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Vascularizing engineered tissues for *in vivo* and *in vitro* applications

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Abstract: Engineering human tissues to enhance, replace or simulate physiological function has enormous potential to significantly impact human healthcare. A fundamental feature of human tissue is a vascular supply. The creation of a living dynamic circulation has proved to be a significant challenge. The past decade has brought several new approaches to create a vascular supply for three-dimensional human tissues for both *in vitro* and *in vivo* applications. However, future challenges remain, and new innovation in the areas of cell sourcing, the extracellular matrix, and controlling the immune response will be required to fully harness the potential of tissue engineering.

Key words: microcirculation, regenerative medicine, blood supply.

11.1 Introduction

The field of tissue engineering is broad, and includes both the creation of new organs for those damaged by disease or trauma as well as three-dimensional (3D) *in vitro* models that can better replicate *in vivo* biology compared to two-dimensional (2D) monolayer culture (Griffith and Swartz, 2006). Regarding organ replacement, the field, in general, has woefully underperformed since the initial excitement nearly 15 years ago when a scaffold resembling a human ear appeared on the dorsal surface of a mouse (Cao *et al.*, 1997). Early laboratory successes at creating artificial skin struggled in the clinical and commercial sectors. While artificial skin is a US Food and Drug Administration (FDA) -approved medical device, cartilage is the only other engineered artificial tissue that has reached this lofty status. The underlying reasons for the lengthy delay in developing successful replacement tissues are many, but can generally be captured in the accepted paradigm: (1) a cell source is expanded *in vitro*, (2) the cells are seeded onto a scaffold, which supplies support and structure, and (3) the engineered tissue is introduced to the host to restore function. This paradigm requires a cell source(s) that is compatible with the host, and that, once implanted, requires removal of waste products and delivery of nutrients at rates that are compatible with tissue survival. The transport of nutrients and waste products in thin tissues (<2 mm, e.g. artificial skin) can occur by simple diffusion (Brownian motion), at least initially, until full integration is achieved. However, thicker tissues will

require convection as the primary mechanism of transport to enhance nutrient and waste exchange. A primary challenge for the field of tissue engineering is to create a thick (≥ 1 cm) metabolically active 'tissue' that can survive implantation into a host. This will require rapid (< 24 hours) delivery of blood to the full thickness of the graft.

While 2D cell culture systems have long been the mainstay to understanding cell biology due to simplicity, reproducibility and affordability, these systems do not capture the rich cell–cell and cell–matrix interactions of the 3D *in vivo* tissue microenvironment that are so crucial to understanding tissue biology. The concept of creating *in vitro* 3D models of human organs can be traced back nearly four decades with the advent of 3D tumor spheroids to better represent growth and progression of cancer (Dalen and Burki, 1971; Sutherland *et al.*, 1971; Folkman and Hochberg, 1973). These early systems, as well as the systems that followed, which attempted to mimic complex organs such as the heart (Bursac *et al.*, 1999; Papadaki *et al.*, 2001; Naito *et al.*, 2004; Radisic *et al.*, 2004), lungs (Agarwal *et al.*, 2001; Choe *et al.*, 2003, 2006; Thompson *et al.*, 2006, 2007; Malavia *et al.*, 2009), and liver (Liu Tsang *et al.*, 2007; Domansky *et al.*, 2010) were relatively simple and limited in physical size by the lack of convective transport through a vessel network. In order to replicate the complex 3D arrangement of cells and extracellular matrix (ECM), new human microphysiological systems must be developed, and must include a vascular supply. The vasculature not only provides the necessary convective transport of nutrients and waste in 3D culture, it also couples and integrates the response of multiple organ systems.

This chapter will cover in detail the primary methods employed to vascularize engineered tissues, discuss some of the *in vivo* and *in vitro* applications of these vascularized tissues, and propose significant challenges that must currently be overcome for these tissues to reach their full potential.

11.2 Strategies to vascularize engineered tissues

11.2.1 In-growth of native vessels

The most common approach to vascularize a tissue-engineered construct for implantation is to stimulate the in-growth of host vessels. The first method includes degradable microcarriers, cellular transfection, or soft hydrogels as sources of pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF). For example, degradable microcarriers [e.g. poly-(D,L-lactide-co-glycolide or poly(lactic-co-glycolic acid)] can be used to release angiogenic growth factors such as VEGF or basic fibroblast growth factor (bFGF) to stimulate *in vivo* angiogenesis (Cleland *et al.*, 2001; Perets *et al.*, 2003). Alternatively, a mammalian cell can be transfected to overexpress an angiogenic cofactor and then be placed within the engineered tissue (Ajioka *et al.*, 2001). Similarly, gelatin-based or alginate-based hydrogels have been preloaded with either bFGF

or VEGF to stimulate angiogenesis *in vivo* (Lee *et al.*, 2000; Doi *et al.*, 2007; Silva and Mooney, 2007).

