7 Microfluidic Systems for Engineering Vascularized Tissue Constructs

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Abstract:

A major challenge in tissue engineering is the lack of proper vascularization of the fabricated tissue constructs. Microscale technologies, especially microfluidic systems, provide new opportunities to overcome the challenge of developing artificial micro-vasculatures. In this chapter, the application of microfluidic systems for generating vascularized tissue constructs by using microfluidic platforms has been outlined. Mathematical modeling has been shown as a powerful tool for optimizing the microfluidic system design parameters. Future challenges for the microfluidic-based tissue engineering constructs have also been discussed for success in clinical application.

7.1 Introduction

Organ failure is one of the most serious problems faced by the healthcare industry in developed nations. Each year in the United States alone, millions of people suffer from end-stage organ failure and tissue loss, resulting in more than $400 billion in health care costs [1]. Only 10% of these patients benefit from organ transplantation, while the majority of patients perish due to the severe shortage of available organ donors [2]. To address this problem, the field of tissue engineering has emerged with the aim of generating tissues that restore, maintain, or enhance tissue function [1]. Tissue engineering is an interdisciplinary research field, which leverages both biological understanding and engineering approaches. Commonly-adopted tissue engineering approaches incorporate (i) isolated cells or cell substitutes, (ii) bio-compatible materials for cellular support and regeneration, or (iii) cell-biomaterial (i.e. scaffold) composites [3]. Transplantable cells are derived either as autografts (from the patient), allografts (from a human donor) or xenografts (from a different species). Isolated cells are then cultured on biocompatible scaffolds, which provide physical and chemical support and guide the cell growth and organization into three dimensional (3D) tissues with mimicry of living tissues in vivo (inside the body).

Despite the enormous advances in tissue engineering, which have resulted in clinically viable products such as skin and cartilage, several challenges still prevent the widespread clinical application of tissue engineering products. These challenges include a number of business, regulatory and ethical issues as well as scientific barriers. These scientific issues include (1) how to acquire adequate source of cells, (2) how to engineer complex vascularized tissues that mimic the complexity of native tissue architecture and (3) how to generate tissues ex vivo (outside the body) with the biomechanical and metabolic functions that mimic normal tissues. Among all these scientific issues, one major challenge of engineering tissues in vitro (in tissue culture) is lack of a proper vascularization [4]. Development of an artificial microvasculature is critical to move tissue engineered organs into the clinics to benefit patients with end-stage organ failure. Oxygen and other nutrients can only diffuse through a short distance

before being consumed (a few hundred micrometers at most). Without an intrinsic capillary network, the maximal thickness of engineered tissue is approximately 150-200 μm because of oxygen diffusion limitations [5]. To date, the most successful tissue engineering applications in clinics are skin and cartilage, which have relatively low requirements for nutrients and oxygen and rely on vascularization from the host to provide permanent engraftment and mass transfer of oxygen and nutrients [6]. However, such techniques encounter difficulties when applied to thick, complex tissues, particularly those comprising the large vital organs such as liver, kidney, and heart [7].

Living tissues are ensembles of different cell types embedded in complex and well-defined geometries and within an extracellular matrix (ECM) that is unique to each tissue type. The generation of tissues requires tools to control the biological, chemical and mechanical environment experienced by cells in culture. Conventional techniques for scaffold fabrication, such as solvent casting, particulate leaching, gas forming and fiber bonding cannot be used to fabricate scaffolds with controllable pore geometry, size, and interconnectivity, that can be used to creat a vasculature [8]. Several microscale technologies, especially microfluidic systems, provide new hope to overcome this challenge to build tissues with vascularized structures in a reproducible manner. Microscale technologies can achieve a resolution of 0.1 μm, which is two orders of magnitude smaller than the dimensions of the capillaries, and span of five orders of magnitude ranging from overall dimensions of tens of centimeters down to cellular dimensions of 5 μm [7]. In this chapter, we discuss the applications of microfluidic systems in tissue engineering to generate vascularized tissue constructs.

The use of microfluidics for generating tissue constructs has been progressing through multiple stages of complexity over the past few years. The initial designs for the fabrication of microfluidic vascular patterns were performed in two-dimensional (2D) systems and more recently have evolved to more biomimetic systems. Here we will provide a view of this field starting with 2D systems of microchannels with non-tissue engineering materials and progressing to

more advanced systems with hydrogels and additional levels of control.

