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Engineering a microcirculation for perfusion control of ex vivo–assembled organ systems: Challenges and opportunities

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Abstract

Donor organ shortage remains a clear problem for many end-stage organ patients around the world. The number of available donor organs pales in comparison with the number of patients in need of these organs. The field of tissue engineering proposes a plausible solution. Using stem cells, a patient's autologous cells, or allografted cells to seed-engineered scaffolds, tissue-engineered constructs can effectively supplement the donor pool and bypass other problems that arise when using donor organs, such as who receives the organ first and whether donor organ rejection may occur. However, current research methods and technologies have been unable to successfully engineer and vascularize large volume tissue constructs. This review examines the current perfusion methods for ex vivo organ systems, defines the different types of vascularization in organs, explores various strategies to vascularize ex vivo organ systems, and discusses challenges and opportunities for the field of tissue engineering.

Keywords

Organ grafts, transplantation, perfusion, angiogenesis, vascularization

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Introduction

Ex vivo organ system assembly is the production of organ systems outside of the human body. Primarily, tissue engineering is the principal method by which ex vivo organs are assembled. Tissue engineering uses the combination of pluripotent and mesenchymal stem cells (MSCs), engineering materials, and suitable biochemical and physicochemical factors to improve or replace biological functions in efforts to improve clinical repair of damaged tissues and organs.[1] The overall goal of tissue engineering is to provide another source of organs and organ materials for patients in need of organ transplants or replacements.

In the event of death, the deceased may, at times, contain viable organs. However, the body, in response to the trauma of death, creates a harsh environment by increasing the number of circulating inflammatory cytokines, catecholamines, and activated leukocytes. Thus, organs that can be used for donation need to be removed as soon as possible in order to avoid prolonged exposure to this environment. Inadequate perfusion of these organs results in a decrease in the number of viable donor transplant organs for patients in need.

Of course, in today's world, the dawn of three-dimensional (3D) scaffolding material has advanced the field of tissue engineering and regenerative medicine and has provided organ recipients a potential alternative for an organ source. 3D printing scaffolds of organs that are then seeded with the recipient patient's autologous cells of the organ in need bypass many of the problems that occur with transplantation, such as donor organ shortage, ethical issues of which patient receives which organ first, and organ rejection. At the same time, since 3D scaffolding is a relatively new phenomenon, much more research is needed in order

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Table 1. Preservation methods of various organs.

Organ	Static storage	Dynamic storage
Kidney	SCS	HMP
Liver	SCS	N/A
Lung	SCS	N/A
Pancreas	SCS	N/A
Heart	SCS	N/A

SCS: simple cold storage; HMP: hypothermic machine perfusion.

for this technology to reach its full potential of capturing the majority of the donor organ market. Currently, tissue engineering is limited by the ability to adequately vascularize scaffolded tissues.

Oxygen is carried by hemoglobin, a protein inside red blood cells that has four symmetrical subunits, from the lungs to vascularized tissues. Specifically, the heme groups within hemoglobin bind to oxygen. In order for oxygenated blood to be transported from the lungs to tissues and deoxygenated blood to be transported from the tissues to lungs, a vascular network is needed. Engineering and controlling the growth of intricate blood vessels, such as capillaries, arterioles, venules, arteries, and veins, in tissue-engineered constructs has been a major hurdle preventing transplantation of these ex vivo constructs. Perfusion-based bioreactors that allow for continuous feeding and nutrient circulation are used to provide both mechanical and electrical stimuli to promote proliferation, differentiation, and ultimately vascular network formation.[2]

Our discussion begins with an in-depth look at the underlying mechanisms for the preservation of any ex vivo organ. From there, we will explore current methods in creating a microcirculation for perfusion. Then, we will look into studies pertaining to kidney, liver, lung, heart, and skin grafts to observe the current landscape and evaluate the advantages and disadvantages of current strategies. Finally, we will take a step back and discuss the challenges faced by ex vivo organ perfusion control and explore any opportunities that may shine light on to this revolutionary field.

General mechanism for preservation of ex vivo organ systems

When a donor is declared brain dead, the recommendation is to maintain body functions, correct organic dysfunctions, and expedite organ removal for transplantation within 12–24 h of the diagnosis.[3–5] Some organs can survive outside the body longer than others. With current preservation methods, according to the US Department of Health and Human Services, the recommended time frames for organs to be transplanted once explanted from the donor are up to 4–6 h for heart, 4–6 h for lungs, 8–12 h for livers, 12–18 h for pancreas, 24–36 h for kidneys, and 8–16 h for intestines.

Organ preservation affords time to organize resources to transport organs, perform tests, and make any necessary adjustments. In order for organ systems to receive oxygen they need for survival, a continuous supply of blood is needed. Modern organ preservation originated from the use of low temperatures to cool and slow biological deterioration of donated organs. At the same time, since cold temperatures also have a detrimental effect on organs removed from their normal physiological environment, newer techniques have been developed to avoid these issues.

Currently, there are two types of preservation methods for most transplantable organs. These include static and dynamic storage methods. The main method for static storage is simple cold storage (SCS), while the main method for dynamic storage is hypothermic machine perfusion (HMP). Table 1 shows which methods have been approved for transplantable organs.

SCS combines low temperatures with special preservation solutions aimed to reduce graft injury and suppress ischemia-reperfusion injury (IRI). In contrast, HMP is largely dependent on providing an oxygen supply for aerobic metabolism via vascular perfusion. Figure 1 presents the schematic representation for a traditional HMP system.

Perfusion is the delivery of blood to the capillary beds of organs. When perfusing an organ, one can assess and evaluate organ function prior to transplantation for longer periods of time.[7] During this assessment period, organs can be resuscitated and potentially repaired if once deemed suboptimal for transplantation. Since there is a huge difference between the demand for organs for transplantation and the number of donor organs available,[8] increasing the number of donor organs available via ex vivo perfusion can go a long way in decreasing the transplantation waiting list mortality rates. The understanding of organ perfusion has only been possible by observing the potential effects of hypoxic injury on donated organs.