The second approach involves the use of degradable polymer scaffolds that can provide bulk and porosity for an implantable device, and also encourage the in-growth of vessels *in vivo* (Freed *et al.*, 1994; Loebsack *et al.*, 2001). The polymeric scaffolds can also release pro-angiogenic growth factors (Plate XIX, see color section between pp. 234 and 235). For example, degradable polylactic glycol scaffolds have been shown both to release VEGF and bFGF and to stimulate angiogenesis when implanted into a host (Perets *et al.*, 2003; Sun *et al.*, 2005; Ennett *et al.*, 2006). The primary disadvantage of this approach is the reliance on diffusion to deliver nutrients and oxygen while waiting for the in-growth of new vessels from the host. This generally takes > 3 days, which may be too long to allow hypoxia-sensitive cells to survive in a truly thick tissue.

11.2.2 Microfabricated microfluidic channels

Microfabrication technology has led to the creation of precise microchannels on non-biological substrates (e.g. silicon or polydimethyl siloxane) (Barenstein *et al.*, 2002; Shin *et al.*, 2004; Hsu *et al.*, 2010), or within biological substrates such as collagen (Chrobak *et al.*, 2006; Price *et al.*, 2010; Zheng *et al.*, 2011) or fibrin (Miller *et al.*, 2012), and a recent review has covered this topic in detail (Wong *et al.*, 2012). These approaches offer several distinct advantages including control over the physical size (diameter, length) of the fluidic channels, the branching characteristics (e.g. angles) of the channel network, and the essentially instant introduction of advection as a mechanism of transport. These advantages are offset by the lack of connection to the native circulation and pump, and the inability of the channels to respond to the dynamic metabolic needs of the tissue, even when 'endothelialized' (Myers *et al.*, 2012). Nonetheless, the most recent example of this approach described a 3D network of 'vessels' cast from a sacrificial carbohydrate, surrounded by a cellularized fibrin matrix (colour Plate XX) (Miller *et al.*, 2012). The channels were lined by endothelial cells, and were shown to nourish nutrient-sensitive primary hepatocytes, and began to initiate new sprouts. The presence of endothelial cells clearly allows the potential for significant remodeling of the network over time to meet changing metabolic needs, and so create a dynamic vascular network.

11.2.3 Spontaneous self-assembly (vasculogenesis) of capillaries from endothelial cells

Human endothelial cells (hECs) can be sourced from multiple tissues including, but not limited to, the umbilical vein (Dosne *et al.*, 1978; Gospodarowicz *et al.*, 1978), umbilical cord blood (Fan *et al.*, 2003; Murga *et al.*, 2004), peripheral adult blood (Ingram *et al.*, 2004; Fuchs *et al.*, 2006) and skin (Sherer

et al., 1980), as well as human embryonic stem cells (Levenberg *et al.*, 2002). When hEC are combined with a matrix such as collagen, fibrin, matrigel, or synthetic polymer, stromal cells (e.g. fibroblast, mesenchymal stem cell), and appropriate growth media, they spontaneously form a 3D network of human capillaries over the course of 3–7 days. The potential advantage of this strategy is that, if the vessel network is continuous following *in vitro* development, upon implantation, a minimal number of anastomostic sites with the host will result in rapid perfusion of the network and tissue. The vessel network could then remodel (pruning and extension) in response to the needs of the tissue.

This technique has been used by numerous groups to 'prevascularize' an engineered tissue before implantation in an immune-compromised host (Levenberg *et al.*, 2005; Schechner *et al.*, 2000, 2003; Melero-Martin *et al.*, 2007, 2008; Kang *et al.*, 2009; Chen *et al.*, 2009, 2010; Lin and Melero-Martin, 2011). The hEC vessels anastomose with the host circulation within 1–7 days depending on the *in vitro* culture time and other factors such as hEC type and fibroblast concentration, demonstrating the potential for this approach (colour Plate XXI). Our group has recently reported significant clotting of blood following initial anastomosis using a window chamber and multispectral imaging (White *et al.*, 2012) highlighting the need to visualize flow dynamically and not rely solely on histological analysis of red blood cell-filled lumens. In this strategy, the vascular supply of the tissue is largely developed *in vitro*, thus overcoming the limitations of other strategies that rely on ingrowth of new vessels or the formation of new vessels following implantation.

