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Engineering of bypass conduits to improve patency

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Abstract. For patients with severe coronary artery and distal peripheral vascular disease not amenable to angioplasty and lacking sufficient autologous vessels there is a pressing need for improvements to current surgical bypass options. It has been decades since any real progress in bypass material has reached mainstream surgical practice. This review looks at possible remedies to this situation. Options considered are methods to reduce prosthetic graft thrombogenicity, including endothelial cell seeding and developments of new prosthetic materials. The promise of tissue-engineered blood vessels is examined with a specific look at how peptides can improve cell adhesion to scaffolds.

INTRODUCTION

The causes of graft occlusion can be divided into early, mid-term and late (Conte *et al.* 2002). Early failure occurs within 30 days of surgery and tends to be a result of technical problems, poor inflow and/or outflow and acute thrombosis caused by activation of the clotting cascade; it is related to Virchow's classic triad of coagulability of the blood, vessel wall damage and blood stasis (Virchow 1860). Mid-term occlusion from 3 months to 2 years, is a result of narrowing of the lumen of the graft (principally around the distal anastomosis) because of 'neointimal hyperplasia' (Szilagyi *et al.* 1973; Sottiurai *et al.* 1983). Late occlusion (after more than 2 years) is because of underlying atherosclerotic degeneration.

Neointimal hyperplasia consists principally of a proliferation of vascular smooth muscle cells (SMC) associated with synthesis of extracellular matrix (ECM) (Sottiurai *et al.* 1983; Ross 1993, 1999). This is a particular problem for the prosthetic grafts currently used – polytetrafluoroethylene (PTFE) and polyethylene terephthalate (Dacron) – in both low blood flow and narrow diameter (under 6 mm) arterial circulation (Christenson *et al.* 1985, 1988; Whittemore *et al.* 1989; Pomposelli *et al.* 1998; Byrne *et al.* 1999; Faries *et al.* 2000a,b). The exact cause of this is still not truly understood, but current hypotheses indicate the following risk factors – disturbed flow (Imparato *et al.* 1972), damage to the vessel wall (Ross 1993) and/or compliance mismatch

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between the elastic artery and the relatively inelastic prosthetic graft (Gozna *et al.* 1974). This mismatch occurs both along the graft length and at the anastomosis as a result of non-compliant suture material and suturing technique (Tiwari *et al.* 2003a). The difference in compliance results in haemodynamic changes, including altered flow, increased shear rates, downstream turbulence and cyclic stress, and culminates in the release of growth factors which stimulate neointimal hyperplasia (Howard *et al.* 1997). The benefit of interposition vein collars (Bell 1998) is partly related to their superior compliance because of improved pulsatile flow profile propagation.

Inherent thrombogenicity is also a major cause of failure of prosthetic grafts. Clinical trials have reflected attempts to improve on graft thrombogenicity and poor compliance of existing prosthetic grafts.

REDUCING GRAFT THROMBOGENICITY

Approaches include lining or bonding the prosthetic graft with anticoagulant chemicals like heparin (Devine *et al.* 2001; Begovac *et al.* 2003), or lining the graft with endothelial cells (EC) in a process known as seeding.

Chemical engineering

By bonding anticoagulant chemicals to the conduit surface, surface thrombogenesis should be reduced. In theory, these chemicals, like heparin, have only a finite lifespan and so may only work in the short term. However, recent animal studies have shown some promise with improved patency in canine models, by immobilizing heparin onto PTFE grafts (Begovac *et al.* 2003). Furthermore, collagen-coated, heparin-bonded Dacron has shown superior patency to PTFE in a clinical study of above- and below-knee arterial bypasses (Devine *et al.* 2001). Perhaps part of the benefit may be the inhibitory properties of heparin on smooth muscle cell proliferation (Hoover *et al.* 1980; Fager *et al.* 1988) and thus inhibition of development of neointimal hyperplasia. More recently, investigators have begun to look at the potential for anti-platelet agents – dipyridamole was effective in an *in vivo* goat model (Aldenhoff *et al.* 2001). Kidane and colleagues have provided a detailed review of this subject (Kidane *et al.* 2004).

