the outer layers of the graft (Greisler, 1982). Additionally, ingrown cells appeared to differentiate towards an SMC phenotype as they

approached the lumen, and at the lumenal surface, a spontaneous formation of a monolayer of endothelial-like cells was observed at 1 month postimplantation. Similarly, mixtures of polyurethane and PLA (95/5) implanted into rats as acellular aortic replacements were reported to form intimal layers with longitudinally oriented ECs, and medial layers containing SMCs and histologically identifiable elastic laminae, 6 weeks after implantation. After 1 year, however, many of the constructs became aneurysmal, and failed possibly due to degradation of the PLA matrix (van der Lei *et al*., 1987). While foreign body immune responses will continue to create concerns, cell-seeded manmade polymeric scaffolds are among the most popular of TE platforms, and are currently being pursued by several research groups.

Are we there yet?

Heralded as a sure thing by the end of the 20th century, the small-diameter TEBV remains a challenge more than 20 years later. Questions regarding material design, immune acceptance, and endothelialization strategies remain at the crux of the issue. It is also important to note that conclusions drawn from experimental models are inherently dependent on the suitability of the model used. Among others, species differences can have a great influence on the outcome of interventions. For instance, the distinctively different values obtained from the characterization of platelet and coagulation function in dogs suggest that this species is not an ideal animal model for the evaluation of blood–surface interactions (Sato & Harasaki, 2002).

Since both the configuration and composition of matrix materials can affect cell ingrowth, phenotype, and viability, significant efforts have been directed at developing an 'ideal' structural support. In general, scaffolds with large surface areas, porous microstructures, and interconnected pores best facilitate cell attachment and tissue growth. Consequently, TE fabrication methods have favoured the production of salt-leached foams and electrospinning of fibres to generate porous polymeric scaffolds. Recently, electrospinning was adapted to synthesize nonwoven meshes of collagen and collagen-polyethylene oxide (PEO) fibres (Huang *et al*., 2001) producing nontoxic, mechanically robust structures without denaturing the collagen fibres. New polymer systems, affectionately termed 'bio-smart', possess the ability to respond to glucose concentrations and other environmental cues (Brahim *et al*., 2002). While controlled insulin delivery and electroactive biosensors remain the current targeted applications, future developments may extend these benefits to vascular graft applications.

Vital issues of graft acceptance and survival must be addressed in any successful TE therapy. While autologous grafts provide the only current method to ensure immune compatibility, cell-seeded allogenic and acellular xenogenic implants have been successfully utilized in a number of applications. Furthermore, studies related to organ transplantation are elucidating the signals involved in T-cell activation. Consequently, it may be possible to engineer immune acceptance by regulating factors in the costimulatory pathways, such as the binding of CD28 on T cells to B7 ligands on antigen-presenting cells, or creating immunochimerism (Bour-Jordan & Blueston, 2002).

Complications associated with surface thrombogenecity are thought to result from the lack of intact endothelial linings. In an ongoing clinical study, *in vitro* endothelialization was used to seed almost 200 ePTFE femoro-popliteal bypass grafts with autologous ECs (Zilla *et al*., 1994). Although each intervention required approximately 30 days to expand ECs from venous biopsies prior to implantation, their 5-year patency rates increased to 74% from 33%, with nonendothelialized controls (Merzkirch *et al*., 2001). Alternatively, vascular grafts may eventually be designed to spontaneously recruit ECs *in vivo*. Peptide sequences and protein domains related to both cell adhesion and angiogenesis stimulation are currently being used to line TEBVs to promote EC migration (Lin *et al*., 1993; Walluscheck *et al*., 1996; Stone *et al*., 2002).

The interdisciplinary efforts put into developing small-diameter arterial replacements have led to significant biotechnological advances, and the prototypes have taught us a lot about our own arteries. While we have reached a stage where we may know 'how' to build an artery, the major obstacle remains as to 'what' needs to go into it, knowledge that will come with the elucidation of all the *sine qua non* details of nature's clever design.

Smallest vessels

Metabolically active cells must be situated within 150–200 μm of a blood supply in order to function properly (Colton, 1995). Therapeutic angiogenesis, or directed growth and regulated assembly of microvascular beds, would greatly benefit an estimated 300 million patients living in Western nations with chronic wounds, peripheral arterial disease, and tissue ischaemia (The Angiogenesis Foundation). Furthermore, the integration and survival of TE organ replacements themselves also depend on their proper vascularization, which may require recreation of a supporting microvascular network and the ability to connect it to the body's circulation. Not surprisingly, the highest level of clinical success among implanted TE products to date are the skin and bladder patches, thin enough to survive largely on diffusional mass transport processes. Thus, even the small-diameter blood vessels, with walls ranging from 300 to 1000 μm, as well as their TE replacements, require some degree of subservient microvasculature for proper oxygenation (Folkman & Hochberg, 1973; Colton, 1995).