Our group has recently used this technique to create a perfused vascular network of microvessels in a microfabricated device (Moya *et al.*, 2012). The vessels demonstrate physiological permeability (impermeable to 70 kDa fluorescent dextran). Furthermore, flow includes a wide range of velocity (maximum 4000 μm/s, mean 690 μm/s) and shear (maximum 1000 1/s, mean 320 1/s) that encompass the physiological range which can be easily manipulated by controlling the pressure drop across the network.

11.2.4 Sprouting (angiogenesis) vessels from endothelial cell lined channels

An alternative method to create a functional vessel or vessel network in an *in vitro* tissue uses a combination of microfabrication technology and hECs. Recently, it has been demonstrated that hECs lining fabricated channels directly within (Zheng *et al.*, 2012) or adjacent to (Song *et al.*, 2012) a collagen or fibrin (Yeon *et al.*, 2012) network can sprout into the adjacent matrix and become functional (colour Plate XXII). These recent reports describe only a single functional vessel, but highlight the potential of this technique to create functional and dynamic vessel networks in an *in vitro* engineered tissue construct.

11.2.5 Microvessel fragments

An alternative strategy to prevascularize a tissue before implantation is to seed small isolated microvessel fragments into an appropriate extracellular matrix such as collagen (Hoying *et al.*, 1996; Shepherd *et al.*, 2004). The microvessel segments demonstrate angiogenic potential following 5–7 days in culture. The microvessel fragments are isolated from the epididymal fat pads of rats, and, following implantation into an immune compromised mouse, the rat microvessels anastomose with the host vasculature. Of particular interest relative to other prevascularization strategies is the observation that this method results in a wider range of vessel types including arterioles, capillaries and venules, and so affords the possibility of a hierarchical vessel network more rapidly with perhaps less remodeling on the part of the host. This method has been used to augment vascularization of the myocardial wall following induced infarction in a mouse model (Shepherd *et al.*, 2007).

11.2.6 *In vivo* conditioning

An intriguing method to vascularize an engineered tissue is to use the complex *in vivo* environment to condition or vascularize the engineer tissue. In this technique, the engineered tissue is placed inside the host, and essentially employs the host as the 'bioreactor' to develop the vascular supply in the engineered tissue, with the potential to then move the engineered tissue to a more permanent location. The most common method of *in vivo* conditioning is to create an arteriovenous (AV) loop (colour Plate XXIII) in which a vascular graft is surgically inserted as a bypass (or loop) from a small artery to a nearby venule (Mian *et al.*, 2000; Kneser *et al.*, 2006; Lokmic *et al.*, 2007; Manasseri *et al.*, 2007; Morritt *et al.*, 2007; Ren *et al.*, 2008; Dong *et al.*, 2010; Beier *et al.*, 2011; Boos *et al.*, 2013). Within the space created by the loop, a tissue engineered construct is placed, and small vessels can sprout from the AV loop and enter the engineered tissue to provide nutrients and remove waste products. The potential drawback of this method is the time necessary for the in-growth of vessels, but a major advantage is the ability to remove the tissue once it is vascularized and have a convenient large vessel (from the AV loop) for surgical anastomosis at a permanent site.

More recently, an alternative method of *in vivo* conditioning was presented in which tissue engineered cardiac tissue was placed initially on the highly vascular omentum of the host to become prevascularized, and then subsequently removed and placed within an infarcted region of the heart (Dvir *et al.*, 2009). Although this approach to prevascularization led to functional tissue 4 weeks after transplantation from the omentum and into the heart, there remain several major disadvantages including: (1) the tissue dimensions remain in the order of 2–3 mm, (2) the technique requires two surgical procedures and (3) the techniques relies on

the in-growth of host vessels over a 7-day *in vivo* vascularization process, which is likely to be too long for thicker tissues > 1 cm.

11.2.7 Extracellular matrix

Any vascularized engineered tissue requires an ECM to support the vascular network. There are numerous models of the ECM that can generally be separated into naturally occurring and synthetic; they have recently been reviewed by Tian and George (2011). The naturally occurring ECM mimics include: collagen, fibrin, starch, matrigel, silk fibrion and decelluarized ECM from the tissue of interest (e.g. cardiac tissue). The synthetic materials that have been successfully used to vascularize engineered tissues include functionalized polyethylene glycol and poly(lactic glycolic) acid. Although all of these materials can support vascularization, each has advantages and disadvantages. Broadly, the naturally occurring ECMs have inherent chemical and physical cues that support cell adhesion and vascular growth, and are easily degraded or remodeled by the host, but suffer from lot-to-lot variation, the potential for disease transmission, and are difficult to 'tune' mechanically or chemically to address more mechanistic questions. In contrast, the synthetic matrices are chemically defined, and so have little lot-to-lot variability and can be specially designed to address mechanistic questions; however, these matrices must be functionalized with binding sites (e.g. RGD sequence) for mammalian cells, and may be difficult for the host to degrade or remodel.

