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The daunting quest for a small diameter vascular graft

'...the (this) quest for a clinically acceptable small diameter vascular graft has persisted for over half a century and continues today with even more vigor.'

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Due to inadequate vascular perfusion brought about by vascular disease or trauma, there is a significant worldwide demand for a small diameter (<5 mm inner diameter [ID]) vascular graft for use as a bypass or replacement conduit. As a surgeon, it is preferable to use autologous vascular material (i.e., veins or arteries) for arterial bypass surgery, since they show, by far, the lowest failure rates. However, the use of autologous tissue is hampered by limited availability and suitability due to extensive peripheral vascular disease and/or use in a previous bypass surgery. Today's clinically-used synthetic vascular graft materials, when utilized as small diameter substitutes, are associated with high-occlusion/failure rates. Thus, it is imperative to develop innovative technologies targeted to the fabrication of a small diameter vascular graft.

Since there is currently no clinically acceptable nonautologous, small diameter vascular graft, perhaps one of the innovative technologies currently being evaluated may someday revolutionize the field of vascular surgery. The innovative technologies utilized in the development or improvement of small diameter vascular grafts can be classified into the following groups, with each presenting a host of concerns, limitations and/or design issues:

- Biostable, synthetic vascular grafts
- Synthetic vascular grafts – active coatings or drug elution
- Endothelial cell seeding/sodding
- Bioresorbable vascular grafts
- Tissue engineered vascular grafts

In general, the ideal vascular graft would have the following characteristics:

- Ease of surgical handling
- Suture retention
- Flexibility with kink resistance
- Biocompatibility (nontoxic and non-thrombogenic)
- Postimplantation durability after tissue ingrowth
- Infection resistance
- Leak resistance, but with adequate porosity for healing/regeneration
- Appropriate remodeling response
- Compliance matching that of the native artery
- Resistance to aneurysm formation

Other concerns with the ideal vascular graft are that it must be easily manufactured, sterilized and stored, available in a variety of sizes (lengths, diameters and tapers) and economical. Basically, surgeons are demanding the off-the-shelf availability of current synthetic grafts without the short- and long-term complications. Fulfilling these requirements creates a daunting challenge in the development of a vascular graft worthy of clinical acceptance.

The use of a biostable, polymeric vascular graft was first published in 1952 by Voorhees and colleagues who presented the concept of a synthetic conduit as a replacement for deficient natural blood vessels [1]. This advance sparked the race to develop additional, off-the-shelf polymeric vascular grafts. Two polymeric compositions for large (>10 mm ID) and medium (6–10 mm ID)

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vascular grafts were developed, and continue to be used clinically with great success. The first, and most satisfactory in terms of mechanical strength and thromboresistance, of these two compositions is expanded poly(tetrafluoroethylene) (ePTFE). The second is poly(ethylene terephthalate) (PET), better known as Dacron®. Unfortunately, the patency rates for small diameter (<5 mm ID) vascular prostheses configured from these materials are clinically unacceptable due to acute thrombus formation and chronic anastomotic (excessive tissue formation in adjacent arterial tissue) and/or intimal (excessive tissue ingrowth through the graft wall) hyperplasia. Thrombogenicity is inherent to the surface characteristics of the implanted graft materials that lack a functional intima, that is, endothelial cells (ECs). Thus, this is a critical aspect to be considered when engineering the next generation of biomaterials for use as a vascular graft. Intimal hyperplasia is theorized to initiate via a combination of compliance mismatch (mechanical properties of the graft differing significantly from those of the adjacent native vessel) and an 'abnornmal cellular microenvironment' (vascular smooth muscle cells interfacing continuously/permanently with a synthetic material and not the typical cellular microenvironment of neighboring cells and extracellular matrix [ECM]). One additional concern of these nondegradable polymeric materials during the long-term implantation is the continuous potential for catastrophic infections. Thus, one is left wondering, is it possible to engineer a graft structure from a nonbiodegradable polymer to meet the stringent mechanical properties, mechanical durability and biocompatibility required? While probably not impossible, it seems unlikely that this can be achieved without the use of a synthetic polymer structure combined with biologic components, including EC seeding/sodding or tissue-engineered constructs, in order to more closely match both the cellular environment and compliance of native vasculature.

