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IN VIVO ENGINEERING OF BLOOD VESSELS

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1. ABSTRACT

The inadequacy of conventional synthetic grafts has led to efforts to construct a superior vascular graft. *In vivo* tissue engineering is one approach to this problem that has been investigated for half a century and enables the construction of autogenous vascular prostheses. Three types of *in vivo* engineering are explored: remodelling of implanted scaffolds, fibrocollagenous tubes, and the artificial artery generated in the peritoneal cavity. Scaffolds designed to be remodelled may be synthetic or biological and have been remodelled in animal models to form vasoactive neoarteries with arterial morphology. The differences in vascular remodelling ability, particularly spontaneous endothelialisation, between animal models and humans may impair the effectiveness of this approach in the clinic. Fibrocollagenous tubes such as the Sparks' Mandril have demonstrated poor performance in the clinic and are prone to aneurysm formation. The artificial artery generated in the peritoneal cavity is a novel addition to the ranks of *in vivo* engineered vascular prostheses and combines many of the best features of scaffolds designed to be remodelled and fibrocollagenous tubes. However, understanding and manipulating the vascular remodelling process will be the key to producing the ideal arterial prosthesis.

2. INTRODUCTION

Atherosclerosis is the leading cause of mortality and morbidity in the developed world (1). In this disease, eccentric atheromatous plaques form in large and medium sized arteries predisposing to arterial occlusion by thrombosis. Occlusion may lead to ischaemic events such as myocardial infarction, or stroke or to limb ischaemia and subsequent amputation. Medical management of atherosclerosis is focused on the reduction of risk factors that contribute to the aetiology of this disease. These risk factors include hypercholesterolemia, smoking, diabetes, hypertension and obesity. Only the control of hypercholesterolemia has been demonstrated to promote favourable changes in the atheroma itself. However, these changes are often inadequate and surgical measures are often required to treat the arterial occlusion produced by atherosclerosis. Options in the surgical treatment of atherosclerosis include atherectomy, balloon angioplasty (with or without stent placement), endartectomy and bypass grafting. Unfortunately, restenosis is a common complication of all of these approaches.

In the field of bypass grafting, restenosis is particularly problematic in small vessel, low-flow settings. Under these conditions conventional synthetic vascular grafts perform poorly and the best results are obtained with autologous blood vessels (2). However, autologous vessels for grafting are often not available in individuals suffering from vascular disease (3), and those blood vessels that are available may be diseased or possess dysfunctional endothelia. Thus synthetic vascular grafts, despite their

inadequate performance, are the only widely available option under these circumstances.

The lack of a prosthetic vascular graft capable of performing adequately as a small diameter conduit has led to numerous investigators pursuing different avenues to address this holy grail of vascular biology. These approaches largely fall into the following categories: modification of existing synthetic vascular grafts, production of alternate synthetic vascular grafts, seeding of synthetic vascular grafts with endotheliod cells, *in vitro* tissue engineering and *in vivo* tissue engineering. This review will focus on the *in vivo* tissue engineering approach to produce a superior vascular graft.

3. *IN VIVO* TISSUE ENGINEERING

In vitro tissue engineering is a well known approach to the production of a superior vascular graft. The general theme underlying this approach is the addition of cells to an acellular scaffold, of a biological or synthetic material, and exposing this structure to mechanical influences to promote the maturation and strengthening of the vascular prosthesis. Though variations on this theme exist within the field, the general approach holds true.

In vivo tissue engineering is less well known and the approaches taken fall into two main categories: the implantation of acellular scaffolds designed to be remodelled *in vivo* and the generation of autogenous fibrocollagenous tubes of tissue. Within these categories various interpretations exist, however they all derive from the central tenet that autologous tissue will be able to best perform the function, and be most capable of undergoing further adaptation to perform the role of a high pressure vascular conduit.