Prior to organ procurement, regardless of preservation modality, the desired organ is thoroughly washed with about 4–6 L of chilled preservation solution into all major vascular channels in order to clear out the blood and achieve moderate cooling. The current gold standard preservation solution for which all new solutions are compared to is the University of Wisconsin (UW)-lactobionate-based solution.[9] This UW solution was originally formulated to improve pancreas preservation but was then found to also have improved liver and kidney preservation as well.[10] Once washed, the organ is then procured and transferred to a sterile table, where the final desired preservation solutions can be achieved. Specifically, for SCS, the organs are then immersed in fresh sterile solutions, double-bagged, and buried in melting ice in transport containers.[8]

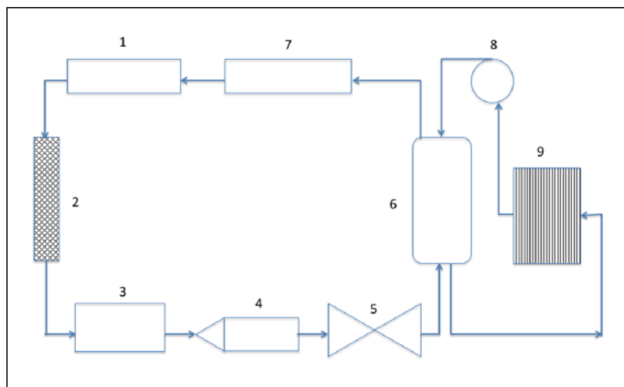

Figure 1. Schematic representation for a traditional HMP system. 1: Compressor, 2: condenser, 3: store room, 4: drying room, 5: expansion value, 6: heat exchanger, 7: gas–liquid separation, 8: cyclic pump, and 9: organ box. A single compressor regulates temperature of the organ, which is placed in a separate box. The optimal preservation temperature that will provide a balance between cooling injury and hypothermic protection is about 4°C.
Adapted from Shen and Yan.[6]

When deciding between SCS and HMP for preservation of ex vivo organs, efficacy, long-term survival rates, delayed graft function (DGF), and cost must be taken into consideration.[11] Studies have so far shown mixed results. For example, in a meta-analysis comparing 175 machine-perfused kidney grafts with 176 cold storage grafts, the perfused kidneys suffered less DGF than the cold storage grafts.[12] However, no differences were found in 1-year graft or patient survival rates. One study found decreased incidence and duration of DGF in 82 pairs of kidney grafts after machine perfusion, while another study showed no beneficial effect on DGF.[13,14] Since HMP costs are greater than those associated with SCS and similar intermediate-term graft and patient survival rates have been reported by numerous studies for SCS and HMP organs, the utility of current machine perfusion methods remains uncertain.[11]

At the same time, HMP has recently been given a renewed interest as a preservation method for both thoracic and abdominal organs from non-heart-beating donors (NHBD). Since these organs come from marginal donors, greater importance is attached to the preservation methods used during procuration of the organs in order to expand the donor organ pool.[10] Machine perfusion systems are designed to continuously pump cold preservation solution throughout the organ, providing nutrients, removing toxic metabolites, and absorbing lactic acid build up. At the same time, only kidneys are approved clinically for HMP as of November 2016. Clinical trials for perfusion of other organ systems are currently underway.

HMP of kidneys requires the dissection of the renal artery in order to attach the kidney to the machine. Additional dissection of the kidney is performed to make the seal watertight. Although HMP preparation takes longer than that for SCS, more kidney testing can be performed to assess for abnormalities. Machine-perfused renal allografts, when compared with renal allografts preserved by SCS, had a lower risk of graft failure during the first year after transplantation and showed an improved 1-year graft survival.[15]

In order to securely assume successful transplantation, optimal preservation of the liver from the time it is procured from the donor until the time it is implanted in the recipient is necessary to prevent rapid deterioration of the graft.[16] The current standard for liver preservation is SCS is shown in Table 1. However, machine perfusion preservation is an emerging technology that limits possible IRI sustained during allograft preservation. At the same time, no guidelines exist on how to assess viability of high-risk organs that might otherwise be rejected for transplantation.

Creating a microcirculation for perfusion

For organs that are procured from organ donors, creating microcirculations is unnecessary, since the blood vessels are already present. However, when it comes to organs that are engineered ex vivo, one major challenge is the ability to induce and control vascularization for perfusion control. Although vascularization occurs in many of these engineered constructs, the newly formed blood vessels are not big enough to circulate materials of large volumes. Highly metabolic organs such as the heart, lung, and liver require capillaries to reach within 100–200 μm of each cell within the growing tissue to provide sufficient gas, nutrient, and metabolite exchange.[17] In order to increase the viability of tissue-engineered grafts, the ability to stimulate extensive vascularization to avoid necrosis is required.[18] Formidable structure, functionality, and stability of these vascular networks result in sufficient tissue function.

A clear understanding of vascularization is needed when considering strategies for vascularizing engineered tissues. Vascularization can occur by vasculogenesis, angiogenesis, or arteriogenesis. Vasculogenesis is the process by which endothelial progenitor cells (EPCs) proliferate and coalesce into a primitive network of vessels known as the primary capillary plexus. This network of vessels is remodeled by sprouting and branching of new vessels from preexisting ones via angiogenesis. Arteriogenesis is the process by which arteries and arterioles are remodeled into larger vessels. Figure 2 presents how the processes of vasculogenesis and angiogenesis help create a mature vascular network.

Angiogenesis relies on the coordination of many different activities in several cell types, including endothelial cells (ECs), pericytes, fibroblasts, and immune mediators.[19] The cytokines and growth factors expressed by these cells affect EC migration, proliferation, tube formation, and vessel stabilization. Pro-angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), and angiopoietin 1

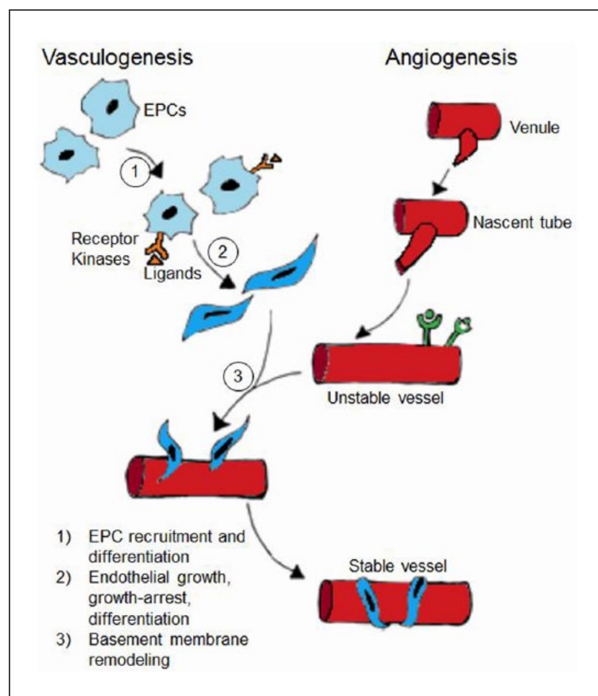

Figure 2. Vasculogenesis vs angiogenesis. Vasculogenesis is the major mechanism of vessel formation during embryogenesis and development. New blood vessels are created from the differentiation of mesodermal endothelial progenitor cells (EPCs) into endothelial cells and the de novo formation of a primitive vascular network. Additional blood vessels are created from preexisting vessels by either forming new branches or remodeling the existing vessels via angiogenesis. Vascular endothelial growth factor (VEGF) is a major contributor to both vasculogenesis and angiogenesis and is aided by many other pro-angiogenic factors, including fibroblast growth factor (FGF) and angiopoietin 1 (ang-1). Angiogenic stimuli that promote the formation of new blood vessels include exercise, ischemia, and infarction followed by regeneration.