11.3 *In vitro* applications

11.3.1 Biological understanding of the tissue microenvironment

An important *in vitro* application of vascularizing engineered tissues is to simply enhance our general understanding of the dynamic and 3D human tissue microenvironment, as recently reviewed by Griffith and Swartz (2006). This application is a natural extension from the enormous understanding of cell biology (e.g. signal transduction, migration, proliferation) that simple 2D monolayer culture has provided over the past five decades. *In vivo*, cells live in a 3D environment, and interact with a multitude of neighboring cells and extracellular matrix proteins, as well as the microcirculation. The interactions are non-linear, and include both chemical and mechanical components. Hence, our understanding of human biology will be dramatically improved as 3D models of the microenvironment are developed that include the microcirculation.

11.3.2 Drug discovery and chemical toxicity screening

While the actual cost of bringing a new drug to market is controversial, the magnitude remains enormous and significantly impacts the cost of medication, and

the number of new drugs approved by the FDA each year. Since 1950 the number of new drugs approved by the FDA for human use per billion US dollars invested has dropped in a linear fashion (Munos, 2009). This trend has been dubbed 'Eroom's Law' (Scannell *et al.*, 2012), and the cost of bringing a single drug to market now exceeds one billion US dollars. In 2004, pharmaceutical companies spent approximately $37 billion in all phases of drug development. The two most costly steps (each ~25% of total) are preclinical testing of thousands of compounds to assess potential efficacy and side effects, and phase III clinical trials in humans. Only about 20% of drugs entering clinical trials make it to market, with 50% of drug candidates failing in expensive phase III trials due to lack of either safety or efficacy (DiMasi *et al.*, 2003). Hence, reducing the cost and improving the success rate depend strongly on our ability to both rapidly and cost-effectively screen compounds, and to better predict success in large-scale human trials.

Chemotherapeutics to treat cancer, which typically have a shorter time to market, are particularly prone to failing in phase III trials (DiMasi and Grabowski, 2007), and major cardiac side effects have become apparent only after approval and large-scale clinical use for several important drugs (Herman and Ferrans, 1998; Chu *et al.*, 2007). Flat, 2D monocultures of tumor cells or normal cells do not begin to capture the complexity of the 3D tissue microenvironment. An ideal system for screening potential new drug candidates would be: 3D geometry; composed of tissue-specific cells, stromal cells, vasculature and matrix; affordable; rapid; reproducible; and suitable for high-throughput screening applications. In addition, a perfused vasculature would allow physiological nutrient and drug delivery.

The impact of creating *in vitro* 3D vascularized tissues that mimic the human tissue microenvironment is potentially paradigm-shifting for the field of chemical toxicity screening. Humans are exposed to chemical toxins primarily through absorption through the skin, inhalation into the lungs, or ingestion. While acute toxicity to the initial organ of exposure can occur, toxins have the potential to be absorbed by the microcirculation and either damage the cardiovascular system directly or be delivered by convection to a distant site (e.g. liver, brain) of toxicity. Hence, the ability to screen chemical toxins to determine the permeability across human capillaries or direct toxicity to the microcirculation is of enormous importance in understanding the integrated effect of an individual toxin. Finally, the 'decision' by a tissue following insult (e.g. toxic chemical) to revitalize or undergo programmed death is poorly understood, yet is fundamental for our success in advancing human health, and must depend on a functional (i.e. perfused) capillary bed.

11.3.3 Personalized medicine

The response of humans to drugs is heterogeneous, and depends on numerous factors such as age, race, body mass and genotype. Some of these factors are well