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In an attempt to overcome the inherent limitations of synthetic polymers, Malcolm Herring in the late 1970s pioneered the technology of EC seeding, followed by others with the concept of EC sodding, with the overall hypothesis that a small diameter synthetic vascular graft could be made more biocompatible by creating or transplanting a 'disguise' derived from one's own natural vascular luminal lining (ECs) [2,3]. Why EC seeding/sodding? The endothelium, once thought of as a simple cell lining, in actuality represents a complex 'biofactory' that produces components critical to controlling or inhibiting the failure modes exhibited by the synthetic vascular grafts. Since inception of the concept of EC seeding, numerous methodologies for EC seeding/sodding have been developed. These techniques can be differentiated and categorized based upon the unique physical force utilized in each seeding/sodding process: gravitational, hydrostatic and electrostatic [4]. Unfortunately, this overall concept has yet to be embraced by the surgical community. It should be noted, however, that the basic technology still holds promise in that it has been shown to reduce, or at least slow the failure rate of the synthetic polymer grafts, primarily ePTFE, in human trials. While the technology is far from perfect, this most basic form of vascular tissue engineering – imparting some degree of biologic activity to the luminal surface of a synthetic construct – could potentially be, at a minimum, the bridge technology to the next generation of vascular prosthesis. One major concern that remains, especially when utilizing ePTFE grafts due to their nonstick surface characteristics, is the potential for delamination of the EC lining, neointima, upon implantation due to surgical, mechanical manipulation and subsequent exposure to blood shear stress. It is also speculated that there may be a potential for spontaneous and catastrophic delamination of the neointima at anytime after implantation. Besides the labor- and cost-intensive procedure to implement this method, another major limitation of the seeding procedures is that many of them require a substantial cell culture period to allow for cell adhesion and neointima formation, which introduces concerns regarding cellular genotypic and/or phenotypic changes, and a risk of infection (cell culture contamination) [4]. Surgeons do not want any additional steps in the operating room – they want to take the graft 'off-the-shelf' and implant it. During surgery any additional steps, for example, harvesting ECs or waiting for completion of the procedure (because it can be time consuming, often requiring multiple procedures on different days), would be burdensome. The take-home message is, keep it simple. Despite these drawbacks, Peter Zilla (Cape Town, South Africa) continues to champion EC seeding as a clinical tool with an ongoing multicenter clinical trial.

Over the years, several groups have proposed different approaches which alter the inner surface layer of the vascular prosthesis either physically, chemically or by a drug-eluting coating which would prevent thrombogenicity [5–8]. The stent industry has developed, as coatings on metallic stents, several slow-releasing drugs, which also have potential application on synthetic vascular prostheses. The first trials used heparin coating on the luminal surfaces, and showed an improvement in patency, as well as a reduction of neointimal hyperplasia in experimental models. However, clinically, these results could not be confirmed, potentially due to the rapid release of the heparin coating. Additionally, immunosuppressive agents such as rapamycin or cytostatic drugs have shown excellent results with regard to limiting neointimal proliferation and restenosis in experimental models and large clinical trials. Similar compounds have been used for the coating of ePTFE grafts in experimental settings and

showed some reduction of intimal hyperplasia. However, before clinical application, the adverse effects of such potent immunosuppressive drugs and the incidence of thrombus formation have to be clarified. More recently, attempts have been made to repopulate the inner surface of implanted vascular grafts with endothelial or progenitor cells. For this purpose, the grafts were incubated with growth factors or anti-CD34 antibody.