are all currently used in vascular growth. Although therapeutic vascularization using growth factors and gene delivery have proven effective, they have a few problems, which include depending on the host for vessel ingrowth and determining the exact number of molecules necessary and delivering them with the right release pharmacokinetics. In addition, hypoxia-inducible factor 1α (HIF-1α), sonic hedgehog (SHH), and bone morphogenetic protein (BMP), delivered growth factors, might actually harm the engineered tissue construct.[20]

Control mechanisms for growth factors are still under development to prevent hypervascularization of tissues. When tissues are subjected to hypoxia, HIF-1α induces the expression of VEGF and its receptor. The binding of VEGF to its receptor activates relay proteins that signal EC nuclei to prompt genes to make products needed for new EC growth.[21] Matrix metalloproteinases (MMPs) produced by ECs break down the extracellular matrix (ECM) and permit the migration of ECs. With the help of integrins α and β, the ECs organize into hollow tubes and evolve into a mature network of blood vessels.[22,23] The maturation and development of newly formed vasculature have been found to be governed by angiotensin 1, angiotensin 2, and their receptor Tie-2.[24]

Ex vivo experiments revealed that erythrocytes act as oxygen sensors that autonomously regulate their own deformability and thereby flow through capillaries in response to physiological decreases in oxygen tension.[25] Current strategies for determining the viability of newly constructed blood vessels depend on the use of these erythrocytic properties.

Currently, there are three foundational strategies for vascularizing engineered ex vivo tissues, which include prevascularizing the constructs prior to implantation, inducing vascularization upon implantation, and using reconstructive microsurgery to enhance vascularization.

Prevascularized constructs—in vitro vascularization

Producing a vascular network within a tissue-engineered construct prior to implantation is one strategy currently under development. It has gained interest due to its versatility and its utility for integration into various tissue-engineered constructs. The functionality of the vasculature of the engineered constructs is to provide nutrient balance for the entirety of the organ when ex vivo and connected to a bioreactor. The goal is for the vascular network to anastomose with existing vessels in the host, thus accelerating vascularization and improving viability.[26] Once implanted within the host, various growth factors and cytokines along with microenvironment modulation by the ECM would promote graft survival.[27]

The basic principle of inducing vascularization prior to implantation is to provide the right culture conditions and microenvironment in vitro to induce ECs to form stable and functional vascular networks.[20] Specifically, the endothelium has been shown to play an active role in cell–cell communications that support islet survival and engraftment.[26] EC spheroids produce capillary like sprouts, especially in the presence of angiogenic factors such as VEGF and bFGF.[28] It has also been shown that fibroblasts modulate the EC network formation, forming and maintaining the microvasculature associated with neosynthesis of ECM.

ECM components, such as fibronectin, collagen, and Matrigel, have all been used widely as materials for inducing in vitro vascularization due to angiogenic properties.[19] In fact, the first report of in vitro prevascularization was reported using a triculture of human keratinocytes, human dermal fibroblasts, and human umbilical vein endothelial cells (HUVECs) in a collagen hydrogel scaffold to reconstruct prevascularized skin.[20] The fibroblasts secreted a large amount of human ECM, while the

keratinocytes secreted VEGF, spontaneously forming capillary networks.

Human pluripotent stem cells can be differentiated into vascular progenitor cells using hydrogels. Marrow-derived stem cells can also form functional blood vessels. When cocultured, EPCs and MSCs in a Matrigel plug were able to produce robust, functional networks.[20] There is a growing body of evidence showing the role of mesenchymal prevascular cells in supporting stable and long-lasting functional vasculatures.[20]

Dermal fibroblasts, skeletal myoblasts, adipose-derived MSCs, and bone marrow–derived MSCs can induce by coculture an EC network structure.[29] MSCs, in particular, can differentiate into vascular ECs, demonstrating their involvement in promoting angiogenesis within engineered 3D tissue.[30] More evidence of MSC involvement in angiogenesis was seen when MSC-containing 3D tissues displayed greater regeneration than those without MSCs by inducing macrophage infiltration.[31] When macrophage infiltration due to inflammation occurs, the in vitro EC network structure changes and in vivo angiogenesis occurs.[32] While coculture and growth factor–guided techniques have shown their ability to vascularize tissue-engineered constructs, challenges still remain in controlling the precision by which these networks form and their overall architectural specification, thus inhibiting the ability to generate spatially controllable vascular networks.[33] In addition, a random network does not offer clear locations for surgical anastomosis, which could delay vascular network perfusion.[34]

In order to precisely engineer vasculature to form desired geometrical features, one technique is to surround a non-sacrificial material around a sacrificial component, followed by removal of the sacrificial component.[33] Perfusable, vascular tubes that mimicked native vessels by displaying endothelial barrier function were formed by surrounding a 120-μm diameter stainless steel needle with collagen I. Once removing the needle, the collagen microchannel was seeded with primary endothelial and perivascular cells, which led to the formation of a precisely formed vessel.[35] Although this technique addresses the concern of precision, the residual materials left behind by the sacrificial component that is either removed or even dissolved can be cytotoxic or harmful to the recipient.[33]

Recent technology that was pioneered by Vacanti and Borenstein combines microfabrication and biodegradable microfluidics into a polymer-based biomicroelectromechanical system (BioMEMS). Traditionally, etching and lithographic techniques are used to produce a desired polydimethylsiloxane (PDMS) cast with micron-sized precision. Subsequently, stacking single-layer microfluidic networks, considering oxygen limitations, can produce complex vascular microchannels as well. More recent technologies involve direct-write assembly, laser-guided direct writing, and soft lithography, which can be applied to various hydrogels, including alginate, collagen, and fibrin.

Non-inflammatory, non-immunogenic high-strength silk fibroin, the same biomaterial that is used in surgical sutures, was found to be a suitable biomaterial for the construction of a biodegradable microvascular scaffold, based on its unique mechanical properties, biodegradability, and biocompatibility. The silk microfluidic devices supported suitable flow rates and formed leak-proof, patent microchannel networks. The silk surface was found to support the formation of a confluent endothelial layer. The microfluidics-based construction of microvascular networks from lithographically fabricated master molds was found to enable the formation of an integrated microvasculature for tissue engineering applications.[36]

While there has been success in producing microfluidic microvessels, most of the studies have only sustained single-cell suspensions in hydrogels. It remains unclear how these microvessels fare in vivo. In addition, perfusing microfluidic microvessels along with blood and integrating them into 3D-engineered tissues have yet to be demonstrated.[2]

Polyethylene terephthalate (PET), also known as Dacron, expanded polytetrafluoroethylene (ePTFE), and polyurethane have been used to successfully make vascular prostheses for arterial grafts with a diameter larger than 8 mm.[37] However, due to surface thrombogenicity and intimal hyperplasia, vascular grafts with a diameter less than 6 mm in carotid and common femoral arteries have not been successful.[37] Surface thrombogenicity impairs vessel patency and occurs due to the lack of EC coverage of the inner surface of the vessel.[38] To reduce thrombogenicity, anticoagulants and chemicals, such as hirudin, dipyridamole, tissue factor pathway inhibitor, and non-thrombogenic phospholipid polymers, have been used.[39] Venous cuffs between an artery and the synthetic graft have been used to partially alleviate the problems caused by intimal hyperplasia.[40]