Cellular engineering

ECs are capable of synthesizing anti-thrombotic chemicals themselves and inhibiting SMC proliferation – and so offer a potential long-term solution to graft thrombogenicity and neointimal hyperplasia. Difficulties with seeding ECs are: obtaining them from an appropriate source, expanding their numbers in culture, applying them to the graft and retaining them in position.

Sources for ECs (Tiwari *et al.* 2001) include veins (Zilla *et al.* 1987; Ortenwall *et al.* 1989, 1990; Zilla *et al.* 1990; Kadletz *et al.* 1992; Magometschnigg *et al.* 1992; Jensen *et al.* 1994), adipose tissue capillaries (Watkins *et al.* 1984; Jarrell *et al.* 1986; Anders *et al.* 1987; Rupnick *et al.* 1989; Sharp *et al.* 1989; Meerbaum *et al.* 1990; Pronk *et al.* 1993; Vici *et al.* 1993; Scott *et al.* 1995; Williams 1999; Karube *et al.* 2001; Tiwari *et al.* 2002a,b), blood-borne cells (Boyer *et al.* 2000) and CD34+ bone marrow cells (Bhattacharya *et al.* 2000a,b; Shi *et al.* 2002) – the latter two sources being putative stem cells or, rather, endothelial progenitors. Methods to improve cell attachment onto the graft surface include the use of chemical coatings, pre-clotting, chemical bonding and surface modifications. This subject has been reviewed comprehensively by Salacinski *et al.* (2001).

Two principal methods for applying ECs to grafts have been identified: single- and two-stage seeding. Single-stage seeding (Herring *et al.* 1978) involves extraction and seeding cells at the same time as implanting the graft in the patient. Whilst representing an ideal from a clinician's viewpoint, trials have not proven this method to be of benefit (Zilla *et al.* 1987; Herring *et al.* 1994). Reasons for this include, first, that it is difficult to obtain directly a sufficient number of cells to cover the graft surface and, second, that on exposure to arterial pressures and blood flow the ECs are largely washed away (Rosenman *et al.* 1985; Kesler *et al.* 1986; Kent *et al.* 1992; Falk *et al.* 1998; Giudiceandrea *et al.* 1998).

In comparison, two-stage seeding has shown itself to improve markedly the patency of prosthetic (normally expanded PTFE) grafts to levels seen previously only in vein bypasses (Deutsch *et al.* 1999; Laube *et al.* 2000). This method involves initial extraction of ECs from a parent vein, followed by a period of cell culture *in vitro* to generate sufficient ECs to achieve supra-confluent levels to cover the appropriate area of the graft. Once sufficient ECs are produced (maybe requiring the subject's own blood serum in the culture medium) they are seeded onto a 70-cm ePTFE graft pre-lined with fibrin glue in a specialized rotating device for 1 week. The graft is then implanted into the subject at a separate operation. Clearly, this method requires two operations, with a waiting period of a month or so between culturing and subsequent seeding of the ECs (Zilla *et al.* 1990, 1999). In addition, such a graft is commercially expensive as it has to be pre-lined with a fibrinolytically inhibited fibrin glue (Zilla *et al.* 1989; Deutsch *et al.* 1999). This is not an option for emergency bypass, which is the main indication in peripheral vascular disease as there is little indication for doing such a demanding procedure for intermittent claudication (Burns *et al*. 2003).

CONDUITS ENGINEERED FOR SUPERIOR COMPLIANCE

A further area of research would be to improve upon the poor compliance of PTFE and Dacron. Yet, in the past few decades, apart from research into polyurethanes, there has been little success in providing alternative prosthetic materials.

Polyurethanes

Polyurethane grafts are known to be more compliant than PTFE and Dacron, but historically have been associated with a significant rate of thrombosis and infection, with patency rates sometimes worse than PTFE grafts (Ota *et al.* 1989; Nakagawa *et al.* 1994); also, they suffer from aneurysm formation (Brothers *et al.* 1990). Tiwari and colleagues have compiled an excellent review of this (Tiwari *et al.* 2002b).