Despite the obvious need for targeted angiogenesis, and the deceptively simple design of the capillary, essentially made up of tubular structures of a single layer of ECs and their associated basal lamina, the size and frailty of the vessels raise formidable complications for *in vitro* fabrication attempts. Only a few attempts have endeavoured to generate capillary beds *de novo*, while most strategies for therapeutic angiogenesis have aimed at directing the body's natural regenerative ability to educe neovascularization (Figure 1). On the other hand, TE devices are also being designed to promote neovascularization, tissue integration, and device function following implantation. Finally, recent advances in nanotechnology have rendered the construction of designer microvascular beds a viable, albeit somewhat future, possibility.

Assisted angiogenesis – helping the body help itself

Some of the most investigated EC mitogens include the vascular endothelial growth factor (VEGF) and basic fibro-

blast growth factor (bFGF). While secreted by a number of cell types when exposed to ischaemia, the paracrine effects of VEGF seem to be highly specific to ECs, thus making it a good candidate for angiogenesis-targeted delivery systems. However, subjects treated with intracoronary injections suffered from hypotensive complications (Hariawala *et al*., 1996). Local delivery methods including intramyocardial and intraepicardial inoculations have been explored using canine, porcine, and rodent ischaemic models. Direct injections of VEGF protein to regions rendered ischaemic by resection of rabbit common femoral arteries, improved the blood flow to distal tissue (Takeshita *et al*., 1994), but such delivery is susceptible to concentration spikes.

Controlled drug-delivery systems aim to administer efficacious dosing levels of therapeutic agents to targeted sites, without the spikes associated with injections or the systematic side effects and GI tract barriers associated with oral administration. Typical controlled delivery systems range from skin patches to reservoir pumps. Alginate beads and porous biodegradable polymer scaffolds are two controlled release systems that have been specifically investigated with regard to angiogenesis. For instance, VEGF was added to poly(lactide-co-glycolide) through flash freezing and lyophilization processing. These mixtures have subsequently been processed into porous disks using CO_2 gas foaming and NaCl leaching. *In vitro* release studies showed that bioactivity could be maintained, and that the release kinetics were a function of the polymer composition and porosity (Sheridan *et al*., 2000).

Although angiogenic proteins can be incorporated in polymer scaffold delivery systems, the high temperatures and solvents frequently used in processing steps may potentially denature these therapeutic agents. Furthermore, in spite of having some degree of control, the majority of the growth factor release generally occurs within the 48 h. This pharmacokinetic profile may be adequate in cases where establishment of revascularization is desired immediately following implantation, but the inability to provide a truly 'controlled' delivery system limits the prospects of this technique. Finally, significant quantities (up to 30%) of protein remain in the device even after extended periods of time, creating a potential risk should the device fail (Sheridan *et al*., 2000). On the other hand, better control of delivery may be achieved by designing additional features. For instance, alginate hydrogels implanted subcutaneously were engineered to release VEGF in response to mechanical loading (Lee *et al*., 2000a).

Genetic and cellular engineering were also used to deliver angiogenic factors. Adenoviral injections directly into an ischaemic porcine myocardium resulted in a 3-week window of elevated VEGF expression, during which tissue perfusion was enhanced without the hypotensive effects frequently observed following systemic delivery (Mack *et al*., 1998). Although the effects were independent of dosing, similar neovascularization results were observed when naked plasmids were delivered to rabbits through hydrogel angioplasty balloon coatings (Takeshita *et al*., 1996).

Cells have also been used as vehicles for delivery of biologically active factors. Myoblasts can be genetically engineered *ex vivo* into bioartificial muscles (BAMs). When implanted in nonmuscle and/or muscle sites, BAMs were shown to provide predictable long-term delivery of growth hormone, insulin-like growth factor, erythropoietin, and other biologically active factors (Payumo *et al*., 2002). For instance, implantation of murine retrovirally transfected BAMs producing VEGF led to a 30-fold increase in PECAM-positive capillary cells after 1 week, while systemic VEGF levels were not affected (Lu *et al*., 2001). However, long-term VEGF gene delivery has been linked with haemangioma complications. Injection of stably transduced myoblasts into normal mouse hearts prompted the development of fatal haemangiomas after 2 weeks of persistent VEGF delivery (Lee *et al*., 2000b). Use of inducible delivery vehicles may help control such problems. Stimulation of neovessel stabilization by delivery of multiple factors may also help.