The ideal autologous tissue for vascular reconstruction is autologous vasculature. In the absence of autologous vasculature of sufficient quality the only options available for the use of autologous tissue are to harvest existing non-vascular tissue or to generate non-vascular tissue. The use of autologous non-vascular tissue as a vascular graft has been investigated in the past, but the practice has for the most part been discontinued, due to the harvest morbidity and the general poor performance yielded by such materials. Thus only the generation of tissue within the host remains. This involves the implantation of a foreign body, either into the vasculature where it undergoes remodelling to form an *in situ* vascular interposition graft, or into another part of the body, where a tissue tube forms that can be harvested for subsequent grafting. Both of these approaches necessitate an inflammatory reaction and cellularisation of either the implanted material or the area surrounding the implanted material. This review will initially discuss the implantation of acellular scaffolds into the vasculature and then progress to the generation of tissue external to the vascular system.

4. SCAFFOLDS DESIGNED TO BE REMODELLED

Scaffolds designed to be remodelled come in two basic varieties, decellularised tissue tubes and synthetic materials. Decellularised tissue tubes such as xenogenic small intestinal submucosa or decellularised arteries encourage host infiltration and remodelling due to their composition of natural elements recognisable to host cells, predominantly collagen. These tissues require a treatment process that removes cellular and antigenic material to minimise immunogenicity while preserving protein structure to enable ready remodelling. Synthetic materials tend to be polymers or polymer combinations such as polyurethanes and polylactic acid that are readily manipulated in order to increase or decrease biodegradation times, porosity, elasticity or other mechanical properties. This enables improved interaction with the host and allows the best management of the transition from a construct dependent on synthetic material for its mechanical strength to one with sufficient tissue strength to tolerate the demands of the arterial circulation. Successful management of this transition is essential as these synthetic scaffolds are designed to be absorbed by the host during the remodelling process.

Remodelling is the central process essential to the further development of both of these graft types. This process occurs in the following sequence (4). Initially there is formation of a thrombus layer, comprised of platelets and fibrin, as a result of the contact of blood with the non-haemocompatible graft surface. After thrombus layer formation endothelial and smooth muscle cells from the adjacent artery grow into the thrombus layer. Subsequent proliferation and matrix production by these cells leads to healing. Exposure to the mechanical stimulation of blood pulsation stimulates smooth muscle cells to orientate and produce extracellular matrix, including elastin. Perivascular granulation tissue infiltrates the external surface of the graft and histiocytes and fibroblasts resorb the graft scaffold and produce the adventitia. This process of remodelling occurs in both appropriately treated decellularised tissue tubes and synthetic absorbable scaffolds.

Many decellularised tissue tubes have been investigated as potential vascular grafts. Examples include human and xenogenic arteries (5), human (6) and xenogenic ureters (7) and human umbilical vein grafts (8). The majority of investigators attempted to produce stable constructs designed to maintain structural integrity with little attention paid to maintaining the ability of the graft to be remodelled. However, two examples of decellularised tissue tubes that undergo extensive remodelling exist. These are the Synergraft bovine ureter graft (9) and the small intestinal submucosa graft (10-15).

4.1. Synergraft bovine ureter

The Synergraft bovine ureter graft is distinguished from other bovine ureter grafts by the decellularization process it undergoes (9). This method enables removal of cellular material while leaving a functional extracellular matrix. This process enabled Synergraft processed bovine ureters to undergo remodelling and cellular infiltration by host endothelial and smooth muscle cells over a 90 day period in a canine abdominal aorta interposition graft model. The grafts remained patent for up to 43 weeks and there was minimal intimal

hyperplasia. Thus the Synergraft processing method represents a novel decellularisation method for the production of acellular tissue tubes.

4.2. Small intestinal submucosa

The small intestinal submucosa is the best example of a decellularised tissue tube implanted for its remodelling capabilities. Small intestinal submucosa has been investigated as an arterial graft since at least the mid 1960s (12). Early studies focused on the use of autogenous small intestinal submucosa. The processing of these early grafts prevented their infiltration by host cellular material and the use of autogenous small intestine also limited the clinical applicability of this approach. Subsequent studies utilised xenogenic decellularised porcine small intestinal submucosa in canine models. The investigations of Badylak *et al.* (14) were the first to demonstrate complete remodelling of xenogenic small intestinal submucosa and infiltration of host cellular material in all layers of the graft in a canine model. This investigation demonstrated complete endothelialization of the graft and infiltration of smooth muscle cells by 28 days. By 90 days the grafts had a histological appearance similar to that of an artery. Over the 180 day duration of the study the small intestinal submucosa grafts demonstrated a total patency of approximately 88%. The remodelled SIS grafts were stronger than the normal artery with one-third the compliance (15). This investigation was the first time complete healing of the small intestinal submucosa vascular graft had been demonstrated. In a subsequent investigation by Huynh *et al.* (10) a small intestinal submucosal collagen layer graft with a luminal layer of type I bovine collagen, added to reduce thrombogenicity, developed vasomotor reactivity following two months implantation as an arterial interposition graft. The histological features of this vascular graft were similar to those of an artery at 90 days, with complete endothelialization of the lumen and smooth muscle cell infiltration of the media. During the 90 day investigation the 4mm diameter grafts all remained patent. This was the first demonstration of remodelling of the SIS graft with the development of vasomotor reactivity. As such it represents a significant step forward towards production of the ideal vascular graft.