Tissue-engineered blood vessels (TEBVs) are another successful engineered vascular conduit in which cell sheets that promote collagen synthesis are wrapped around porous cylindrical mandrel so that all cells have access to nutrients during maturation ex vivo.[38] To construct the adventitial layer of the vessel, a fibroblast cell sheet layer is wrapped around the tunica media made of smooth muscle cells. After weeks of maturation, the support mandrel is removed and ECs are seeded on the liminal surface of the vessel. While the medial fiber orientation of TEBVs are different from that found in native arteries, the TEBVs exhibit abundant ECM in the medial layer, characteristic of arteries.[41] TEBVs between 400 and 800 μm were created more recently and exhibited vasoreactivity, mechanical strength, and stability with a burst pressure above 1777 mm Hg and contractile and ECM protein expression of collagen IV and laminin when subjected to laminar flow perfusion.[42]

Overall, these approaches result in the production of precise vascular networks with control over oxygen and nutrient transport in tissue-engineered constructs. The scaffold material provides the physical template for cells to organize into a functional vasculature.[36] Mechanical forces and cues can be designed within the microfluidic network to ensure healthy organ-specific physiology.[36] Once these networks are formed, the 3D scaffold then anastomoses to the ingrowing host vasculature, thus supplying the graft with nutrients and oxygen. Since the host does not have to expend much energy in vascularizing the graft, the process is reduced from weeks to days.

"Organs-on-chips"—microfluidic devices. According to Bhatia and Ingber, organs-on-chips, which are microfluidic devices for culturing living cells in continuously perfused, micrometer-sized chambers, offer the possibility of creating predictive models of human physiology and disease. The goal is to synthesize minimal functional units, such as hepatocytes cultured in vitro in a perfused microfluidic chamber, in order to mimic tissue- and organ-level functions.[43] This allows for intricate modeling of physiological function of tissues to occur at a cellular level. Physical forces, such as physiologically relevant levels of fluid shear stress, cyclic strain, mechanical compression, and recruitment of circulating immune cells, allow for analysis of these systems in likely microenvironmental situations and investigation of basic mechanisms of organ physiology and disease. Results from these models can then be applied to larger scale systems and contribute to tissue engineering. On the other hand, there are limitations for many of these 3D models. Variability in size and shape of organoids makes functional, biochemical, and genetic analyses of entrapped cells extremely difficult.

The fabrication of the "chip" originates from the manufacturing process of microchips, which uses a modified form of photolithographic etching.[43] Soft lithography is utilized in making microfluidic culture systems, providing control over many system parameters that are not easily controlled in 3D static cultures or bioreactors. PDMS and glass are common materials used for the fabrication of microfluidic channels, making the chips compatible with live-cell microscopy and high-throughput screening methodologies.[44] While stereolithography uses a liquid resin material that is readily removed from the channels post-printing, channel resolution is limited with this method.[45] On the other hand, extrusion printing offers high resolution but requires the use of support materials that must be removed post-printing. The challenge of producing biocompatible 3D-printed microfluidic platforms more suitable for implantation is currently addressed using nontoxic printable materials that facilitate cell attachment on the printed surface.[45,46]

Since these systems are finely manufactured, microsensors can be installed within the system to study real-time microenvironmental effects on tissue barrier integrity, cell migration, and fluid pressure.[47–49] While chips have been fabricated for study of the liver, kidney, intestine, lung, heart, smooth and striated muscle, fat, bone, marrow, cornea, skin, blood vessels, nerve, and blood–brain barrier, many of these devices cannot be considered models of organs since only one cell type was cultured in one microchannel. Interactions between many different types of cells are crucial for maintaining tissue structure and function and, ultimately, modeling the multiorgan interactions that occur in vivo. Integrating 3D culture system ingredients, such as the ECM and synthetic polymer gels, into the microfluidic channels provides a way to incorporate greater complexity of the tissue microenvironment on chips. Microfluidic circuits may soon be 3D printed and seeded with cells or bioprinted with cell lining to mimic human vasculature and move toward "body-on-a-chip" constructs.[45]

In terms of applying the chip model to vascularization, biodegradable chips composed entirely of ECM that contained internal networks of microchannels filled with sacrificial material were created to model angiogenesis and microvascular function. The ECM was dissolved before plating cells either inside the channels (ECs) or in the surrounding perfused ECM (tumor cells, fibroblasts, etc.).[50,51] Interestingly enough, the newly formed microvessels became perfused only once they functionally integrated into the existing vascular network, shining light on how high-resolution analysis can help determine how spatial diffusive gradients influence angiogenic sprouting and inhibition.[50,51] Recently, multiplexed arrays of nearly identical ECM gels containing well-formed microvascular networks were fabricated on a single chip, which may potentially uncover the effects of multiple perturbations on angiogenic responses.[52]

Another approach to prevascularized constructs is to prevascularize the site of the transplant instead of prevascularizing the graft itself prior to implantation. The ability to confirm adequate vascularization of a transplant site can be used to determine whether it is optimal for use. In diabetic rats, a PET mesh bag, containing gelatin microspheres loaded with FGF-2, was used to induce angiogenesis at the intermuscular space. Islets mixed in 5% agarose were transplanted into the same site as the PET mesh bag after confirming new vasculature formation. Vascularization was further observed around the cells within 10 days posttransplantation, and normoglycemia was achieved and maintained.[53]

Inducing vascularization upon implantation—in vivo vascularization

Another strategy to develop vasculature is to stimulate and guide the body to accelerate the invasion and organization of vessels upon implantation via the release of growth

factors. The stimuli are provided by the polymer scaffold itself. Through scaffold design and engineering, issues of oxygen gradients and flow regimes, as well as cell alignment and angiogenesis, may be controlled.[28] The engineered tissue construct is usually implanted in a region that is enriched with blood vessels, such as the omentum, where VEGF and bFGF levels are high.[20] A key difference between this strategy and prevascularizing constructs before implantation is that upon implantation, inducing vascularization requires a longer perfusion time in vivo and a shorter construction time in vitro.