understood, while others are not. For example, it is well known that older people metabolize some classes of drugs slower. A growing interest in medicine is the concept that the specific drug and dosage can be tailored for an individual – 'personalized medicine'. This potentially requires an inordinate amount of information (e.g. genotype for an individual), and could be laborious and cost prohibitive. However, the rapidly developing fields of high-throughput screening and stem cell technology provide hope that this approach may be used in the near future, at least for specific applications such as chemotherapy for cancer. A paradigm for personalized medicine already exists for some forms of cancer. Breast cancer is now widely recognized to have molecular subtypes based primarily on estrogen receptor and HER2 receptor status (Perou *et al.*, 2000). Cells procured from a biopsy can be tested for their estrogen receptor and HER2 status and the results dictate therapy (Harbeck *et al.*, 2010). More recently, it has been shown that endothelial growth factor receptor tyrosine kinase receptor inhibitors are more effective in lung cancers with endothelial growth factor receptor mutations (Mok *et al.*, 2010). The advent of induced pluripotent stem cell technology allows cells such as skin fibroblasts, which are easily harvested from an individual, to be driven first into a pluripotent stem cell, and then into essentially any cell type in the body. While differentiation protocols are still actively being developed and validated, this technology creates the possibility of creating *in vitro* disease models that are derived directly from a specific patient. In parallel, microfabrication technology (as described above) facilitates the creation of 3D organ mimics that are patient-specific.

11.4 *In vivo* applications

11.4.1 Whole organ replacement

Replacing a failing or diseased organ has long been the most obvious promise of tissue engineering. Examples include organs such as the kidney, liver and heart, all of which represent major sources of mortality in the USA, and for which there are active donor programs that fall far short of the demand (Sharing, 2012). When these organs fail, a person's life is at stake. These organs are biologically complex, not only involving a multitude of different cell types with specialized functions, but also being physically large, cell dense, and with their viability and function dependent wholly on a vascular supply. Our ability to replace these complex organs with tissue engineered equivalents will undoubtedly depend on our ability to advance the field of vascularized engineered tissues.

11.4.2 Organ enhancement or partial replacement

A more reachable goal of *in vivo* tissue engineering is the enhancement of an organ whose function is compromised by disease or aging. This might also be termed partial replacement. Early success stories of tissue engineering such as the 'skin equivalent' are just that – they replace a portion of the skin that has been

damaged by trauma or disease. Other examples of organ enhancement that are making rapid progress are tissue engineered cartilage (Moreira-Teixeira *et al.*, 2011; Kock *et al.*, 2012) and small arteries (Dahl *et al.*, 2011; Quint *et al.*, 2011). These approaches do not attempt to replace all of the cartilage or the entire arterial vessel tree; rather, these constructs attempt to replace specific parts of these organs that are diseased or failing. In this approach, the tissue engineered constructs can be smaller, less complex in their biological function, and so less dependent on a vascular supply. This approach to tissue engineering may extend lives, but the most obvious benefit is the improvement in the quality of life.

11.5 Major barriers and future trends

11.5.1 Hierarchical vascular structure

Prevascularization strategies for implantable tissues, as well as vascularization of *in vitro* tissue models currently include only small vessels that most closely represent capillaries. Although some of the *in vitro* vessels may reach a diameter of 50–100 μm (Chen *et al.*, 2010; Hsu *et al.*, 2012), this does not include the rich hierarchical structure of the vascular system that is present in larger (in the order of centimeters) tissues that includes arterioles, capillaries and venules. In addition, the methods to currently create *in vitro* vascular networks tend to 'over-vascularize' tissue, and result in a density of capillaries and small vessels that far exceeds that present *in vivo* (White *et al.*, 2012). This is likely due to the presence of exogenous growth factors such as VEGF, but other factors such as the ECM and oxygen tension may also contribute. Future *in vitro* vascularization strategies must plan for a hierarchical structure that more closely resembles the *in vivo* vascular structure to avoid clotting and extensive vessel remodeling, which could compromise tissue function.

11.5.2 Extracellular matrix

The extracellular matrix used by most *in vitro* vascularization strategies includes fibrin and/or collagen. Collagen is the most prevalent protein in the extracellular matrix, and fibrin is present in blood clots and so is present in healing wounds. While these proteins tend to stimulate angiogenesis, they do not replicate the extracellular matrix and so extensive remodeling is necessary to fully replicate the tissue of interest. More recently, decellularizing xenogeneic tissues offer an intriguing option to recover an ECM that more closely represents the proteins and other macromolecular content of native human tissue (Ott *et al.*, 2008; Petersen *et al.*, 2010).