More recently, several variations of polyurethane graft have been investigated (Sonoda *et al.* 2003) and are available commercially (Eberhart *et al.* 1999). Our own group has developed a poly(carbonate-urea)urethane commercial product, MyoLink™, for haemodialysis access, and has also begun pilot studies of its use as a bypass graft for peripheral vascular surgery. This material offers several potential key advantages over PTFE and Dacron, including superior compliance and tissue and blood compatibility. Furthermore, MyoLink™ has undergone *in vitro* degradation tests and has been successfully implanted in a dog model for 36 months, demonstrating very high biostability (Salacinski *et al.* 2002; Tiwari *et al.* 2002b; Seifalian *et al.* 2003). An added potential advantage is its superior ability to attach ECs for potential seeding applications (Giudiceandrea *et al.* 1998; Stansby *et al.* 1994), which is further enhanced by bonding the attachment peptide RGD and heparin to its surface (Tiwari *et al.* 2002c).

TISSUE-ENGINEERED BYPASS CONDUITS

Tissue engineering (TE) is a multidisciplinary field combining biology, materials science and surgery to provide living tissue products to restore, maintain or improve tissue function (Langer & Vacanti 1993). Hopefully, this will meet the needs for donor organs and tissues, but TE also offers the promise of being able to dramatically expand our ability to repair tissues, improve surgical procedures and thus significantly improve the quality of patients' lives.

It is felt that TE would be particularly valuable in the production of vascular grafts. The reason for this is the massive need and precarious supply of natural graft material for both coronary artery bypass grafting (CABG) and lower limb bypass grafting in peripheral vascular disease (PVD).

Scaffold–cell–bioreactor model

Implantation of scaffolds seeded with cells is the most commonly used method for proposed tissue-engineered solutions and can be subdivided into open and closed systems (Langer & Vacanti 1993; Rabkin & Schoen 2002). In a closed system, the cells are separated from the body by a membrane which permits passage of nutrients and wastes but blocks transit of larger elements such as immune cells. The system can be implanted or used as an extra-corporeal device. Examples include delivery of drugs to restricted anatomic sites and for renal, hepatic or pancreatic assist devices (Rabkin & Schoen 2002). In contrast, for an open system, the *cell–scaffold–bioreactor* is the classical model. Cells are attached onto scaffolds – either natural such as collagen or synthetic such as polytetrafluoroethylene (PTFE) – *in vitro* and the proto-tissue is matured in a mechanically and biochemically supportive environment within a bioreactor. The resultant prosthesis is then implanted into the body in the anatomically appropriate position where *in situ* native remodelling can occur. This is a popular approach (Rabkin & Schoen 2002). The open approach then can further be subdivided into the type of matrix or scaffold that is used – either natural or synthetic. Synthetic matrices can then be further segregated into biodegradable or permanent.

The application of TE to blood vessels should ideally result in conduits with the properties outlined in Table 1 (Rabkin & Schoen 2002; Thomas *et al.* 2003).

To successfully produce a tissue-engineered vascular conduit, there are several key components that need to be assessed, as summarised in Table 2. First, the use and type of mandrel or scaffold. A mandrel confers a physical presence around which cells and tissues develop, but it is ultimately removed from the final graft, whereas cells and tissues must grow into or onto scaffolds, and as such the scaffold is a critical component of the final graft. Second, the use and type of extracellular matrix, which may be a component of the scaffold or may be added to it. Finally, the last element of variability is the type of cells added. However, equally critical to this 'mix' are the signals to which these cells will be exposed. Signals which affect the behaviour of cells are from three main sources. First, from the fluid and chemicals flowing through the vessel – *in vivo* this, of course, is blood. Next, those from the extracellular matrix – the ECM is not merely a collection of proteins serving as a biological glue but is also a supplier of regulatory signals. Finally the mechanical environment of the vessel provides signals imposed by the haemodynamics of the vascular system (Ziegler & Nerem 1994).