To maintain suitable oxygen tensions and nutrient diffusion throughout the graft, tissue engineers have utilized channeled scaffolds, which incorporate phosphate-based glass fibers into collagen scaffolds or using a laser cutting system to create holes into the scaffold. The phosphate-based glass fibers are used to help standardize the size and distribution of the channels. Once the fibers have degraded, the microchannels are left with the potential for improved flow and cell viability.[28]

Vascularization, in general, is an intricate temporal and special orchestration of many growth factors. FGF-1, FGF-2, VEGF, and PDGF are a few growth factors that have demonstrated the ability to enhance vascularization in both clinical and basic research.[54] Methods in which angiogenic proteins are used to treat ischemic tissues primarily use large bolus injections and are found to be ineffective. In addition, since proteins have short half-lives, these methods must depend on high levels of injected proteins, which may lead to abnormal vasculature and severe side effects.[55]

Better timing and delivery mechanisms are needed in order to successfully control vascularization. As for timing, several studies have indicated that a time frame may exist for which these growth factors are deemed optimal.[56] Typically, neovessels are observed 7 days after administration of angiogenic growth factors.[56,57] Administering PDGF-BB can disrupt EC–mural cell interactions but only when administered to immature vessels.[58] Also, when removing VEGF prior to the formation of a mural cell coat, it was found that the EC detached from the vessel wall and that the vessels regressed, indicating that the timing of both growth factor addition and removal may be critical for successful vascularization.[59]

Growth factor dose is also important for successfully stimulating proper vascularization responses. When VEGF was administered at high dosages, it was found that the vessels formed had disordered structure and hyperpermeability, showing that the key determinant of normal VEGF-induced angiogenesis is the microenvironmental amount of growth factor secreted, rather than overall dose. Long-term continuous delivery of VEGF can lead to normal angiogenesis without other exogenous growth factors when maintained below a threshold microenvironmental level.[60]

Using one growth factor alone may not be sufficient enough to generate a mature, stable microvascular network in a large engineered tissue, even though single proteins have been shown to enhance vascularization. Dual-protein strategies have been investigated in further improving the vascularizing response. It was found that supplementation with angiopoietin 1 (ang-1) after delivery of VEGF can inhibit vessel regression and maintain increases in vascular growth.[61] Larger vessels and greater mural cell interactions resulted from rapid release of PDGF-BB after administration of VEGF, clearly indicating the importance of controlled delivery of multiple growth factors in vascularization.[62] At the same time, the means of delivery still need improvement, as it is not clear how many growth factors are needed and in what combinations they should be used.

Cell-based strategies have also been used in accelerating vessel assembly. Stem cells have been especially attractive because of their regenerative potential and their ability to differentiate into many cell types. Microvessels were found to have formed on Matrigel scaffolds in nude mice when human embryonic bodies differentiated into endothelial-like and smooth muscle–like vascular progenitor cells after exposure to PDGF and VEGF.[63] These vessels also appeared to anastomose efficiently with host vasculature.

When compared to injecting genes for ANG-1 and VEGF, transplanting stem cells may be more effective at promoting angiogenesis.[64] Human bone marrow–derived mesenchymal stem cell (hMSC) transplantation in cardiac muscle was found to have superior effects in cardiac function, angiogenesis, myogenesis, and myocardial performance than angiogenic growth factor gene injections. To improve upon stem cell use in inducing vascularization, stem cells that were transfected with growth factor genes for VEGF[65] or FGF-2[15] demonstrated improved vascularization over control groups that were not transfected with growth factor genes. While the stem cells help proliferate vascularization, the growth factors help provide targeted local delivery of angiogenic proteins. Long-term studies are still needed to examine the clinical efficacy and safety of gene modification and the effects of cells actively remaining and secreting factors after desired vessel formation.

Researchers have also used traditional approached to perfuse 3D tissues, including transplanting the engineered organs into highly vascular sites, such as the kidney capsule.[30] At the same time, since these organs need to be retransplanted into the correct location for it to provide functionality to the recipient, arteriovenous (AV) loops have been used to make flaps for promotion of vascularized 3D-engineered tissues.[66] By having AV loops, the 3D tissue can be retransplanted via vascular anastomosis into another site for further maturation. This approach allows capillary sprouting to occur, either before or concurrently with implanted cell differentiation and tissue growth.[67]

Surgical approaches

Techniques in the surgical sciences can be used to improve vascularization on implantation for treatment of large volume defects of organ systems. Reconstructive microsurgical prefabrication approaches have been exploited to induce and enhance vascularization.[68,69] Scaffolds are implanted in a donor tissue location that would promote greater vascularization than the defect location. After a period of prefabrication time, the vascularized engineered tissue can then be transferred to the defect site. An optimal incubation period of 9 weeks was observed to have resulted in scaffold vascularization consistent with the implantation site.[69]

Highly vascularized donor locations can be used for initial implantation of tissue engineering constructs in order to guide fabrication of large volumes of vascularized tissues with complex 3D shape.[69] Prefabricated and prelaminated flaps have been widely and successfully used in clinical cases to immensely ease the transfer of vascularized tissue from the prefabrication location to the defective recipient location.[70] Flap prefabrication is the implantation of a separated vascular pedicle in a new territory, followed by a transfer of the flap vascularized by this pedicle. Vascular induction by implantation of a pedicle allowed designing the flaps in discrete donor sites or in areas of skin excess.[71] Establishing new flaps with any desired anatomical component is possible in regions that do not have defined axial vessels.

Prefabrication allows almost any tissue volume to be transferred to any specified recipient site, thus expanding the options of microsurgical reconstructions.[72,73] At the same time, it may be difficult to clinically apply this pedicle model. However, large volumes of vascularized tissues can be created by implanting materials around microsurgically created vessel loops.[74]

In order for these constructs to remain functional and survive in the host, all of the cells within the construct require sufficient oxygen and nutrients as well as the removal of waste products. Simple diffusion only allows cells that are no more than 100 and 200 µm from the nearest capillary to survive.[28] A challenge that remains is inducing enough vascularization so that gas, nutrient, and waste exchange can occur in the inner cells of large tissue-engineered patches. Therefore, it is suggested that large constructs are prevascularized prior to implantation.

Overall, the use of preexisting surgical approaches has received little to no attention in applications for enhancing vascularization in tissue engineering. Combining this strategy with growth factors or other cell strategies, surgical science may help enhance the volumes of scaffolds vascularized.[15]

Vascularizing kidney grafts

The supply of transplantable kidneys has been unable to keep up with increasing numbers of people approaching and living with end-stage renal disease, creating an urgent need for alternative sources of new organs.[75] While adult kidneys are currently too large and anatomically complex for current tissue engineering methods to construct, immature kidneys could be engineered and transplanted into hosts so that maturation can occur. Major problems that tissue engineering faces are vascularizing kidney grafts and connecting the nephrons of kidney grafts to flowing blood.