7.2 Generating 2D vascularized tissue constructs using microfluidic systems

Most of the initial work to generate vascularized tissue constructs utilized microscale technologies (microfabrication or micromolding) to fabricate 2D vascularized patterns (normally starts from a single channel that branches out multiple times into thinner and thinner channels) on various biomaterials (silicon, glass, or polymer). Drs. Jeffrey Borenstein and Joseph P. Vacanti were among the pioneers to build vascular tissue constructs in this manner. In their early work, photolithography was utilized to generate 2D bifurcating patterns on silicon and Pyrex, reminiscent of the branched architecture of vascular and capillary networks (Fig. 7.1A) [9]. Endothelial cells and hepatocytes (primary liver cells) were cultured and subsequently lifted from these 2D patterns as single-cell monolayers, which were subsequently shown to maintain proliferation and functionality. Single endothelial cells were aligned to form a branched network (Fig. 7.1B). In their subsequent work, soft lithographic techniques were applied to mold polydimethylsiloxane (PDMS) on silicon wafer with the impression of bifurcating vascular networks [4]. These patterned layers of PDMS were irreversibly bonded to flat PDMS layers to create enclosed network channels that were seeded with endothelial cells. To generate highly uniform flow patterns, which mimic both large-scale physiologic properties (total flow rate, i.e. ~100 ml/min in femoral artery) and small-scale phenomena (fluid velocity in the capillaries, i.e.~1 ml/min in skeletal muscle) [10], three network designs were fabricated to approximate the fluid dynamics (Fig. 7.1C). The first one provided a stepwise scaling from arteries to capillaries, but the flow resistance was much higher than physiological goals, limiting the flow under practical pressure drops to sub-optimal values. The second design provided high capillary cross section, and substantially increased the flow rate at realistic value of input pressure, but the flow was non-uniform. The third design demonstrated both uniform flow as well as reduced resistance

of the network. These scaffolds have been successfully seeded with endothelial cells in PDMS channels with dimensions on the order of capillary diameters (Fig. 7.1D)

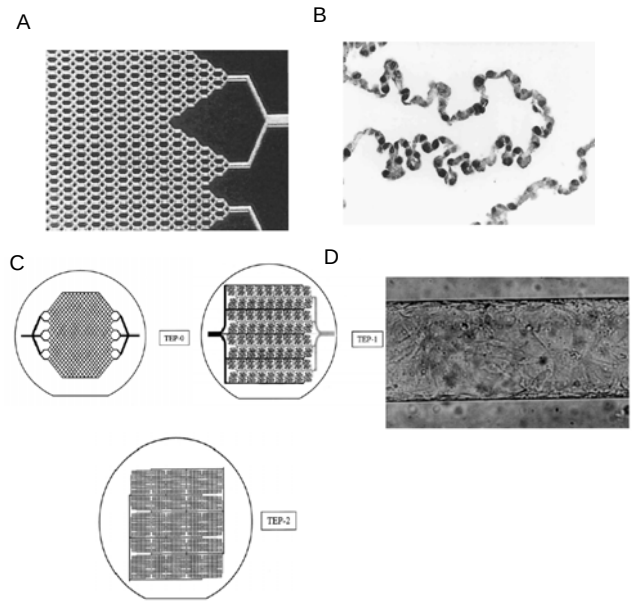

Fig. 7.1 Non-biodegradable microfluidic systems to generate 2D vascularized tissue constructs: (A) Optical image of a capillary network etched into silicon wafer (reproduced with permission from [9]); (B) H&E staining for detached monolayers of endothelial cells from the silicon capillary network (reproduced with permission from [9]); (C) Three vascularized network designs. (TEP-0: 35 x20, TEP-1: 30x40, TEP-2: 25x35, Dimensions in μm) (reproduced with permission from [4]); (D) Image of confluent endothelial cells in a PDMS microfluidic vascularized structure (width of the channel: 200 μm) (reproduced with permission from [4]).