Natural scaffolds

These are scaffolds derived from human or animal tissue itself. Lantz *et al.* (1993) developed a biological vascular graft material made from small intestinal submucosa (SIS) and tested it in

Table 1. Ideal properties of a tissue-engineered blood vessel

Biological	Mechanical	Commercial	Physical
Vasoreactive: dilate/ constrict to neural and chemical stimuli	Strength to resist burst pressures	Can be tailored to an individual's requirements, for example, length and diameter	Leak-proof: avoids haemorrhage through its walls
Non-thrombogenic	Avoids kinking even over joints	Inexpensive to manufacture	Porosity for healing and angiogenesis
Biostable: does not weaken *in vivo* to result in aneurysms and/or rupture	Hold sutures under circumferential and longitudinal tension	Short time period from request to implantation	
Biocompatible: not inflammatory, toxic, carcinogenic or immunogenic	Retains axial and radial compliance and pulsatility		
Infection-resistant			

dogs. The graft was prepared by removing a jejunal segment, from which the luminal mucosa, the muscularis externa and the serosa were then removed by abrasion. This left the submucosa with attached stratum compactum (dense collagen layer) and muscularis mucosa intact. The derived material was shown to be usable as an autograft, an allograft or as a xenograft, demonstrating biocompatability and high patency rates (75% overall) in the aorta, the carotid and femoral arteries and in the superior vena cava (SVC). After 90 days the grafts were seen to be similar to either artery or SVC (as appropriate) under histological examination. Furthermore, when challenged with a bacterial load, the infection was much more successfully cleared than ePTFE matrix (Lantz *et al.* 1993). Similarly, Huynh and colleagues constructed a scaffold from a collagen biomaterial derived from the submucosa of the small intestine and type I bovine collagen. The inner lining was treated with heparin and this acellular graft was implanted into rabbit aortas with good patencies (Huynh *et al.* 1999).

Decellularized natural scaffolds have been used by a number of researchers; Bader and colleagues used porcine aorta, decellularized by using trypsin. The xenografts were then repopulated with human myofibroblasts and endothelial cells from saphenous vein biopsies (Bader *et al.* 2000). Clarke and colleagues decellularized bovine ureters (using hypotonic water and ribonucleases), which were then grafted into dog aortas with 100% patency and no aneurysms at 10 months (Clarke *et al.* 2001). Conklin *et al.* (2002) decellularized porcine carotid arteries using detergents and enzymes. These scaffolds were then covalently linked to heparin, resulting in reduced *in vitro* thrombogenicity. Furthermore, the compliance was similar to natural vessels with excellent burst and suture-retention strengths. Implanted as xenografts into dog carotid arteries, by 2 months, smooth muscle cells had repopulated the walls and endothelial cells lined the lumina. Unfortunately, these animal studies were too short to draw significant conclusions. Kaushal and colleagues used decellularized porcine iliac vessels on which to seed ovine endothelial progenitor cells (EPC). As carotid interposition grafts, these remained patent for 130 days and once explanted these grafts exhibited contractile activity and nitric-oxide-mediated vascular relaxation that were similar in properties to native carotid arteries. In comparison, non-seeded grafts occluded within 15 days (Kaushal *et al.* 2001).

The obvious advantage of a natural scaffold is that it is composed of extra-cellular matrix proteins typically found in the body and, when derived from a vessel, the three-dimensional architecture is very similar to that of the vessel it is replacing, thus conferring appropriate