Bioresorbable vascular grafts are analogs to the biostable, synthetic polymers, with one major exception. The structural materials utilized in the design of the graft are capable of acting as a vascular graft, while at the same time promoting arterial regeneration *in situ*. The challenge in these designs is coordinating the time of degradation (loss of mechanical strength) with the time of significant tissue regeneration (which must take up the loss of strength accordingly) in order to prevent catastrophic failure (aneurysm formation/rupture). Substantial work has been performed over the past 40 years in regards to design and testing of bioresorbable vascular grafts, and this historic work suggests that this approach to vascular grafts is promising. As best stated by Howard Greisler and associates, 'The resorbable graft represents a paradigm shift in vascular prostheses. Unlike conventional prostheses whose effectiveness depends by and large upon an inherent resistance to a normal healing process, resorbable grafts work with the body, providing a framework for well-established healing patterns. It seems intuitive that by augmenting rather than opposing these patterns, a more efficacious vascular graft should result' [9]. One potential downside to this innovative work was the use of large diameter fibers (140 or 250 µm) of various biodegradable polymers knitted to form the graft walls – the large diameters are probably not optimum for promoting arterial regeneration, but were adequate in animal models. Whether or not regeneration capacity would translate to human success is unknown, and probably questionable.

Bionanotechnology, or more specifically electrospinning, is a polymer processing technique that may be able to address the limitations of the knitted, large diameter fibers used in creating bioresorbable vascular grafts. Electrospinning is currently being utilized in the authors' laboratory to revitalize the concept of a bioresorbable vascular graft, and could potentially prove to be a vital technology in the development of a tissue-engineered blood vessel [10–12]. Electrospinning allows for the generation of nonwoven fibrous structures that mimic the nanoscale fiber dimensions of the native ECM, the fiber composition (i.e., collagens and elastin) of the ECM and the mechanical properties of the native structures. This establishes a well-defined architecture capable of guiding cell migration, proliferation, differentiation and function (tissue development and maintenance). Thus, the authors' group is conducting extensive research regarding the application of electrospun, nonwoven seamless tubular structures composed of natural and synthetic biodegradable polymers as acellular vascular grafts capable of *in situ* regeneration [10–12]. One major advantage of this acellular, *in situ* regeneration approach is that it removes all concerns regarding cell sourcing for development, as well as cellular genotypic and/or phenotypic changes and a risk of infection (cell culture contamination) during cell/tissue culture. We can then address the question, 'How much *in vitro* fabrication, if any, is really required beyond scaffolding?' Aside from fabrication technique, the bioresorbable graft will have comparable concerns regarding storage, distribution and 'off-the-shelf' availability to the current biostable grafts.

While EC seeding is considered by many to be the first and simplest form of vascular tissue engineering, it does not form a neoartery. More complex attempts have been conducted by combining synthetics (biostable and biodegradable) with natural materials and cells, to incorporate a higher degree of biologic activity, thereby reducing the biostable polymeric structures to minimize the associated potential failure modes. In general, three classic models of vascular tissue engineering have been prominent. The earliest, originated by Weinberg and Bell in 1986, and most widely studied model, is the use of collagen gels with encapsulated cells [13]. Generally, in this model the outer layer of the graft wall is composed of a collagen gel containing fibroblasts to form an adventitia, which surrounds a collagen gel layer containing smooth muscle cells to form the media, and a monolayer of ECs forms a neointima. In the majority of studies on this model, one major limitation is the requirement of a synthetic graft-like external support structure, often a Dacron graft, to allow the overall structure to withstand arterial forces (i.e., pressure). One concern with the clinical application of such a model would be the continuous potential for delamination of part or the entire biologic structural portion, as well as the threat of nontreatable infections due to the synthetic graft wrapping. The second model utilizes a bioresorbable tissue-engineering scaffold for seeding of the arterial cellular components, and allows those cells to proliferate and fabricate a native ECM in place of the resorbable structure over time in culture (often in a dynamic bioreactor for construct conditioning/strengthening) [14,15]. The major disadvantage of this model is the weeks to months (labor and cost intensive) required to develop any substantial mechanical integrity. The final model could be referred to as sheet engineering, or self-assembly model where the individual cellular components (fibroblasts and smooth muscle cells) are grown in culture under conditions that allow excessive ECM production [16]. This model of assembly requires several weeks to produce a continuous sheet-like structure which can be lifted from the culture dish and wrapped around a mandrel to form tubular layers of a vascular graft. These constructs, unlike collagen gel structures, do exhibit substantial mechanical integrity and can withstand arterial forces. One major down side with sheet engineering and the

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scaffold-derived structures are the long culture periods required, raising the concerns of potential genotypic and/or phenotypic changes and the introduction of infection (cell culture contamination) during the extended development stages. Another concern is the continued potential for delamination and unraveling. All of these models raise the concerns of the cell source for mass production required to allow widespread clinical use. Currently, protocols for future clinical applications are based on prior harvesting of autologous cells followed by *in vitro* and bioreactor cell expansion. For these tissue-engineered cellularized products, in terms of manufacturing, storage, distribution, operating and handling, the 'off-the-shelf' availability may turn out to be as daunting a task as the original design has proven to be. The question is raised, 'can this type of graft ever be economical and provide wide-spread availability, especially in emergency situations, to the large population requiring them?' This will be the challenge to vascular tissue engineers.