One limiting factor of microfluidic scaffolds in above two studies has been the choice of material. Microfabricated silicon and PDMS, although ubiquitous and inexpensive, are not biodegradable and have limited biocompatibility, and are therefore not suitable biomaterials for implantation. To address this limitation, a new approach for cell and tissue engineering, known as biodegradable microfluidics has been explored to fabricate potential implantable microfluidic tissue constructs [8]. King et al. have produced highly branched microfluidic scaffolds from poly (L-lactic-co-glycolic acid) (PLGA) (Fig. 7.2A) [8]. Biodegradable PLGA was heated to melt on PDMS molds and then pressed with constant force between two parallel metal plates to create the vascularized patterns. The microvascularized PLGA membrane was laminated by thermal fusion bonding to construct the microfluidic systems. Although this approach of building a biodegradable microfluidic scaffold is rapid, versatile and low-cost, the potential limitations of PLGA-based biodegradable microfluidic scaffold are the cytotoxic by-products and inflammatory immune response generated during implantation [11]. Furthermore, PLGA scaffolds are brittle, hard and lack the desired mechanical elasticity of many native tissues. To address these limitations, Wang et al synthesized a novel biodegradable polymer known as poly(glycerol sebacate) (PGS) or bio-rubber [12], a tough, elastomer that is biocompatible, inexpensive, and easy to synthesize. The sebacic acid-containing polymers, which have already been approved for use in medical applications, showed reduced inflammatory response relative to PLGA [13]. Unlike PLGA, bio-rubber dissolves through a surface erosion process that is far more linear in terms of mechanical strength degradation over time, and therefore provides the resulting scaffolds more consistent mechanical integrity before degradation [12]. PGS was used to fabricate microcapillary networks by using patterned silicon micromolds as templates to fabricate PGS molds [6] (Fig. 7.2B). A patterned PGS film was then bonded with a flat film to create capillary networks that were perfused with a syringe pump at a physiological flow rate. The devices were endothelialized under flow conditions, and part of the lumens reached confluence within 14 days of culture (Fig. 7.2C). This approach may lead to tissue-engineered microvasculature that is critical in organ engineering.

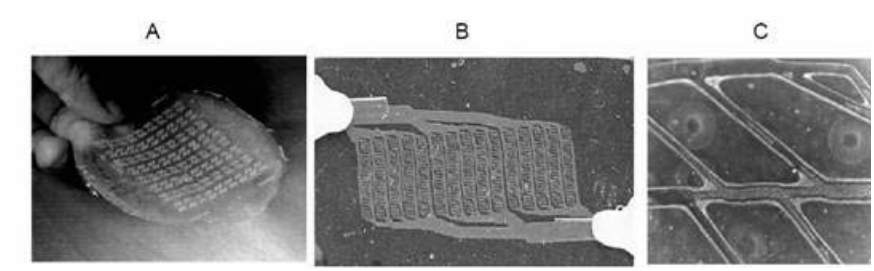

Fig. 7.2 Biodegradable microfluidics to generate 2D vascularized tissue constructs: (A) A PLGA scaffold with 2D vascularized patterns made by micromolding (reproduced with permission from [8]); (B) A PGS scaffold with 2D vascularized patterns made by micromolding and (C) An expanded view of the endothelialized capillary network in PGS: Endothelial cells reached confluence at various portions of the PGS capillaries within 14 days (reproduced with permission from [6]).

Besides PLGA and PGS, other biodegradable polymeric materials have been used to build vascularized microfluidic scaffolds. Sarka et al. developed and characterized porous micropatterned polycaprolactone (PCL) scaffolds as a functional small diameter blood vessel analog, using a novel technique that integrates soft lithography, melt molding and particulate leaching of PLGA micro/nanoparticles [14]. PCL was chosen because of its ease of processing (melting temperature 58-63^{o}C), biocompatibility and the ability to manipulate its mechanical and degradation properties by the formation of PCL copolymers. Vascular smooth muscle cells have been aligned on these porous micropatterned PCL scaffolds. Poly(lactide-co-glycolide) (PLG) has also been used to build artificial capillary networks [15]. When coated with fibronectin, endothelial cells grew to near confluence within the enclosed networks. Recently, Bettinger et al. built microfluidic devices made of the silk fibroin protein, which was from the Bombyx mori silkworm and approved by the FDA for medical applications [16]. Hepatocytes grown in the silk fibroin-based microfluidic devices exhibited similar morphology and cell functions to their counterparts cultured on other biodegradable scaffolds such as PGS.