Table 2. Previously used tissue-engineered bypass conduits

Scaffold/ mandrel	ECM	Cells	Peptides	Patency	Researcher
Porcine SIS & Col.		None	Heparin	100% ≤ 90/7	(Huynh *et al.* 1999)
Porcine/Dog SIS		None	None	≥ 75% ≤ 5 year	(Lantz *et al.* 1993)
Decell. porcine aorta		MyoFB & EC ≈ 2 × 10^6/cm length	None	Not assessed	(Bader *et al.* 2000)
Decell. porcine iliac vessel		EPC: confluent layer	None	100% ≤ 130/7	(Kaushal *et al.* 2001)
Decell. bovine ureter		None	None	100% ≤ 10/12	(Clarke *et al.* 2001)
Decell. porcine carotid		None	Heparin	100% ≤ 67/7	(Conklin *et al.* 2002)
PGA	None	SMC: 5 × 10^6/ml EC: 3 × 10^6/ml	None	100% ≤ 24/7	(Niklason *et al.* 1999)
PGA-PHA	None	≈ 10^6/cm^2 mixed SMC, EC & FB	None	100% ≤ 150/7	(Shum-Tim *et al.* 1999)
PU	None	≈ 2 × 10^6/cm^2 SMC	None	92% ≤ 1/52	(Yue *et al.* 1988)
PTFE	Peritoneum		None	80% ≤ 21/7	(Sparks *et al.* 2002)
Dacron	Col.	0.2–30 × 10^5 SMC/ml & 10^5 EC/cm^2	± FN	N/A	(Weinberg & Bell 1986) (Baguneid *et al.* 2004)
PU/Dacron	Col. + dermatan sulphate	6.6 × 10^5 EC/cm^2 ± 7.5–20 × 10^5/ml SMC ± 7.5 × 10^5/ml FB	None	75–100% 16–26/52	(Miwa *et al.* 1993a; Miwa & Matsuda 1994; Ishibashi *et al.* 1996)
PU	Col.	EPC	None	92% ≤ 3/12	(He *et al.* 2003)
PU	None	SMC & EC	None	100% ≤ 4/52	(Ratcliffe 2000)
ST	ECM + MyoFB & mesothelial cells secondary to inflammatory reaction		None	67% ≤ 4/12	(Campbell *et al.* 1999)
Fascia-wrapped ST			Protamine & heparin	73% ≤ 8/52	(Tsukagoshi *et al.* 1999)
PTFE	None	SMC & FB sheets ± EC	None	50% ≤ 1/52	(L'heureux *et al.* 1998)
Glass	Col.	FB: 10^6/ml & EC: 25 × 10^3/cm^2	None	N/A	(Berglund *et al.* 2003)
Glass + mesh	Col.	5–15 × 10^5/ml SMC & 4 × 10^5 EC/cm^2	None	64–100% ≤ 6/12	(Hirai and Matsuda 1996; Kobashi and Matsuda 1999a; He and Matsuda 2002a)

Col., collagen; Decell., decellularized; EC, endothelial cells; FB, fibroblasts; PGA, polyglycolic acid; PHA, polyhydroxyalkanoate; PU, polyurethane; SIS, small intestinal submucosa; SMC, smooth muscle cells; ST, silicone tube.
Timing: x/7 = x days; y/52 = y weeks; z/12 = z months; year = years.

mechanical and physical properties. However, despite the encouraging results above, there remain concerns over transmission of endogenous retroviruses, though there is some reassuring evidence on this issue (Kallenbach *et al.* 2004). Even so, potentially infective proteins such as prions remain a concern even when human vessel-derived substrates are used.

Synthetic biodegradable scaffolds

These are scaffolds not found in nature, but which over a period of time degrade in the body so that ultimately they are no longer part of the graft. Yue and colleagues used a microporous biodegradable scaffold made from a polyurethane-based material which they seeded with rat SMCs. These were then implanted into the rat aorta where they demonstrated superior patency compared with non-seeded grafts (Yue *et al.* 1988). Shum-Tim and colleagues used a copolymer of polyglycolic acid (PGA) and polyhydroxyalkanoate (PHA) as a scaffold onto which they seeded a mixture of SMCs, ECs and fibroblasts – cultured as explants from lamb carotid arteries. When grafted into the lamb abdominal aorta, these were all patent at 150 days compared with controls composed of acellular PGA-PHA copolymer only, which all thrombosed (Shum-Tim *et al.* 1999). Niklason and colleagues used PGA scaffolds, chemically modified with sodium hydroxide, onto which were pipetted bovine aortic SMCs in suspension. The grafts were then exposed to pulsatile pressure before seeding with ECs. When grafted into swine, the pulsed grafts showed 100% patency up to 4 weeks (Niklason *et al.* 1999). This latter model demonstrated how conduits may develop excellent mechanical properties under suitable *in vitro* conditions. However, there remains an absence of long-term outcomes. The overall time period involved in such protocols is of importance; first the freshly harvested cells need to be expanded in number in culture and then further cultured with the conduit *in vitro* before they can be applied as a surgical prosthesis.

Synthetic permanent scaffolds

These are scaffolds made of substances not found in nature, which persist indefinitely as part of the subsequently prepared graft. Usually they are based on materials already used for bypass grafting and therefore with established clinical track records. In their landmark paper, Weinberg & Bell used Dacron as their scaffold, which was embedded into collagen. SMCs were then cultured in the graft before ECs were seeded onto the inner lining (Weinberg & Bell 1986). This resulted in a graft which had an *in vitro* burst strength of 323 mmHg. Baguneid *et al.* (2004) used a variation of this: porcine SMCs were allowed to contract collagen around a Dacron scaffold. It was demonstrated that luminal pre-coating with fibronectin and 1 week of low shear stress preconditioning enhanced the retention and viability of seeded ECs.