Although the metanephrogenic mesenchyme of kidney rudiments contains endogenous endothelial progenitors, these progenitors do not differentiate well in normal culture, as they form large masses of ECs rather than organized vessels.[76] However, if an immature kidney graft is cultured with an external source of vessels capable of angiogenesis, it is capable of attracting exogenous blood vessel branches and gaining a blood supply.[77,78] Regardless of the underlying mechanisms, the ability of kidney rudiments to acquire vascular systems when placed near host vessels offers the possibility of equipping cell-derived reaggregate kidneys with a functional blood supply.[75]

In a study performed by Xinaris, renal organoids constructed from single-cell suspensions were implanted below the kidney capsule of a living rat host. Upon implantation, the kidney tissue further matured, forming vascularized glomeruli with fully differentiated capillary walls, including the slit diaphragm and appearance of erythropoietin-producing cells. The implant exhibited tubular reabsorption of macromolecules and gained access to the tubular lumen on glomerular filtration. Long term, the goal for tissue engineering will be to replace renal function with a constructed kidney graft.[79]

Another alternative treatment option includes recellularizing decellularized native kidney scaffolds with autologous cells.[80] The decellularization process begins with the removal of biological materials using a combination of detergents, which include Triton X-100 and sodium dodecyl sulfate (SDS) or SDS combined with peracetic acid.[81] The tissue is washed throughout the process with Hanks' balanced salt solution (HBSS). Between washes, a DNAse step is required to further remove any remaining DNA debris.[80]

To decontaminate the matrix, the tissue is thoroughly washed with saline solution, either phosphate-buffered or sodium chloride.[82] The overall decontamination of kidney scaffolds presents many challenges. The method chosen should not interfere with the cell adhesion and growth occurring within the scaffold as well as not change the structure of the ECM.[83] Immersing the scaffold in acid solutions and perfusing it in a combination of antibiotics or different types of irradiation are currently the main strategies used for sterilization.[84–86]

The goal of recellularization is to resupply the kidney graft with all of the major cell types that make up the nephron. These cell types include mesangial cells, podocytes,

juxtaglomerular cells, parietal cells, medullary tubular cells, columnar epithelial cells, principal cells, and intercalated cells.[80] Recellularization of the native kidney scaffold involves use of a combination of cells, which include both parenchymal and non-parencymal cells. ECs are necessary to replenish the vascular architecture of the scaffold.[82] Of the cell types and sources available, induced pluripotent stem cells (iPSCs) and primary human renal cells are considered to be the most promising and clinically relevant based on their differentiation and expansion potentials.[80,87] Creating fully functional renal constructs depends on having optimal recellularization parameters, such as cell seeding numbers, methods, and bioreactor culture.

Once implanted, the long-term challenge of engineered kidney constructs is vascular potency.[87] Thrombosis will likely occur in the absence of complete endothelial reseeding of vascular matrices, thus rendering the construct nonfunctional.[88] Antithrombogenic strategies such as efficient and functional reendothelialization of acellular kidney scaffolds have been explored to address this critical issue. In a study performed at the Wake Forest Institute for Regenerative Medicine, an EC seeding method was used to permit effective coating of the vascular matrix of a decellularized porcine kidney scaffold using a combination of static and ramping perfusion cell seeding.[89] CD31 antibody was conjugated to the vascular lumen to enhance the efficiency of reendothelialization of vasculatures within decellularized porcine kidney scaffolds.[89] While this method had led to the longest duration of patency shown by a recellularized kidney scaffold, more studies are required in order to establish methods for isolation and expansion of autologous ECs.[87]

Vascularizing liver grafts

For tens of thousands of patients who suffer from end-stage liver disease, liver transplantation is the only curative treatment option. Another potential solution that end-stage liver patients can have in the future lies in the field of tissue engineering. As discussed above, vascularizing high-level constructs remains a challenge for many researchers. Despite many reports describing functional cell differentiation, no studies have succeeded in generating a 3D-vascularized liver.[90]

However, a study performed by Takebe showed the generation of vascularized and functional human liver from human iPSCs by transplantation of liver buds created in vitro (iPSC-LBs).[90] Immature endothermal cells that were destined to become hepatic cells organized themselves into 3D liver buds. Through immunohistochemistry and gene expression analyses, a resemblance between in vitro–grown iPSC-LBs and in vivo liver buds was revealed. Upon iPSC-LB transplantation into the tissue, functional vasculatures were formed and stimulated the maturation of the iPSC-LBs into adult liver resembling tissue that performed protein production and human-specific drug metabolism without recipient liver replacement.[91] Further research efforts must ensue to translate these techniques into viable treatments for patients.

Another strategy to vascularize liver tissue constructs is to subcutaneously implant-engineered hepatocyte cell sheets. In a study performed at the Nagasaki University Graduate School of Biomedical Sciences, vascularized subcutaneous human liver tissues (VSLTs) were produced in vivo by transplantation of engineered hepatocyte/fibroblast sheets (EHFSs) without the addition of stem cells or ECs. The EHFSs showed superior expression levels of the vascularization-associated growth factors (VEGF), transforming growth factor (TGF) beta 1, and HGF in vitro and showed significantly higher liver-specific protein synthesis rates in vivo than tissues from hepatocyte-only sheets. While this strategy is safe and minimally invasive, the functionality and efficiency of these engineered hepatocytes require further improvement and investigation.[92]

A layer-by-layer cell (LbL) coating technique investigated by the Osaka University Graduate School of Medicine was used to construct a 3D-vascularized functional human liver tissue.[93] The LbL cell coating is based on the interactions between the ECM proteins, fibronectin and gelatin, with the cell surface. Fibronectin can bind to integrin a5b1, which is expressed on most cell surfaces, and also to gelatin. When the LbL coating is performed repeatedly, multiple layers of fibronectin and gelatin are formed on the cell surface. These ECM nanofilms, therefore, serve as molecular adhesive that promote the binding of adjacent cells to each other and form a 3D dense vascular network. Using this approach, the experimenters of this study coated both human primary hepatocytes and normal human dermal fibroblasts with fibronectin and gelatin using the filter-based LbL procedure. The resulting liver tissue demonstrated higher albumin production and cytochrome P450 activity in vitro. Upon subcutaneous implantation into immunodeficient mice, the vascularized liver tissue showed greater albumin production in the early stage than non-vascularized tissue or a hepatocyte suspension.[93]

Vascularizing lung grafts

Tissue engineering efforts in developing lungs have only begun gaining momentum despite a significant unmet need of lung transplants. The end goal of lung grafts is constructing a surface area for gas exchange. Alveoli in lungs are responsible for exchanging oxygen inspired from the air with the carbon dioxide carried by blood. One of the tissue engineering strategies utilizes a prevascularized construct, consisting of a thin, porous membrane that separates the vascular network from an adjacent alveolar chamber containing continuous oxygen flow. At the same time,

these constructs are too thick to achieve the required lung surface area to support the respiratory needs of an individual. Currently, these prevascularized lungs are being developed as external temporary lung replacement devices for preterm infants.[94]

Microfluidic platforms are finding increasing applications in respiratory studies. Biomimetic microsystems have been reported to reproduce the alveolar–capillary interface of the human lung as an alternative to animal and clinical studies for drug screening and toxicology applications.[95] Since this finding, two approaches using microfluidic devices to study the differentiation of lung stem and progenitor cells in the view of future lung tissue engineering applications have emerged.[96,97] The first strategy obtains alveoli-like structures after seeding isolated mouse pulmonary stem and progenitor cells in a compatible gelatin/microbubble scaffold using a two-channel fluid jacket microfluidic device.[96] The second strategy isolated mouse lung multipotent stem cells for further characterization upon development of a microfluidic magnetic-activated cell sorting system.[97]

Similar to the aforementioned decellularization and recellularization technique explored for vascularizing kidney grafts, bioengineered lungs have also been explored using this strategy. However, the resulting engineered lungs have shown a limited and temporary function, mainly due to blood clotting and pulmonary edema.[98] To address the need for functional vascularized lung scaffolds, an airway-specific approach to remove the pulmonary epithelium was developed while preserving lung vasculature, ECM, and other supporting cell types.[98] The epithelial cells were removed via a mild detergent solution that was delivered intratracheally to an isolated single lung. The resulting de-epithelialized vascularized lung grafts were shown to support the attachment and growth of human adult pulmonary cells and stem cell–derived lung-specified epithelial cells.