Results from small phase I human clinical trials using plasmid-based VEGF therapy through injection directly into the myocardial tissue during anterolateral thoracotomy operations indicate decreased angina and improvement of perfusion, as determined *via* coronary angiography (Losordo *et al*., 1999). Subsequent studies reproduced the results with minimal toxicity using elevated doses (Symes *et al*., 1999; Vale *et al*., 2000). Recent trials are investigating replication-deficient adenoviruses as alternative delivery vehicles (Rosengart *et al*., 1999). Unchecked angiogenesis, however, facilitates pathological conditions such as tumour growth and metastasis. Consequently, a delicate balance of stimulating signals and inhibitory factors must be maintained to drive therapeutic neovascularization without triggering pathological responses.

Angiogenesis necessary for TE devices – nature supporting the machine

Since implanted tissues need adequate vascular perfusion to function properly, the majority of TE devices will require some degree of angiogenesis and/or inosculation – the process where the vasculature of the host is connected to that of the implant – to succeed. Consequently, angiogenesis has been investigated in TE devices ranging from myocardial patches and skin grafts to bioartificial livers to bone grafts and orthopaedic devices (Mueller-Klieser, 1997; Kellar *et al*., 2001; Eckardt *et al*., 2003). Similarly, transplanted organs would benefit from improved integration with the host's vascular network, following implantation.

A major strategy to guide the formation of new blood vessels, including the proper architecture of the supporting microvascular network, relies upon providing the appropriate scaffolds, either natural or synthetic. ECMs are known to affect cellular processes including cell phenotype, proliferation, and migration. Besides providing specific anchors or paths to cells, natural scaffolds are also the reservoirs soaking the biologically active factors released by cells. The scaffolds are also dynamic structures, with the reshaping being a *sine qua non* of tissue development and remodelling. Degradation of matrix also exposes new specific cryptic sites which can further guide blood vessel formation. Synthetic matrices can also be designed to incorporate active factors, and resorb through hydrolytic degradation mechanisms so that interaction with cells will direct the cells down prescribed pathways. Such modifications can be achieved by precise engineering of the scaffolds (Hubbell, 1995), or by exploiting the angiogenic properties of added natural components. For example, culturing tumorigenic rat carcinoma cells on porous implants of ePTFE led to the deposition of matrix coating, which enhanced neovascularization of treated scaffolds compared to untreated ePTFE scaffolds (Kidd *et al*., 2002).

Another essential step for establishing proper vascular perfusion is connection of the microvascular networks formed prior to implantation to that of the host. Studies examining human dermal microvascular ECs cultured on biodegradable polymer scaffolds and implanted into SCID mice found that the implanted cell population reorganized and anastomosed with the existing murine microvessels to form a functional capillary bed (Nor *et al*., 2001).

Devices for therapeutic angiogenesis – smaller is not easier

Recreating the smallest calibre vessels turned out to be a major challenge, calling for the most sophisticated and novel engineering approaches. Advances in miniaturization technologies have provided an opportunity to develop implants with microscopic interfaces and mechanochemical environments. By adapting molecular self-assembly, micropatterning, and other techniques that allow the formation of hierarchical structures on a microvascular length scale, it becomes possible to direct the formation of microvascular beds. Examples of angiogenic devices currently under investigation include self-assembled monolayers (SAMS), microfluidic devices, and angiochips.

Lithography is a general technique in which patterns or images are transferred from one media to another, and can be utilized to generate ordered configurations of proteins and cells on surfaces (Kane *et al*., 1999). Photolithography is a specialized patterning technique that produces specific patterns by photoablating immobilized proteins from silicon or glass surfaces (Hammarback *et al*., 1985). Developed for a number of biological applications, photolithography can pattern surfaces at submicron resolutions. Requirements for expensive equipment and clean room access, however, frequently make this technique cost prohibitive. Soft lithography describes techniques where soft, elastomeric materials are transferred through microcontact printing, laminar flow patterning, and other relatively inexpensive patterning procedures. Microcontact printing (μCP) utilizes elastomeric stamps to 'ink' materials onto specific regions of a substrate, and has been traditionally combined with SAMs to generate patterns of alkanethiols (Mrksich *et al*., 1997).