7.3 Generating 3D vascularized tissue constructs using microfluidic systems

In vivo, cells reside within 3D environments in close proximity to blood vessels that supply tissues with nutrients and oxygen and remove waste products and carbon dioxide. In vitro, numerous studies have identified critical features that allow 3D cultures to replicate physiology better than 2D cultures [17-20]. Thus, building 3D vascularized microfluidic scaffolds is of vital importance for the success of tissue engineering applications. Although vascularized microfluidic systems are readily constructed in 2D by photolithographic or soft-lithographic techniques, their construction in 3D remains a challenging problem. So far, the most commonly used approach is to stack and assemble 2D vascularized polymer films (usually made by micromolding techniques) into large 3D devices suitable for transplantation. King et al. developed a scalable fabrication platform for constructing highly branched, multiplayer PLGA microfluidic networks that mimicked tissue microvasculature, for large-scale tissue engineering [8]. In this approach, two or more micro-patterned PLGA films could be bonded by a pure thermal bonding process to form a monolithic, 3D and biodegradable microfluidic device (Fig. 7.3A). Bettinger et al. developed fully 3D microfabricated vascularized constructs by stacking layers of biodegradable PGS patterned films [21]. The 3D microfabricated vascular construct has the unique property of exhibiting constant maximum shear stress within each channel of the device, making it a promising construct for an artificial vascular tissue engineering scaffold. Multi-layered microfludic scaffolds have also been used for liver tissue engineering. Cheung et al. have constructed a 3D scaffold for hepatocyte culture based on a sandwich structure that places each hepatocyte compartment adjacent to a microvascular channel network in a bilayer structure [22]. Between the two chambers sits a nanoporous membrane that separates the cellular components but allows for the free exchange of oxygen, nutrients, and waste products between the compartments (Fig. 7.3B). Furthermore, Leclerc and Fujji have constructed a 3D microfluidic scaffold composed of two stacked layers of PDMS for liver cell (HepG2 cells) culture [23]. The HepG2 cells could be kept

in good condition for nearly 10 days with the completely closed perfusion system.

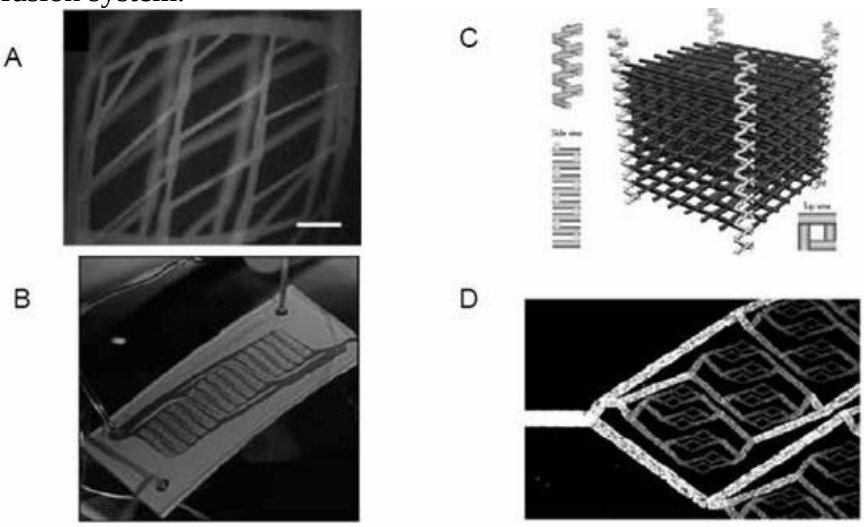

Fig. 7.3 Microfluidics systems to generate 3D vascularized tissue constructs : (A) A multilayer PLGA microfluidic networks perfused with fluorescein dye (reproduced with permission from [8]); (B) A 3D scaffold for liver tissue engineering based on a sandwich structure that places each hepatocyte compartment adjacent to a microvascular channel network in a bilayer structure. (reproduced with permission from 22]); (C) 3D microvascular scaffold with square-spiral tower patterning fabricated by a direct-write assembly (reproduced with permission from [24]); (D) Eight level multi-width and multi-level microvasculature network microchannels fabricated by one-step laser direct writing. Fluorescent image shows the difference in intensity levels corresponding to different channel depths (reproduced with permission from [25]).