11.5.3 Cell source

A major hurdle in the creation of large (> 1 cm) complex tissues is producing an appropriate volume of cells. Cell dense and metabolically active tissues such as

the myocardium or liver will require rapid vascularization, and these tissues contain $> 10 \times 10^6$ cells/mL. Most primary adult human cells have severely limited ability to proliferate *in vitro*, and passaging cells invariably alters cell phenotype. Furthermore, to avoid an immune response upon implantation, xenogeneic or allogeneic sources of cells are not long-term solutions. The relatively recent ability to harvest, culture, expand and differentiate both embryonic and induced pluripotent human stem cells provides an enormous opportunity to create the vast number of specialized human cells for complex tissues. Both embryonic and induced pluripotent stem cells can be produced in very large quantities, and then differentiated into many, if not all, of the cell types in the human body. Further, use of the same induced pluripotent stem cell line as the source of specialized cells in tissue engineered constructs provides histocompatibility for *in vitro* human organ model systems, and the potential of personalized medicine that avoids immune rejection for *in vivo* implantation. Significant challenges remain though, particularly in the differentiation step, which currently leads to cells that are neonatal in phenotype for essentially all specialized cell types (e.g. neuron, hepatocyte, cardiomyocyte) (Hedlund *et al.*, 2007; Navarro-Alvarez *et al.*, 2009; Gherghiceanu *et al.*, 2011; Grade *et al.*, 2012; Synnergren *et al.*, 2012).

11.5.4 Tissue remodeling and immune response

Current technologies do not allow for the creation of a fully functional and vascularized tissue. The paradigm includes a tissue remodeling process once the engineered tissue is implanted, dictated by the host such that the remodeled tissue can meet the specific needs of the host. The remodeling process is initiated by the host and includes an immune response. Managing the immune response and remodeling process such that the implanted tissue becomes fully functional as opposed to degraded and potentially rendered completely dysfunctional remains a challenge. Challenges specific to the vascular supply include maintaining flow in the network of vessels such that remodeling of the vessel network can occur without compromising the delivery of nutrients and removal of waste products (e.g. carbon dioxide). We have previously shown that microvessel networks developed *in vitro* from endothelial cells tend to over-vascularize the tissue with too many and too small vessels.

11.6 Conclusion

Engineering complex 3D human tissues for *in vitro* or *in vivo* applications provides exciting opportunities to advance healthcare and thus quality of life. Early success included relatively thin and simple tissues (e.g. skin) that did not require a vascular supply; however, more complex and cell-dense tissues (e.g. liver) will require a vascular supply to meet the more demanding metabolic needs. Several strategies have been reported to vascularize engineered tissues that take advantage of recent

advances in microfabrication, materials science, and stem cell technology. Discoveries at the interface of these disciplines will likely produce solutions to the remaining hurdles, which include more physiological ECM, a hierarchical vascular supply, an adequate and appropriate cell source, and managing the remodeling process for *in vivo* applications.

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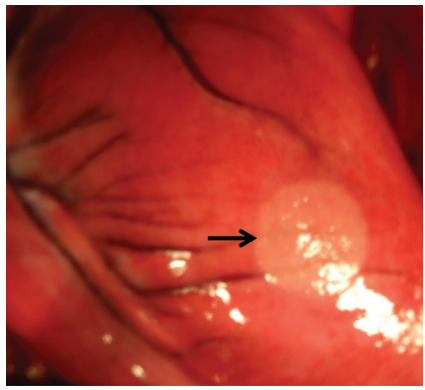

Plate XVIII (Chapter 9) Layered cell sheet (indicated by arrow) onto porcine heart.

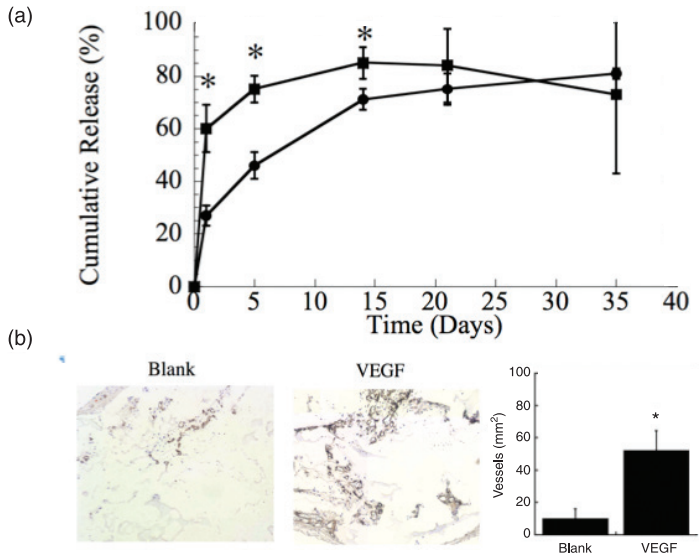

Plate XIX (Chapter 11) (a) *In vivo* vascular endothelial growth factor (VEGF) release profiles. VEGF directly incorporated into scaffolds (■) or pre-encapsulated in 75: 25 PLG (i.v. = 0.59 dL/g, 63 kDa) microspheres (●). Data represent average values (n=5), and error bars indicate standard deviation. Asterisk denotes significant differences (P<0.02) between the values of the two conditions. (b) Vessel density produced in scaffolds containing no growth factor and those rapidly releasing VEGF, following 2 weeks of subcutaneous implantation. Photomicrographs of tissue sections from blank scaffolds (left) and VEGF-releasing scaffolds (middle) following immunohistochemical staining for CD31 (EC marker) (×100 magnification). Quantification of vessel density is shown in the right panel. Values represent averages and standard deviation, and asterisk indicates statistically significant difference (P<0.015) between the two conditions. Reproduced from Ennett *et al.*, (2006) with permission.