Self-assembly processes are essential components of many important natural phenomena, such as gene transcription, protein translation, and ECM organization and remodelling. Under equilibrium conditions, molecules can spontaneously assemble to form stable, organized self-assembled molecular structures. Based on ionic, hydrophobic, and other noncovalent interactions, these aggregates can produce a number of shapes ranging from films and membranes to micelles and capsules to rods and coils. SAMs have been used to preferentially bind fibronectin onto micropatterned surfaces. Alkanethiols adsorbed onto microprinted gold surfaces can be used to fashion structured SAMs with regulated topographies. By varying the alkyl terminal groups, it becomes possible to alter the aqueous properties of the surface, and to control protein binding. Directed attachment of bovine ECs was observed on the fibronectin-bound substrates, independent of surface topography (Mrksich *et al*., 1996). By fabricating self-assembled devices to present channels of specifically adsorbed protein ligands, it is conceivable that one could tailor microvascular networks of preferentially bound ECs. To date, however, the majority of attempts to regulate cell binding through self-assembled protein adsorption have been limited to two-dimensional approaches. Three-dimensional template fabrication methods are needed to extend SAM technologies beyond surface applications.

Microfluidic systems are also being considered for targeted angiogenesis. Complex, elevated surface patterns can be generated by directing mixtures of cells, proteins, and/or polymers through the channels of lithographic molds. This technique has been utilized to spatially divide PC12 nerve and ECs using patterned peptide fragments (Patel *et al*., 1998). As the neurons extended, the outgrowth was focused to areas containing specific peptide sequences. Microfluidic patterned seeding of primary rat cells was accomplished using polydimethylsiloxane (PDMS) as an elastomeric stencil. Hepatocytes and fibroblasts were preferentially seeded onto a range of substrates without additional surface modification. Patterned seeding was obtained on both curved surfaces as well as on hydrogels. Stepwise patterning to produce a 'sandwich configuration' was also investigated (Anderson *et al*., 2000). By layering consecutive microfluidic channel patterns, it is feasible to create a three-dimensional bulk device.

While many of the microfabrication issues are yet to be worked out, some groups have proposed approaches to fashion a true angiogenesis-assist device. Combining micromachined molecular nanofilters into larger silicon-housing frames, angiochips have been put forward as hybrid bioinorganic devices for targeted angiogenesis (Moldovan & Ferrari, 2002; Tao & Desai, 2003).

The question remains as to how to best stimulate neovascularization. While to date, most therapeutic attempts have focused on VEGF, multiple other angiogenic proteins, including placental growth factor (PlGF), bFGF, hypoxia-inducible factor-1α (HIF-1α), and developmental endothelial locus-1, are clearly involved in vessel recruitment and development. Subtle differences between angiogenesis and vasculogenesis may not be fully appreciated. Basic science will shed better light on which factor or combination of factors will function most effectively and at which stage. The perceived dual nature of angiogenesis is a challenge in itself. Angiogenic signals stimulate neovascular growth and thus rescue ischaemic organs, at the same time sustaining the growth and spreading of malignant tumours.

Conclusions

Responding to a serious medical crisis, 'designer blood vessels' could revolutionize healthcare and medical implant industries. In addition, discoveries made in the process of developing these replacements or enhancers of natural vasculature have advanced our basic understanding of the human body and the fields of tissue engineering and organ transplantation as well. In order to truly reach their potential, however, several key issues must be addressed. Some of these are common for all efforts to engineer tissues, while some are very specific to the type of vascular replacement sought.

First, we need a better fundamental biological understanding of the detailed design we are trying to recreate. What is essential and where could we cut corners? How much needs to be prefabricated and how much can we count on recruiting 'on site'? Uncertainties associated with cell sourcing and immune acceptance continue to badger the TEBV community.

While stem cells may provide an answer to many of these queries, much is still unknown regarding their differentiation and long-term behaviour.

Second, we need further advances in materials engineering and fabrication technologies to produce the desired cellular responses. Complex tissue structure and fibre orientation cannot still be mimicked adequately through materials processing. Furthermore, many of the processing steps can damage or denature proteins and cells. While nanotechnology provides an opportunity to organize structures on microscopic levels, many of the techniques are not suitable for three-dimensional or large-scale applications. In addition to providing mechanical support, biomaterials of the future will need to interact both chemically and biologically with the surrounding tissues.

Finally, improved imaging, monitoring, and delivery methods are crucial to generate commercially viable products. Biologically active devices will complicate storage and handling issues and increase costs. Once implanted, it will also be necessary to monitor the device to safeguard against pathological complications. Noninvasive imaging techniques including NMR and ultrasound will be necessary to ensure that the designer blood vessels are functioning properly. Advances in all these areas will continue to benefit tremendously from the conjugated efforts of academic and biotech industry laboratories. The design of blood vessels has been continuously improved through sharing and by incorporating incremental advances made by many scientists, engineers, and medical professionals from around the world.

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