Building 3D vascular microfluidic structures by stacking 2D layers is a cumbersome process requiring multiple fabrication and masking steps that is difficult to scale-up. Therriault et al. demonstrated the direct fabrication of 3D microvascular networks through direct-write assembly of an organic ink [24]. Their approach was based on a 3D microvascular network of cylindrical microchannels, which could be directly assembled by robotic deposition, and then patterned to yield vertically oriented, square-spiral towers within the device (Fig. 7.3C). Sixteen-layer scaffolds were produced by robotic deposition of a paraffin-based organic ink in a layer-wise building sequence. These 3D microvascular networks will provide an enabling platform for a wide array of fluidic-based applications. In addition, Lim et al

demonstrated a faster and more flexible alternative method to fabricate multiple-level microfluidic channels using a maskless laser direct micromachining [25]. Due to the inherent 2D nature of photolithography, microfluidic channels fabricated by photolithography exhibited uniform depth, which generates non-physiological flow conditions and high flow resistances. By using a maskless laser direct micromachining, a multi-width and multi-depth microchannel was fabricated to generate biomimetic vasculatures whose channel diameters changed according to Murray's law (the cube of the radius of a parent vessel equals the sum of the cubes of the radii of the daughters [26]) (Fig. 7.3D). Different depths were fabricated simply by varying the average power from the laser used to micromachine the channels. The multi-depth channels, which obey Murray's law across multiple branching generations, mimic physiological flow patterns with lower overall flow resistances and more gradual changes in the flow velocities across different generations of branching compared to channels of uniform depth.

7.4 Hydrogel-based microfluidic systems for generating vascularized tissue constructs

Tissue engineering scaffolds made from hydrogels have aroused a great deal of interests in recent years [27]. Hydrogels are networks of hydrophilic polymers exhibiting a number of potential advantages compare to other materials such as PDMS, PGS, and PLGA, since their physical properties (i.e. mechanical strength and biodegradability) and biological properties (i.e. the biocompatibility and resemblance to the natural ECM) can be tailored to mimic tissues. Commonly-used hydrogels include natural hydrogels (i.e. collagen, hyaluronic acid, and alginate), synthetic hydrogels (i.e. poly(ethylene glycol)-diacrylate (PEGDA), and poly(vinyl alcohol) (PVA) [16]), and hybrid natural-synthetic composites [28]. Photocrosslinkable hydrogels have been used for the encapsulation of various cells [29-31], which were utilized as building scaffolds for tissue engineering [32-34]. The merger of microengineered hydrogels and microfabrication techniques for microfluidic transport

has been shown of significant potential to generate 3D tissue constructs.

Stroock et al. introduced a hydrogel microfluidic system within calcium alginate hydrogel[35]. They demonstrated that a high level of mass transfer could be achieved within the hydrogel microfluidic system by arraying the channels in appropriate dimensions. These results demonstrated the feasibility of using an embedded microfluidic system to control concentrations of soluble species within the 3D volume defined by a hydrogel. Recently, the same group demonstrated direct fabrication of a functional microfluidic structure within a 3D calcium alginate hydrogel scaffold for tissue engineering applications [36]. These microfluidic channels enabled an efficient exchange of solutes within the interior of the hydrogel scaffold and a quantitative control of the soluble environment experienced by the cells in their 3D environment. More than one independent vascularized network was incorporated within the microfluidic scaffolds. Each network could serve as an independent source for solutes or as a sink for others, such that concentration gradients could be maintained at steady state for both non-reactive and reactive solutes (Fig. 7.4A). This approach is promising for directing cells in the scaffolds with spatial and temporal control and growing thick sections of tissue without necrosis.

Ling et al. built cell-laden microfluidic channels from hydrogels by directly encapsulating cells within the microfluidic channels [31]. Using standard soft lithographic techniques, molten agarose was molded against a SU-8 patterned silicon wafer to build microfluidic channels. Channels of different dimensions were generated and it was shown that agarose was a suitable material for performing microfluidics. Cells embedded within the microfluidic molds were well distributed and media pumped through the channels allowed the exchange of nutrients and waste products. While most cells were found to be viable upon initial device fabrication, only those cells near the microfluidic channels remained viable after 3 days, demonstrating the importance of a perfused network of microchannels for delivering nutrients and oxygen to maintain cell viability in large hydrogels. Cell-laden microfluidic hydrogels could also be scaled up

by stacking the biomimetic vascular patterns to generate multi-layer vascularization in multiple discrete planes.