At the same time, given the complexity and specialized nature of the lung epithelium to support gas exchange at the level of alveoli, many challenges still remain for engineering whole lungs. One challenge includes determining the most effective means to introduce the stimuli facilitated by the right heart.[99] Bioreactors have been developed to mimic the biological, mechanical, and chemical environment of the lung, all while providing means for perfusion and ventilation.[88,100] An optimally designed bioreactor that can induce mechanical, chemical, and biological stimuli will theoretically and ultimately yield a functional graft from a recellularized decellularized scaffold.[99]

Vascularizing heart grafts

The heart itself is an engineering marvel by nature, an organ of extreme structural and functional complexity that is vital for survival.[101] Since the heart is densely packed with myocytes, fibroblasts, vasculature, and collagen-based ECM, the myocardium consumes large amounts of oxygen. In addition, in order to pump blood forward, myocytes, the contractile cells of the heart, form syncytium that propagate electrical signals across intracellular junctions to produce mechanical contractions. This is why, when the heart is subjected to damage and disease, many patients have poor prognoses. Heart disease and stroke are the first and third leading causes of death in the United States, respectively, accounting for 40% of all deaths.[101]

Prevascularized cardiac constructs were able to induce CM maturation into elongated and multinucleated myotubes, which contained gap junctions and organized anisotropically.[102] In general, once cardiac grafts are implanted, the graft must not only be fully anastomosed and perfused but it also must synchronize with the electrical syncytium of the existing myocardium. When engineering a complex and highly vascularized organ, such as the heart, it is crucial that vascularization is linked with tissue differentiation.

To address the issue of low oxygen concentrations and cell viabilities, an elastomeric scaffold was developed with a parallel array of channels. Mimicking the capillary network, the array allowed for flow of culture medium throughout the construct. In addition, perfluorocarbons were added in the medium as oxygen carriers. Significantly improved cardiac tissues were produced as a result based on total cell number, expression of cardiac differentiation, and excitation thresholds.[103]

There still remains debate regarding how much vascularization needs to be provided prior to implanting cardiac grafts. The options include implanting the graft channels without any vascularization, lining the graft channels with vascular cells, or lining the graft channels with primitive channels formed by vascular cells. In addition, it is still unclear which aspects of vascularization are needed early on: coculture vascular and cardiac cells that is important for paracrine signaling, structural design that favors infiltration of host cells and connections with vascular supply, or immobilization of angiogenic growth factors into scaffolds.[101]

Large contractile cardiac tissue grafts constructed in vitro were found to survive after implantation and support contractile function of infarcted hearts. The engineered heart tissue was constructed from neonatal rat heart cells. Upon forming thick myocardial layers when implanted on myocardial infarcts in immune-suppressed rats, the engineered heart tissue showed undelayed electrical coupling to the native myocardium. There was also no evidence of arrhythmia induction. Furthermore, the tissue improved fractional area shorting of infarcted hearts, prevented further dilation, and induced systolic wall thickening of infarcted myocardial segments when compared to the control (nonoperational and noncontractile constructs).[104]

Much progress has been made toward the goal of vascularizing cardiac grafts before implantation.[105] Neonatal rat cardiac cells that were cast in Matrigel in silicone chambers around AV loops of rat epigastric arteries formed well-vascularized cardiac tissue with a defined arterial and venous pedicle. When connected to blood vessels of the neck, the pedicle perfused the tissue fixed to the heart.[106]

Expanding upon this approach in an in vitro setting, an isolated piece of perfused rat grown tissue was integrated into the chamber. A medium perfusion system was connected to the artery and vein of the tissue. After implanting the cardiac cell sheets onto a vascularized surface, blood vessels grew on the sheets and allowed repeated implantations and generation of thicker, fully vascularized grafts. These grafts survived after connection to the circulation in the neck region of rats.[107]

Spontaneous vascularization was found to have occurred in 3D cardiac constructs made from primary cardiac myocytes. The myocytes developed a primitive vascular network with[108] and without[109] the addition of ECs. Although the cellular networks formed lumina, it remains unclear whether the structures only serve a paracrine function. Many of these approaches still lead to only primitive blood vessel structures and low perfusion rates. At the same time, connecting clinically relevant thick cardiac grafts to the coronary circulation seems to be apparent.

Generating cardiac patches based on reendothelialized decellularized scaffolds has been also been studied. ECs were cultured in the vasculature of a biological collagen-based vascularized scaffold. Fibroblasts, MSCs, and iPSC-derived cardiomyocytes were then seeded on the scaffold. Physiological cardiac functions, expression of cardiac-specific markers, physiological beating rates, and responsiveness to drug treatment and electrical stimulation were observed after 14 days.[110]

Another approach to produce a 3D functional vascularized cardiac muscle construct includes the use of human-induced pluripotent stem cell–derived embryonic cardiac myocytes (hiPSC-ECMs) and human mesenchymal stem cells (hMSCs).[111] Human cardiac microvascular endothelial cells (hCMVECs) and hMSCs were cocultured onto a 3D collagen cell carrier (CCC) under vasculogenic culture conditions. These cells then formed extensive plexuses of vascular networks after undergoing maturation, differentiation, and morphogenesis characteristics of microvessels. After prevascularizing the CCCs, hiPSC-ECMs and hMSCs were cocultured onto the CCCs under myogenic culture conditions. Vascular and cardiac phenotypic inductions were analyzed, which revealed neo-angiogenesis and neo-cardiomyogenesis.[111]

An example of "heart-on-a-chip" was given using poly(*N*-isopropylacrylamide) (PIPAAm) and PDMS to engineer an anisotropic rat ventricular tissue and to measure contractility, action potential propagation, epinephrine dose response, and cytoskeletal architecture in a mid- to high-throughput system allowing real-time data collection.[112] If combined with cells harvested from patients, these models could be used as tools for drug screening in individualized medicine. The same major concern as with most ex vivo construct concepts for cardiac grafts applies for microfluidic systems as well. Cardiomyocytes have a peculiar growth attitude, requiring special conditions to adhere and survive while preserving their unique contractile phenotype. Thus, the challenge of finding the most appropriate and representative source of contractile cells still remains.[44]