4.3. Absorbable synthetic grafts

The other approach to production of vascular grafts designed to serve as scaffolds for remodelling is the use of absorbable synthetic materials. One of the earliest examples of this approach was the work of Lommen *et al.* in 1983 (16). This group used a polyurethrane-poly-L-lactic-acid scaffold to generate neoarteries that developed elastic laminae. Around this time Lauritzen *et al,* (17) investigated polyglycolic acid scaffolds and demonstrated their ability to produce neoarteries. In 1985 Van der Lei *et al.* (18, 19) generated a complete neoartery from a polyurethrane-poly-L-lactic acid scaffold similar to that used by Lommen *et al.* (16). The neoarteries generated in these experiments demonstrated complete endothelialisation, formation of a complete neomedia including smooth muscle cells, collagen and elastin fibres, and the formation of a neoadventitia. The neoadventitia consisted of fibroblasts in addition to a range of inflammatory cells, presumably present as part of the inflammatory foreign-body reaction. In a subsequent trial grafts of 1.5mm diameter displayed 100% one year patency but high levels of deformation, 75% were either dilated or aneurysmal, indicating a lack of strength in the vessel walls (20). Other investigations have focused on improving the healing properties of existing grafts, such as ePTFE or polyurethrane grafts, through surface modifications (21), alteration in porosity (22) or addition of growth factors (23). These measures have been demonstrated to lead to improvements in graft healing but their effect on graft patency, especially within the clinical setting, remains to be demonstrated. Furthermore the initial graft material remains within the host and is not remodelled, only healing of the surfaces is improved.

4.4. Translation to the clinic

To date remodelling approaches have led to impressive results in animal models, both in terms of morphology and function. In many ways the remodelling approach offers the most commercially desirable approach to the production of a superior vascular graft. The graft can be readily packaged and stored, contains no living components, and requires only one surgical intervention. However, significant differences between the human and animal models must be taken into consideration. The first and most obvious consideration is the graft size. The longest grafts used in the studies discussed above were approximately 6cm in length. Grafts used in the clinical setting may be 20cm or more in length. Histological studies of the remodelling process have demonstrated that endothelialisation progresses from the anastomotic sites towards the centre of the graft (24), and is often limited in distance. It has been demonstrated that graft healing does occur to a degree in humans (25) but the potential extent of this process remains to be demonstrated. Thus significant differences in the extent of endothelialization of a graft of 6cm and a graft of 20cm length would be expected. Spontaneous endothelialization is also known to be limited in humans compared to most animal models. Limited spontaneous endothelialization is hypothesized to be the major reason for the performance discrepancies between animal and human single-stage endothelial seeded grafts. As the presence of a luminal endothelial monolayer is a significant contributor to graft patency rates (26) the failure of this process could have a significant effect on the performance of these scaffolds. Thus remodelling approaches represent a significant step toward a commercially viable superior vascular prosthesis although performance in the clinical setting remains to be demonstrated.