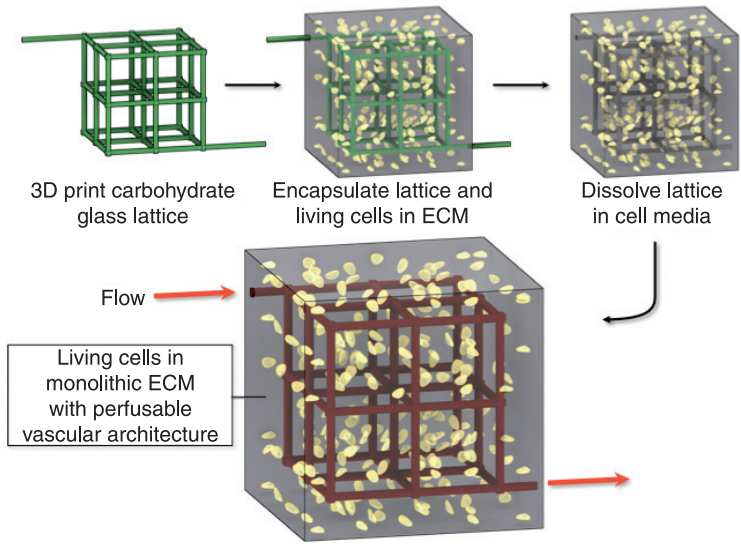

Plate XX (Chapter 11) Schematic overview. An open, interconnected, self-supporting carbohydrate-glass lattice is printed to serve as the sacrificial element for the casting of three-dimensional vascular architectures. The lattice is encapsulated in extracellular matrix along with living cells. The lattice is dissolved in minutes in cell media without damage to nearby cells. The process yields a monolithic tissue construct with a vascular architecture that matches the original lattice. Reproduced from Miller *et al*. (2012) with permission.

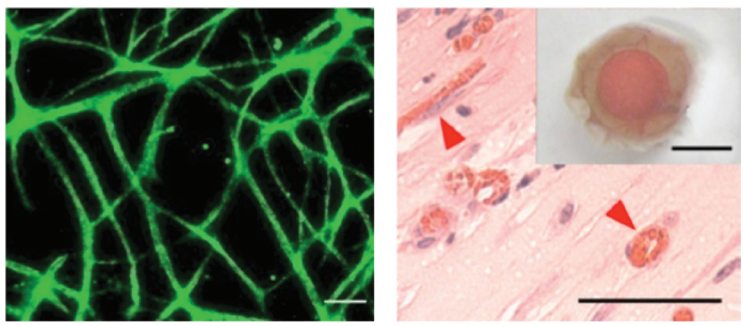

Plate XXI (Chapter 11) (a) Three-dimensional interconnected vessel networks form within 7 days *in vitro*. Endothelial colony-forming cell-derived endothelial cells (ECFC-ECs, 10^6 cells/mL) are culture with a high density of primary human lung fibroblasts (2×10^6 cells/mL). Green fluorescence indicates ECFC-ECs labeled with anti-CD31 antibody. (b) Hematoxylin & eosin stain of implanted tissue constructs harvested at day 1 demonstrate that tissue constructs prevascularized with vessels formed from ECFC-ECs in the presence of a high density of fibroblasts rapidly anastomose with host vasculature and are perfused within 1 day post-implantation. Red arrowheads: vessels perfused by mouse blood. Scale bars: 50 μm; insert, 5 mm. Reproduced from Chen *et al*. (2010) with permission.