More recently, a vascular graft development model has been developed that eliminates many of the concerns raised by the three classic models but creates additional concerns. This model utilizes a mandrel placed in the peritoneal cavity to allow granulation tissue to engulf the mandrel (one's own body as the bioreactor), thereby allowing the natural wound healing mechanisms to produce a hollow tubular structure with myofibroblasts (smooth muscle-like cells) as the major structural cell type, and mesothelial cells lining the external surface [8,17]. Once formed on the mandrel after 2–3 weeks *in vivo*, the hollow tubular structure can be removed and turned inside-out, resembling a blood vessel wall. In short-term animal models, this graft has been successful. The major benefit to this model is that the source of all structural components is autologous, ensuring no rejection. However, there remain concerns regarding long-term stability of this graft, as well as the source and type of cells composing the graft short and long term. Previous studies using a similar technique (subcutaneous implantation), have shown fibrosis after a number of years [18,19]. Since in human trials the mandrel will be held in place by a transdermal access port (for ease of insertion and removal) on the abdomen during the 2–3 week development, there will always be a risk of access site infection. Another major concern with this model will be the potential for abdominal adhesions. Finally, these grafts would not be available in emergency situations.

With the limited successes to date, we must not forget that none of these tissue-engineered models have been evaluated for long-term performance, with an emphasis on anastomotic, and more importantly, intimal hyperplasia. Thus, a better understanding of vascular developmental biology and its application to arterial re-engineering, as well as significant time and financial effort are probably essential prior to clinical application.

In summary, this quest for a clinically acceptable small diameter vascular graft has persisted for over half a century and continues today with even more vigor. It is still, to this day, awe-inspiring for one working in this field to stand back and think, what an amazing engineering achievement our native vascular structures are. Replication of the unique capacity of arterial structures to provide for nonthrombogenicity, strength, and appropriate biologic responses under continuous cyclic loading in the physiologic environment represents a daunting task from a biologic, engineering and material science standpoint. Thus, the ultimate question becomes, 'can we truly replicate an arterial structure in terms of this biologic, engineering and material science perfection?' While, the answer is probably yes, the best we can do at this time is allow the many bright and talented individuals working in these various areas of developing vascular grafts to perform their work and allow the evolving innovations and technology to develop into a clinically acceptable vascular graft.

Where will vascular graft technology be in 5 years? It is hoped that the clinical potential for EC seeding will be elucidated by the multicenter clinical trial currently ongoing under the direction of Dr Zilla. One major concern is that this clinical trial may be the last chance for this technology after over 30 years of effort. As for the more complex tissue-engineered grafts, their clinical potential will hopefully become clear after significant evaluations being undertaken around the world by numerous groups. In particular, a completely autologous, tissue-engineered vascular graft developed by the sheet engineering technique currently in a clinical trial being conducted by Cytograft Tissue Engineering, Inc. will give the tissue engineering community a sense of the capacity of such an endeavor. However, the problem of a universal cell source for the mass production of such products will linger for many more years and creates a major limiting factor. As for bioresorbable grafts, which are currently being revitalized, researchers are hopeful that within a 5-year window the clinical potential for such a technology will also become evident.

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In such a short manuscript it is impossible to mention by name, or even cite all the individuals who have contributed intellectually to this vast field over the past 50 years. Thus, the authors apologize to the many scientists whose work was not cited directly due to space constraints.

Disclosure

Gary Bowlin has several patents and patents pending concerning electrostatic EC seeding and electrospinning technology discussed in this manuscript. The electrospinning technology has been licensed to NanoMatrix, Inc., of which the author has a financial interest.

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