5. FIBROCOLLAGENOUS TISSUE TUBES

The other major approach to be discussed is the *in vivo* generation of fibrocollagenous tissue for the purpose of implantation as a vascular graft. The first published example of a vascular graft consisting of purpose-generated autogenous tissue was in 1950 by Pierce (27). Pierce implanted pieces of polyethylene tubing into the rectus sheaths of dogs then 5 weeks later used the tubes

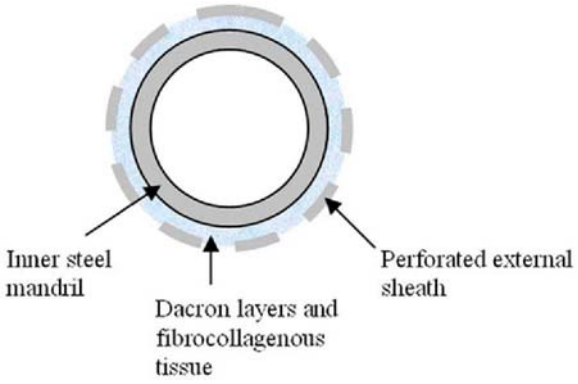

Figure 1. The Sparks' Mandril. This consisted of an inner steel mandril, surrounded by layers of Dacron, and a perforated external sheath. The perforation enabled in-growth of tissue. This tissue grew around and incorporated the Dacron layers. The tissue and Dacron tube was then removed from the Sparks' Mandril to from a vascular graft.

and surrounding fibrocollagenous tissue as abdominal aortic interposition grafts. These grafts demonstrated remarkable patency, 90% over three years. This investigation was the beginning of the field of fibrocollagenous tissue vascular graft development.

Fibrocollagenous vascular grafts are generated as the result of an inflammatory response to the presence of an inert foreign body. This results in the production of a foreign body granuloma. In this structure the foreign body is surrounded by microscopic aggregates of macrophages that may transform to epitheliod or giant cells (28). The presence of epitheliod or giant cells has been noted during histological analysis of some of the fibrocollagenous tubes (29, 30) to be discussed subsequently but may have been missed in other structures due to the delayed time-points for histological assessment, i.e. often several months after implantation. Persistent granulomas develop a rim of fibroblasts and connective tissue. This connective tissue capsule forms the fibrocollagenous tissue tubes that have been used as vascular grafts.

5.1. Eiken's plastic rod

Following the investigations of Pierce, Eiken (31, 32) developed the concept of a host-generated vascular graft further by interposing the fibrocollagenous tissue, without the irritant foreign body, as a vascular graft. In these investigations, plastic rods were inserted into dogs adjacent to the carotid artery. A tube of tissue developed over the next 8 weeks, at which time the plastic rod was removed and the tissue tubes assessed for their potential as vascular grafts. The resultant tissue tube was of collagenous connective tissue with minimal cellularity and cells of endothelial appearance towards the lumen. Neither smooth muscle cells nor elastic fibres were present in the fibrocollagenous tube. Furthermore, the tube was adherent to adjacent tissues and believed dependent on these tissues for strength. Subsequent investigations by Eiken and Norden (32) demonstrated that these constructs lacked sufficient strength for use as arterial grafts. When interposed in the canine carotid artery the connective tissue tubes underwent aneurysm formation and rupture within 1 to 2 days.

5.2. Wire mesh model

Since the initial work by Pierce research into the use of autogenous fibrocollagenous tissue tubes as vascular grafts has continued to the present day. In 1961 Schilling (30) demonstrated that fibrocollagenous tissue derived from subcutaneous implantation of a stainless steel wire mesh cylinder could be used as an arterial graft within the dog. The tissue consisted of lamellae of fibrous connective tissue with minimal cellularity. Each lamella formed over approximately two to three weeks and the lamellae were alternately longitudinally and radially orientated. This was hypothesised to be a result of the tendency of fibroblasts to align along physical planes and also form perpendicular lines of tension (33). There was no evidence of endothelial cells and only minimal inflammatory cell infiltration or vascularisation. The vascular graft took five to six months to develop and was then implanted in the dog as an abdominal aortic interposition graft. Four months after implantation the graft had undergone compaction of the lamellae, acquired vasa vasora and developed an intimal endothelial lining. These changes persisted to three years following implantation. These grafts demonstrated maintained patency for up to three years, with no evidence of aneurysm or dilatation (33). Overall patency for canine autologous fibrocollagenous tube abdominal aortic interposition grafts at three years was 66%. Thus these fibrocollagenous tubes were a promising autologous vascular graft alternative.