Workers in the laboratory of Matsuda have performed considerable amounts of work with synthetic scaffolds, using both Dacron and polyurethane. When an artificial ECM of collagen with dermatan sulphate was pressurized through a Dacron scaffold and then lined with ECs, 100% patency at up to 16 weeks was achieved in canine carotid arteries, though adding SMCs to the ECM improved EC retention and ECM production (Miwa *et al.* 1993a; Matsuda & Miwa 1995). Using a similar model, but including fibroblasts with SMCs in the ECM, resulted in over 80% patency in canine carotids at periods of up to 23 weeks (Ishibashi & Matsuda 1994; Ishibashi *et al.* 1995, 1996). Using segmented polyurethane as the scaffold onto which the ECM of collagen and dermatan sulphate were squeezed, followed by EC seeding, showed 75% patency up to 26 weeks when implanted into canine carotid arteries (Miwa *et al.* 1992, 1993b; Miwa & Matsuda 1994). As with Dacron, the ECM layer could have had SMCs added (Matsuda *et al.* 1989). More recently, endothelial progenitor cells (EPCs) derived from canine peripheral blood, were pre-lined onto a collagen mesh which was then wrapped with a segmented polyurethane film. Out of 12 grafts implanted into canine carotid arteries, 11 remained patent at up to 3 months (He *et al.* 2003). Ratcliffe also used polyurethane scaffolds seeded with SMCs and then ECs which were cultured under fluid flow. They were finally implanted into the carotid arteries of dogs, with 100% patency for up to 4 weeks (Ratcliffe 2000).

Sparks *et al.* (2002) lined PTFE with peritoneum, ensuring that the visceral surface supporting mesothelium was luminal. When implanted into rabbit carotid artery, the 21-day patency was 80% compared with 20% for the contralateral carotid, which had PTFE without the mesothelial lining.

Moulds and mandrels

As mentioned earlier, moulds and mandrels simply confer a mechanical framework so that the physical proportions of the ensuing graft resemble those of the vessel they are mimicking.

Matsuda and colleagues developed a tubular hybrid medial tissue by pouring a cold mixed solution of SMCs and type I collagen into a corresponding tubular mould and by subsequent thermal gelation, followed by 7 days of culture and finally seeding this with ECs (Hirai *et al.* 1994; Hirai & Matsuda 1995). Unfortunately, burst pressures were relatively low at up to 100 mmHg so that, even for use as a venous conduit, an outer Dacron re-inforcement was required. Over a 24-week period, 9 of 14 canine posterior vena cavae remained patent (Hirai & Matsuda 1996). Investigations with more compliant outer re-inforcements, such as segmented polyester and polyurethane-nylon meshes, showed that polyester had better compliance, and burst pressure was maximal when kept on the outside of the collagen-SMC layer rather than inside or integrated within it (Kobashi & Matsuda 1999b). Furthermore, this method could be used to generate branched bypasses (Kobashi & Matsuda 1999a). More recent work from this group using microporous segmented polyurethane as the external reinforcement, especially with a high-pore density, showed patency rates of 100% over a 6-month period in canine carotid arteries (He & Matsuda 2002a,b).

Berglund and colleagues used a variety of cross-linking techniques on type 1 collagen to produce an acellular sleeve around which a second layer of collagen with neonatal dermal fibroblasts was moulded. Mechanical properties such as burst pressure and tensile strength were significantly enhanced (though not to native arterial levels) by all crosslinking treatments, especially glutaraldehyde crosslinking. However, glutaraldehyde limited cell ingrowth into the acellular layer and had a negative morphological impact on the endothelial cells seeded on the inner acellular crosslinked sleeve. Furthermore, glutaraldehyde-crosslinked sleeves ruptured by brittle, defect-based failure modes. The authors therefore hypothesized that physical rather than chemical crosslinking of collagen with ultraviolet radiation and dehydrothermal treatment may offer better results (Berglund *et al.* 2003).