Vascularizing skin grafts

Skin grafts are often used as dermal substitutes for application in full-thickness wounds. Vascularizing these grafts is essential in order for successful outcomes. However, current tissue-engineered skin constructs are used only in preclinical studies due to challenges in promoting vascularization. Generally, autologous approaches are more preferable in clinical situations that allowed for prolonged reconstruction while allogenic therapy is needed in acute cases. In either case, when harvesting cells, they should be available in large amounts and should be safe from forming tumors and transmitting animal diseases.[113]

Comparisons between in vivo and in vitro skin constructs have been examined very closely. The prevascular network in a skin construct could anastomose to the host vasculature within 4 days, while a nonprevascularized construct takes 14 days to anastomose.[19] At the same time, in vitro prevascularization raises the problem of unequal cell distribution in the skin substitute, leading to incomplete vascular networks.[113] Low-pressure centrifugation has been described to help equally distribute cells over matrices.[114,115] Alternatively, in vivo assembly of vascular networks within skin substitutes that have been preseeded in vitro with endothelial (precursor) cells has been shown to have organized into vascular tubes with the help of endogenous ECs upon implantation. However, stable and functional vascular networks depend on recruitment of pericytes and vascular smooth muscle cells around endothelial tubes.[113]

Angiogenesis and vasculogenesis are two mechanisms by which vascularization can occur in skin tissue–engineered constructs. Ex vivo–expanded cells can either secrete angiogenic factors that communicate with endogenous vascular cells to stimulate angiogenesis or directly incorporate into nascent vessels via vasculogenesis.[113] It is unknown whether one mechanism is more favorable over the other mechanism. However, it can be assumed that a combination of the two mechanisms can create a more robust and functional vascular network.

Currently, there are many different types of stem and progenitor cells that are used in methods for vascularizing skin substitutes. When using precursor ECs, autologous cells that are host derived are preferred over allogeneic

cells. Although autologous cells are only available in small numbers and require long ex vivo expansion times to obtain therapeutic amounts, they are not subject to immunologic rejection since they are host derived.[116,117]

Other cell types used for vascularization in skin tissue engineering include human umbilical vein ECs, early EPCs, MSCs, multipotent adult progenitor cells, embryonic stem cells, and iPSCs. Most of these cell types are both vasculogenic and angiogenic in nature. However, human umbilical vein ECs were found to only be vasculogenic, while early EPCs were found to only be angiogenic.[113]

In addition, studies for iPSCs are still in early phases for skin tissue engineering. Thus, iPSC angiogenic and vasculogenic properties are unknown. However, stem cells in general are preferred over other cell types for large wounds due to the immense number of cells produced after expansion.[118] Relatively speaking, even though human dermal microvascular ECs, human umbilical vein ECs, and early EPCs are found to be genetically stable and have vasculogenic properties,[113] they do not produce nearly as many cells as stem cells after expansion, a property that proves crucial for large wounds. In addition, iPSCs are preferred over embryonic stem cells, since iPSCs are host derived. Still, further research needs to be conducted in order to study the efficacy, genetic stability, and long functional effects of such vascularization methods. Continuous hypervascularization will not be necessary in most cases and will thus need to be subsided in order for skin grafts to return to normal vascular profiles.[113] It also remains unclear how to control potential teratogenic properties of stem cells when they are implanted into a host.

Recellularizing decellularized grafts has also been explored when engineering skin equivalents. A decellularized segment of porcine jejunum and a tailored bioreactor system were used to create a biological vascularized scaffold. The scaffold was then seeded with fibroblasts, microvascular ECs, and keratinocytes. Hematoxylin and eosin and immunohistological staining after 14 days found that the scaffold had histological architecture representative of human dermis and epidermis. Also, the scaffold could be perfused with a physiological volume flow.[119]

Challenges and opportunities—recommendations for future research

Creating functional vasculatures remains one of the most fundamental challenges to be overcome before large tissue-engineered constructs can be used in clinical applications. While the strategies discussed above have unique advantages, at present, it is unclear which method will work best to improve in vivo vascularization, and the long-term effects of such strategies are not well-documented.

There remains a significant need for techniques that produce more efficient vascular network architectures, such that the vessels provide adequate perfusion to the engineered construct. The blood flow in the constructs must closely mimic physiological blood flow, so as to when the construct is transplanted into the host and is subjected to mechanical and chemical stress, its functionality still remains. In addition, constructs that are prevascularized prior to implantation should have the controlled ability to remodel their vasculature so that they can be perfused more effectively when implanted in vivo.

Delivering angiogenic factors has proven to be the simplest, most effective method at restoring blood flow in ischemic and other disease models. However, this strategy heavily relies on vessel ingrowth from the host vasculature—a time-consuming process which may not be suitable when rapid vascularization is needed to support and integrate the tissue-engineered constructs.[19] In addition, delivery of growth factors for in vivo vascularization still needs to be improved. It is not clear how many growth factors need to be delivered or in what combination. Polymer systems that deliver multiple proteins with different release kinetics present a significant optimization challenge.[15] Also, scaffolds that anastomose with the host vasculature should be designed to degrade at a rate that is compatible with the time required to achieve mechanical integrity while enabling microvascular stabilization and patency.[36]

An opportunity for ex vivo tissue-engineered constructs that still remains is the ability to effectively use computers and 3D printers to help generate stable, extensive, microvascular networks in large volumes of tissue with fine controlled internal and external architecture, particularly vascular channel elements of different sizes and shapes.[120,121] 3D printing technology that uses computed tomography, magnetic resonance imaging, and optical microscopy can better control porosity, mechanical strength, vascularization, spatial positioning, and morphology of designed scaffolds.[120,121] Thus, a more tight control over the vascular tree, vessel dimensions, and interconnectivity can be established when prevascularizing grafts.[122] In addition, most fabrication approaches employ 3D printing for either microfluidic device fabrication or printing 3D tissues. Future approaches may involve performing these processes in parallel, making organ-on-a-chip platforms more accessible and cost-effective.[45]

Further research in controlling vascularization will be paramount in providing adequate perfusion for engineered constructs when transplanted into the host. Subjecting the vasculature to various mechanical and biochemical cues that occur in the body will also be important in observing how the vasculature responds to physiological stress. While the vessels will need to mature and form new branches to successfully anastomose to the host, it is crucial that these events come to a halt once attached to the host vasculature. In addition, long efficacy and safety trials will need to be conducted once tissue-engineered constructs are in vivo.

Currently, most engineering approaches in tissue engineering are still in the experimental phase and only have been performed in small animal models. Researchers must continue creating new and innovative methods that will successfully control vascularization in ex vivo organ systems. Ultimately, the design goals should allow for quantitative assessment, where minimally sized units could be derived from oxygen transport data and modeling, and functional anastomosis, where the graft successfully fuses with host vasculature and is perfused by blood. Significant advances have been made but a lot more opportunities and challenges still remain before tissue engineering can effectively solve the donor organ shortage problem in the world.

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