Another approach to construction of a fibrocollagenous vascular graft was described by Hufnagel (34). This approach consisted of implantation of a Teflon rod with a thin coating of porous Dacron mesh. This led to the infiltration of cells into the mesh and the formation of a fibrocollagenous tissue tube. The Dacron mesh was contained within the walls of the fibrocollagenous tube. Acceptable performance, as judged by Hufnagel, was produced with grafts of 5mm internal diameter. This was the precursor to the better-known work of Charles Sparks.

5.3. Sparks' Mandril

Sparks followed in the experimental footsteps of Schilling and Hufnagel and became the best-known proponent of autologous fibrocollagenous tissue-tube vascular grafts through his creation, the "Sparks' Mandril" (35, 36). The Sparks' Mandril was a metallic device consisting of an inner highly polished mandril wrapped by knitted Dacron, an outer perforated tube or shell (to allow the influx of cells) (figure 1), a further outer skeleton tube of Lexan, a cap at either end and a central tie-rod through the core of the structure. The assembled construct was implanted either on the rib cage or deep to a muscular plane via a stab wound. Tissue formed over five weeks to six months and the construct was removed through use of another stab wound and a cylindrical cutter. The tissue produced when the construct was immobilised was collagen-rich,

well-vascularised, populated by abundant young fibrocytes and round cells and contained the knitted Dacron within its wall. Sparks then implanted these fibrocollagenous tissue-tubes in dogs and patients as arterial interposition grafts. Grafts recovered from dogs at elective sacrifice at one and two years following implantation as an abdominal aortic or iliac interposition graft demonstrated walls of the same thickness as when implanted, healthy connective tissue with vasa vasora, a glassy smooth luminal surface and no microscopic evidence of aneurysm (35, 36). However, the grafts failed to develop a luminal endothelial surface, in contrast to the work of Schilling (30, 33). The initial trials of these fibrocollagenous tissue tubes as vascular grafts were conducted by Sparks and indicated the tissue tubes had promising performance in both dogs and humans. Patency rates for Sparks' Mandril femoropopliteal bypass grafts in humans were 100% at 1 to 18 months post implantation and 100% as aortic, iliac or femoral interposition grafts for dogs (35, 36).

Sparks also devised the silicon mandril method for production of *in situ* arterial grafts (37, 38). In this method a silicon tube was wrapped with layers of knitted Dacron and implanted in the location of the intended arterial interposition or bypass. After five to six weeks the silicon tubing was removed and the resultant tissue-tube was anastomosed to the vessel to be bypassed. At this point the tissue-tube was densely adhered to adjacent tissue, well-vascularised and consisted of mature connective tissue. Assessment of these tissue-tubes following interposition grafting in dogs revealed the vessels appeared unchanged at sacrifice intervals of two months to one year. There was no evidence of increased vessel wall thickness, vessel calcification or degradation and there was no neointima formation. Femoropopliteal bypass grafts displayed patency rates of 90% at up to 9 months. At that point the Sparks' and Silicone Mandril methods appeared to hold great promise for the production of a superior vascular graft. However, this was not destined to be the case.

Subsequent clinical trials reported the inadequacy of the Sparks' Mandril prosthesis as a vascular graft (39-41). Reasons for failure included aneurysm, dilatation and stretching, and thrombosis. Investigators cited the inherent weakness of the Dacron support and fibrocollagenous wall and subsequent propensity for aneurysm formation (40). Seven year patency rates of Sparks' Mandril prostheses from the original patients implanted by Sparks and colleagues were as follows: ilioiliac and iliofemoral bypass 100%, femoropopliteal bypass 26%, femorotibial and axillofemoral graft 0% (42). However, these results were an optimistic appraisal of the performance of the Sparks Mandril relative to the work of others. A clinical trial by Kretschmer *et al*. reported only 5.1% of Sparks' Mandril femoropopliteal bypass grafts as functioning properly after 4.5 years (41). Christenson and Eklof reported an overall patency rate of Sparks' Mandril prostheses at twelve months as a femoropoliteal bypass as 18% (39). In this same experiment femoropopliteal bypass grafts of Dacron of velour Dacron or autogenous vein had twelve month patency rates of 46% and 70% respectively.