L'Heureux *et al.* (1998) produced an innovative graft exclusively from cultured human cells. First, an acellular lining made by dehydrating a fibroblast sheet was wrapped around a PTFE mandrel and then another sheet, this time made of cultured SMCs, was wrapped around this. After maturing in a bioreactor, an outer sheet of fibroblasts was added before further maturation. Now, the inner PTFE mandrel was removed and seeded with ECs. These grafts were inserted into dogs, though without ECs, which were felt to be too antigenic in a xenotransplant model. Despite this precaution, patency was only 50% at 1 week. Campbell *et al.* (1999) working on rats and rabbits, developed a vascular graft by inducing an inflammatory reaction. The method involved inserting a silastic tube into the peritoneal cavity, which then acted essentially as a mandrel around which layers of myofibroblasts, collagen matrix and a single layer of mesothelium developed over a 2-week period. The tube of living tissue was then extracted and everted after the silastic tube was removed and the graft re-inserted into the aorta of the same animal. The patency rate was 67% over a 4-month period. Tsukagoshi and colleagues working with rabbits, employed a similar method. A piece of fascia from the thigh was excised and wrapped around a silicone rod and then implanted into a subcutaneous pocket in the thigh. After 4 weeks, the fascia-wrapped silicone rod was excised and the rod was removed. The fibrocollagen tube was then treated with protamine, glutaraldehyde and heparin and then anastomosed onto the femoral artery (with patency rates of over 70%) without any subsequent aneurysms (Tsukagoshi *et al.* 1999).

PEPTIDES TO IMPROVE SCAFFOLD-CELL ADHESION

One of the key elements in tissue engineering blood vessels is retaining the cells on the scaffold itself. The initial failure of single-stage seeding of endothelial cells to improve patency of prosthetic vessels was that, upon exposure to pulsatile blood flow, a high proportion of cells were washed off (Salacinski *et al.* 2001). In the first 30–45 min of flow, up to 70% of cells are lost; this is followed by a slower exponential loss over the next 24 h and then normally a levelling off period (Rosenman *et al.* 1985). Some PTFE grafts have shown EC attachment of only 10 ± 7% of applied cells, with only 4 ± 3% of the ECs retained (Kent *et al.* 1992). Therefore, much effort has been expended on improving cell adherence to scaffolds for both ECs and now also SMCs. This includes by both physical and chemical methods.

Historically, the main chemical protocols involved the use of coatings or pre-clotting stages. A detailed review of the various options for improving EC attachment to scaffolds has been composed by Salacinski *et al.* (2001). In essence, the main coatings used so far have been collagen, fibronectin (FN), laminin, poly L-lysin and gelatin. Of these, FN would appear to be the best, whether alone (Budd *et al.* 1989; Thomson *et al.* 1989) or, even better, when in combination with collagen, fibrin, laminin, gelatin or extracellular matrix (Williams *et al.* 1985; Anderson *et al.* 1987; Hess *et al.* 1992). Recently, the immobilization of biomacromolecules like gelatin and collagen has been enhanced by introducing free amino groups (NH_2) onto polyurethane membranes with consequent enhancement of EC proliferation (Zhu *et al.* 2004).

For SMC adhesion, the main advances have been on attachment of cells to plastic culture dishes rather than scaffolds. Here, fibronectin, collagen and, to a lesser extent, laminin and vitronectin have improved adhesion (Hayward *et al.* 1995). Protein modification of a potential scaffold of a biodegradeable hydrogel [consisting of poly(propylene fumarate-coethylebe glycol) and agmatine-modified poly(ethylene glycol)-tethered fumarate hydrogels] enhanced vSMC adhesion and spreading by increasing the amount of the positively charged guanidine group of agmatine (Tanahashi & Mikos 2002, 2003). EC adhesion and retention has been enhanced even more by pre-clotting prosthetic grafts (Salacinski *et al.* 2001) using the patient's own plasma (Vohra *et al.* 1990; Kent *et al.* 1992) or blood (Stansby *et al.* 1991; Vohra *et al.* 1991; Stansby *et al.* 1994). Pre-clotting with serum was less successful (Stansby *et al.* 1991; Kent *et al.* 1992). The best method, to date, seems to be by using a combination of fibrin glue and fibroblast growth factor (Gosselin *et al.* 1996).