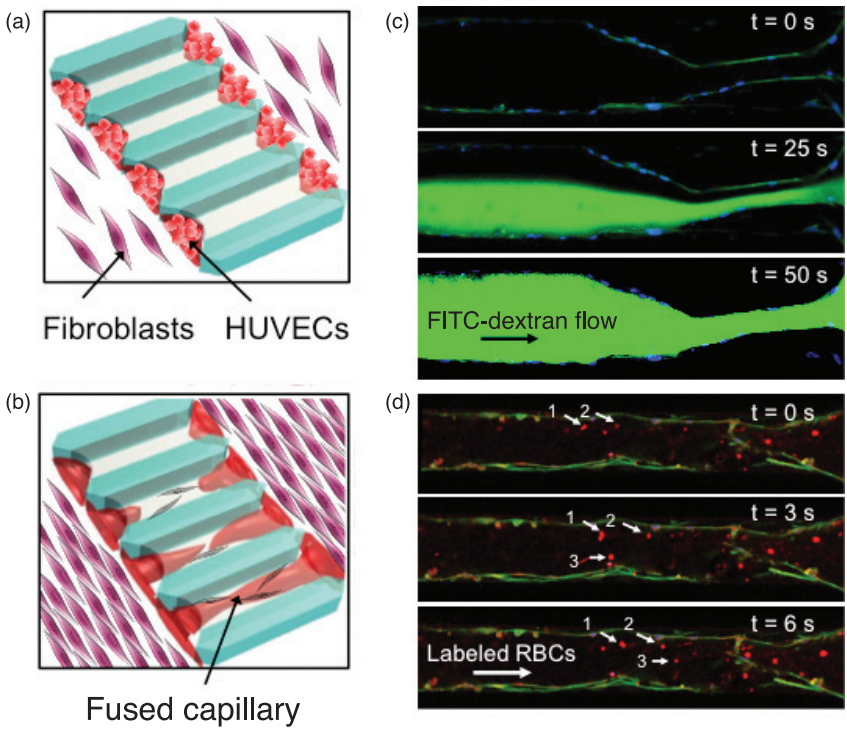

Plate XXII (Chapter 11) (a) Schematic depicting how human umbilical vein endothelial cells (HUVECs) are loaded on the concave sidewalls of a microfluidic device constructed of polydimethylsioloxane (PDMS). Fibroblasts are loaded through the two main inlet channels after HUVEC attachment on the fibrin gel. (b) Schematic demonstrating how capillaries made by HUVECs grow from both sides and anastomose. (c) Images of FITC-dextran flowing through a capillary filled with media for 50 s. (d) Temporal images of labeled red blood cells flowing through the capillary for 6 s. Reproduced from Yeon *et al*. (2012) with permission.

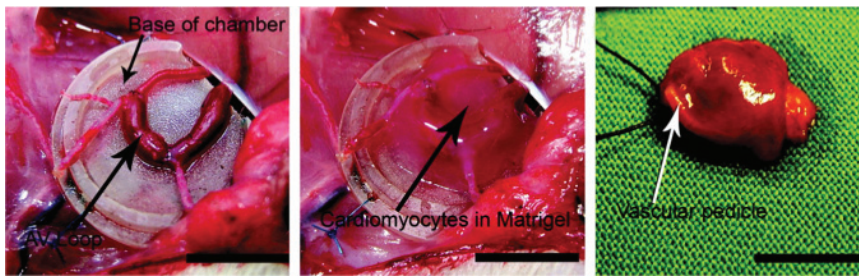

Plate XXIII (Chapter 11) (a) An arteriovenous (AV) loop is constructed and placed into the base of a plastic chamber in the rat groin. (b) Cardiomyocytes suspended in Matrigel are placed around the AV loop. (c) After 4 weeks, new tissue has been generated around the AV loop. Scale = 1 cm. Reproduced from Morritt *et al*. (2007) with permission.

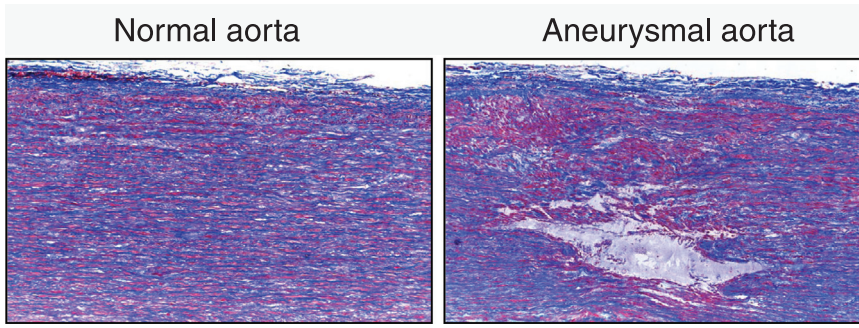

Plate XXIV (Chapter 13) Left and right panels depict histological sections of normal and aneurysmal aortic medial layer, respectively. Note the cystic medial degeneration (center, light blue), fragmented and disorganized collagen (dark blue), and the disrupted lamellae of smooth muscle cells (red) pathognomonic of aortic aneurysm in the right panel.