5.4. Subcutaneous inflatable balloon

Since the clinical failure of the Sparks' Mandril prosthesis a few new approaches to the generation of a fibrocollagenous vascular graft have been explored. One of the most novel approaches was that of Bregman and Wolinsky in 1974 (43). This duo hypothesised that exposure of a developing vascular graft to mechanical influences similar to those occurring during vascular growth would result in a structure with properties closer to those of an artery. Hence, they created fibrocollagenous tubes via subcutaneous implantation of expandable polyurethane balloons that were cyclically inflated and deflated. Four weeks following implantation a thick, fibrocellular tube was retrieved. The tube consisted of two layers. The inner layer was composed of well-vascularised granulation-like tissue with a fibrillar appearance and many plump fibroblast-like cells. The outer layer consisted of dense connective tissue with a minor component of fibroblasts. The structure was highly collagenous and all components, cellular and fibrous, were arranged in a circumferential pattern. Balloons implanted and not subject to cyclical inflation developed a thin, poorly defined tissue that failed to demonstrate circumferential arrangement of cellular and non-cellular components and possessed less tensile strength then pulsed equivalents. When implanted as arterial interposition grafts, the pulsed vascular grafts were able to remain patent for at least eight months and develop vasa-vasora and a luminal coat of fibrinous material and endothelial cells. Altogether these findings suggest that the pulsed fibrocollagenous tubes may have potential as an arterial interposition graft. Unfortunately, no further work was published on this novel vascular graft.

5.5. Perloff, Omniflow and other grafts

One approach to production of a non-autologous fibrocollagenous vascular graft that has achieved a degree of clinical success is that of Perloff *et al.* (44). The Perloff graft was the forerunner of the now commercially available Omniflow vascular grafts (45-47). The Perloff graft was produced by implanting a silicon rod wrapped with a polyester mesh subcutaneously into sheep. A fibrocollagenous tissue tube developed, incorporating the polyester mesh into its wall. This structure was explanted, the silicon rod removed and the structure glutaraldehyde-tanned to reduce immunogenicity and improve structural integrity. The Omniflow vascular graft has a a polymer mesh in its wall and has demonstrated levels of patency superior to ePTFE grafts in below knee applications (45). It also demonstrates low rates of graft infection and aneurysm formation. Investigations into the performance of the Omniflow grafts as arteriovenous fistulae for dialysis patients have demonstrated levels of patency equivalent to those of ePTFE grafts (47). At present, although comprised of xenogenic material, the Omniflow graft is the only fibrocollagenous tissue tube commercially available for use as a vascular graft.

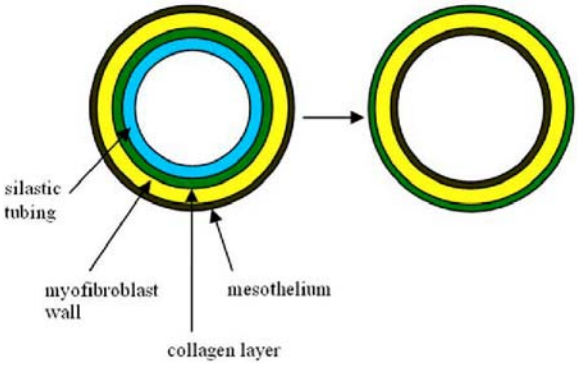

Figure 2. The artificial artery from peritoneal cavity. A length of silastic tubing is implanted into the peritoneal cavity. A myofibroblast rich tissue capsule forms around the silastic and is coated by an external monolayer of mesothelium. Eversion of this tissue capsule with removal of the silastic tubing leaves a structure analogous to an artery. It possesses an internal monolayer of mesothelium and a myofibroblast and collagen rich wall.

In addition to the Omniflow vascular graft a small number of other approaches to the production of a fibrocollagenous vascular graft have been investigated over recent years. Noishiki (48) investigated an autogenous fibrocollagenous tissue tube with a polyester mesh support in the wall. This graft demonstrated a patency rate of 73% up to seven months as a 3mm internal diameter graft and demonstrated good endothelialization in a canine model . Further development of a similar prosthesis with a framework of ultra-fine polyester fibres and a luminal heparin coat demonstrated that thrombogenicity would be the main obstacle in clinical application of such a prosthesis (49, 50). Tsukagoshi *et al*. (51) proposed an autogenous fibrocollagenous tissue tube vascular graft that was developed by harvesting autogenous fascia and wrapping it around a silicon tube that was then implanted subcutaneously. After treatment to promote antithrombogenicity the tissue tubes were implanted and demonstrated a 73% patency rate over an eight week interval in a rabbit model.