Chemical bonding of surface moieties such as peptides has shown some promising results. Heparin was the first to be tried and mixed results have been achieved in improving EC adhesion (Dalsing *et al.* 1989; Walpoth *et al.* 1998). Using lectins has demonstrated excellent results (Ozaki *et al.* 1993), but perhaps the most intriguing possibilities lie with RGD peptides.

Peptides

RGD is a tripeptide sequence (arginine–glycine–aspartate) found in extracellular matrix proteins such as fibronectin. It is the binding motif for cell surface integrin receptors and has been investigated extensively (Walluscheck *et al.* 1996a,b; Mann *et al.* 1999; Salacinski *et al.* 2001; Mann & West 2002). In our laboratory, we have has shown that RGD, when covalently bonded to MyoLink™, and particularly when this was in association with heparin, significantly enhances the retention and viability of seeded ECs (Tiwari *et al.* 2002c). Others have also produced enhanced cell retention onto grafts using RGD peptides(Hsu *et al.* 2003).

RGDs have also been used to enhance SMC attachment to scaffolds. RGDS (Arg–Gly–Asp–Serine), when incorporated into a hydrophilic gel matrix, successfully entrapped SMCs throughout this artificial vascular medium (Moghaddam & Matsuda 1991). RGD peptides when grafted onto dextran as a biomaterial surface coating, demonstrated increased SMC attachment and spreading (Massia & Stark 2001). Workers in our laboratory have found that SMC adhesion to a polyurethane scaffold can be significantly enhanced by using a fibronectin-like protein polymer (Rashid 2004), where multiple copies of the RGD sequence are engineered within a positively charged copolymer (Yang *et al.* 1998). KQAGDV, which is derived from the γ chain of fibrinogen, appears to be a mimic of the RGD sequence; its binding to integrins is inhibited by RGD peptides, though it binds primarily to the α rather than β subunit (Ruoslahti 1996). KQAGDV significantly enhances SMC attachment to modified surfaces and hydrogel polymer scaffolds, though this is at the expense of reduced cell proliferation (Mann & West 2002) and ECM production (Mann *et al.* 1999).

SUMMARY

This review has demonstrated varied approaches that have been undertaken to engineer an alternative blood vessel. All have their advantages and disadvantages. ‘Off-the-shelf’ vessels have their major attraction in being suitable for emergency and urgent cases. The possible options include animal-derived natural scaffolds, but concerns about immunity and transmission of animal diseases, especially via prions, currently restrict their attractiveness.

A fully autologous blood vessel made from human cells, which is both mechanically strong and chemically responsive, has only been demonstrated by L’Heureux and colleagues (L’Heureux *et al.* 1998, 2001). Short-term animal studies have proved difficult because of problems of immunity and consequently there are no long-term studies. Synthetic scaffold systems offer the reassurance of a strong structure resistant to aneurysm – critical for vessels exposed to high arterial pressures over long periods of time. However, that invariably limits their compliance and increases their infection risk. Although two-stage seeding of ECs on prosthetic grafts has proved successful (Deutsch *et al.* 1999; Laube *et al.* 2000), the long time period for culturing cells to expand their number means that it is unsuitable for many urgent cases. Single-stage seeding has so far failed but, perhaps with developments to attachment peptides, linking ECs ably and reliably to scaffold materials, may again become an option. Perhaps the most promising area is that of biodegradable scaffolds, which have already been taken to a clinical level (Shin’oka *et al.* 2001; Isomatsu *et al.* 2003; Matsumura *et al.* 2003), though long-term results are still awaited. All such current approaches have two major limitations in that they all require a separate surgical procedure to be performed to access/extract the cells needed, followed by a long period of culturing/expanding these cell populations. Again, improvements to the scaffold–cell interaction with the use of peptides may improve on the culture period as well as ensure greater cell retention.

Therefore, the ideal TE blood vessel has not yet been found and it is our belief that the ideal will vary depending on clinical situations. Assuming future experiments are successful and repeatable, fully autologous vessels or vessels with a biodegradable scaffold could be offered when there is sufficient time before implantation surgery is required. For more urgent cases, 'off-the-shelf' grafts based on synthetic or animal scaffolds may prove superior to traditional Dacron or PTFE.

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