5.6. Failure of fibrocollagenous tubes

From the above discussion it is evident that much work remains to be done before an autogenous fibrocollagenous tube will produce a clinically superior vascular graft. The only autogenous fibrocollagenous tissue tube to reach the clinic was the Sparks Mandril prosthesis and this was plagued by difficulties. One of the major problems with the Sparks' Mandril was the formation of aneurysms and subsequent thrombosis. This complication was due to a general weakness of the fibrocollagenous tube (29) and the ability of aneurysms to poke through gaps in the Dacron mesh. This tendency was only identified in clinical trials. Aneurysm formation also occurred in the investigations of Eiken and Norden (32) though was not reported for the prostheses of Bregman *et al.* (43) and Schilling *et al*. (30, 33). This may reflect the marked difference in structure among the prostheses. A problem common to all of the prostheses is the difference in compliance between the prosthesis and the anastomosed artery. Lack of compliance is believed to be one of the prime contributors to the failure of conventional synthetic prostheses such as ePTFE and Dacron and as such may play a role in the failure of fibrocollagenous vascular grafts. Lack of compliance is due to the vastly different structural properties of the fibrocollagenous tissue tubes, including the lack of elastin. The lack of an anti-thrombogenic endothelial lining was also central to the failure of Sparks' Mandril prosthesis in the clinic. The consistent absence of spontaneous endothelialization in the dog makes it highly unlikely that this process would have occurred in humans. A further possible contributor to the failure of the Sparks' Mandril prosthesis and those like it is the lack of vasomotor activity. This would impair the ability of the prosthesis to regulate vessel diameter and thus lead to disturbed blood flow through the vessel. In summary the Sparks' Mandril prosthesis and other similar prostheses demonstrated impressive potential in animal models but failed to translate these levels of performance to the clinic due to the inherent weakness of the wall, the difference in compliance between the prostheses and adjacent arteries, the lack of endothelial lining, the absence of vasomotor activity and the inherent differences between animal models and humans, particularly the reduced potential for spontaneous endothelialization in humans.

6. THE ARTIFICIAL ARTERY GENERATED IN THE PERITONEAL CAVITY

There is one vascular graft that possesses many of the desirable characteristics of the Sparks' Mandril prosthesis and other autogenous tissue grafts while also possessing many of the beneficial features of the readily remodelled vascular graft. That graft is the artificial artery generated in the peritoneal cavity.

6.1. History

Years ago the observation was made that foreign material implanted into the peritoneal cavity, e.g. blood clots, egg white, silastic tubing, would either become adhered to the peritoneum or remain free-floating and develop an avascular tissue capsule (52, 53). This tissue capsule consists of several layers of collagen and myofibroblasts and a single layer of mesothelial cells and when of dog origin has a burst pressure in excess of 2500 mm Hg (unpublished data). Upon eversion this tissue capsule has layers homologous to those of an artery (54-57). It consists of an intima of mesothelial cells, a myofibroblast-rich media and an adventitia of collagen (figure 2). This finding led to the implantation of tubes of this tissue capsule into rats and rabbits as arterial interposition grafts. Under these condition the tissue tubes from the peritoneal cavity demonstrated an overall patency rate, up to four months, of 67% in rats and 70% in rabbits, in the absence of heparin or spasmolytics (54). Subsequent investigations have demonstrated patency in dogs up to 6.5 months and in rabbits up to 16 months (unpublished data).

Following implantation in the high-pressure vascular circulation the cells of the artificial artery were seen to differentiate as the artery adapted to the demands of the new environment (54). One month following

implantation, the graft wall was seen to increase in thickness, after which time no further increase occurred. The myofibroblasts of the media increased their volume fraction of myofilaments, a marker of differentiation towards a more contractile state, from a level significantly lower than that of aortic smooth muscle cells to a level equivalent to these same smooth muscle cells over a three month period. Furthermore, from one month post-implantation as a vascular graft, elastic lamellae and vasa vasora were evident within the graft. Vasomotor reactivity, absent prior to implantation as a vascular graft, was also present at six weeks for endothelium-dependent and independent vasoactive factors.

The artificial artery from the peritoneal cavity demonstrates the benefits of autogenous connective tissue tubes, i.e. it is completely autogenous, has cellular constituents, and grafts can be grown to desired dimensions. Simultaneously the artificial artery from the peritoneal cavity possesses many of the desirable traits of the remodelled scaffolds, either prior to or following implantation i.e. possession of endothelia-like and smooth muscle-like cells, gross morphology and histology similar to that of an artery, development of vasomotor reactivity and impressive vessel strength. Furthermore aneurysm formation within the implanted artificial artery from the peritoneal artery, the bane of fibrocollagenous vascular prostheses, has yet to be occur in any trials.

6.2. Origin of the cells

The presence of desirable traits from both fibrocollagenous tissue tubes and remodelled vascular graft scaffolds raises the question of the origin of the cells of the tissue capsule from the peritoneal cavity and that of the cells of the grafted artificial artery. The myofibroblasts of the tissue capsule from the peritoneal cavity have been demonstrated to be of bone marrow origin, via experiments involving chimeric mice (55). The mesothelial cell monolayer is believed to derive from the mesothelial cells of the peritoneal membrane.

The origin of the cells of the one-month post-implantation artificial artery graft remains to be determined. Efendy demonstrated that the myofibroblasts of the tissue tube from the peritoneal cavity were capable of increasing their volume fraction of myofilaments to levels not significantly different to those of abdominal aortic smooth muscle cells in response to cyclical stretching over 24 hours (56). However, as work of Huynh *et al.* (10) demonstrated that decellularised xenogenic small intestinal submucosa underwent cellular infiltration with complete remodelling and vasomotor reactivity within 3 months, a similar process of remodelling may occur to the artificial artery from the peritoneal cavity. Graft healing and remodelling may occur via the migration of endothelial cells and smooth muscle cells into the vascular graft with infiltration of vasa vasora while the original cells may be lost from the graft by sloughing or apoptosis. The finding that the artificial artery from the peritoneal cavity acquires a luminal endothelial lining when implanted non-everted (i.e. the mesothelia is on the external surface of the graft) (unpublished data) may further suggests that migration of endothelial cells from adjacent arteries is occurring.

The origin of the cells of the artificial artery from the peritoneal cavity has significant clinical importance. A criticism of the remodelling approach to production of a superior vascular graft raised earlier is the uncertainty of the extent to which remodelling occurs in humans. To date evidence of extensive remodelling of vasculature within humans exists only for veins, i.e. the process of arterialization. However, this process may result from differentiation of endothelial and smooth muscle cells already present, as opposed to the infiltration of the graft by cells of arterial or other origin. Thus, differentiation of cells within the artificial artery from the peritoneal cavity, as opposed to infiltration of cells, may be essential to the clinical success of this autologous graft.

7. PERSPECTIVE

At present the Holy Grail of vascular reconstruction, the perfect small arterial graft, remains undiscovered. Commercially available synthetic prostheses perform adequately in large vessel, high-flow situations but demonstrate inadequate performance in small vessel, low-flow situations. *In vivo* tissue engineering has been investigated for half a century as a method for the production of a superior vascular graft. To date the only autogenous *in vivo* engineered small arterial graft to undergo extensive clinical investigation, the Spark's Mandril, was a complete failure.

The *in vivo* tissue-engineered approaches that show the greatest potential for the production of a clinical small arterial graft in the near future are the implantation of biological or synthetic scaffolds for remodelling and the artificial artery from the peritoneal cavity. These approaches have all demonstrated the ability to produce vasoactive arterial prostheses with confluent endothelial linings and arterial morphology in animal models. The remaining hurdle is translating this success to the clinic. The differences in remodelling ability and activity between animal models and humans may present the major obstacle on the path to clinical success. As such, understanding and manipulating this process of *in vivo* vascular remodelling should be the focus of future efforts to tissue-engineer the ideal small arterial prosthesis.

8. ACKNOWLEDGEMENTS

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