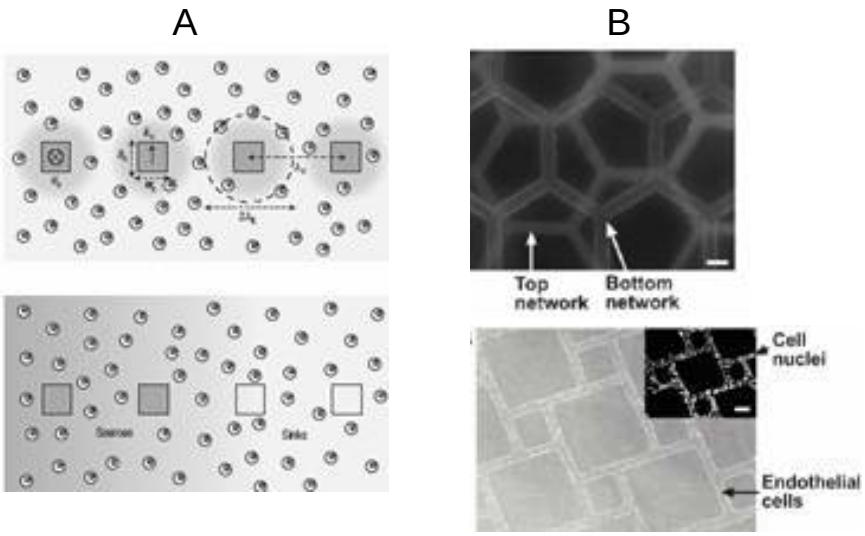

Fig. 7.4 Hydrogel-based microfluidics systems for generating vascularized tissue constructs: (A) Cross-sectional views of cell-seeded microfluidic scaffolds made by calcium alginate hydrogel. Dispersed cells are shown as double circles, microchannels are shown as squares. The shading represents steady-state distributions of solutes: *Top*, reactive solute is delivered via the channels and is consumed by cells as it diffuses into the matrix; *Bottom*, non-reactive solute is delivered via the two channels on the left and extracted by the channels on the right (reproduced with permission from [36]); (B) Microfluidic systems made by collagen gel: *Top*, collagen gels with multi-planar network; *Bottom*, collagen gel with a monolayer of endothelial cells lining along the internal channels. Inset, Hoechst-stained microvascular network (reproduced with permission from [37]).

Golden et al. introduced a general procedure for the formation of microfluidic gels, with an emphasis on gels of native ECM proteins, such as type I collagen and fibrin [37]. In this approach, micromolded meshes of gelatin were used as sacrificial materials, which were encapsulated in a second hydrogel (collagen or fibrin). The gelatin meshes were subsequently removed by heating and flushing, leaving behind interconnected channels in the second hydrogel. The channels were as narrow as 6 μm, and faithfully replicated the features in the original gelatin mesh. 50μm wide microfluidic networks

in collagen and fibrin readily enabled delivery of macromolecules and particles into the channels and transport of macromolecules from channels into the bulk of the gels. By co-encapsulation and melting of two gelatin meshes, microfluidic gels containing two independent networks have been fabricated (Fig. 7.4B top). Microfluidic gels were also suitable as scaffolds for cell culture, which were used to culture human microvascular endothelial cells to form rudimentary endothelial networks for potential tissue engineering applications (Fig. 7.4B bottom).

Growth factors have been used in combination with biomaterials to enhance the formation of microvascular networks. Richardson et al. built microvascular structures by introducing multiple growth factors (platelet derived growth factor, PDGF and Vascular endothelial growth factor, VEGF) into a PLGA scaffold in a stepwise fashion [38]. Both the spatial and temporal aspects of growth factor introduction were critical and must be orchestrated effectively in order to produce sustainable vessels. In another study, human ES (embryonic stem) cells cultured on PLLA/PLGA scaffold, when exposed to insulin-like growth factor, were able to differentiate and organize into a capillary-like endothelial network [39]. It is envisioned that the growth factor–based approaches can be incorporated within the existing microfluidic-based tissue constructs to improve vascularization.

7.5 Mathematical modeling to optimize the microfluidic systems for generating vascularized tissue constructs

Establishing mathematical models of the microfluidic systems usually precedes the fabrication process. Mathematical modeling can provide powerful quantitative tools for theoretical guidance of the microfluidic design and prediction of communications between the microfluidic and biological systems in the vascularized tissue constructs, such as transport and metabolic phenomena. The earliest mathematical modeling of the vascular system could be traced back to 1926, when Murray discovered and mathematically described the

relationship governing the optimum ratio between the diameters of the parent and daughter branches in vascular systems [26]. This relationship is known as Murray's law and states that the cube of the diameter of the parent vessel must equal the sum of the cubes of the daughter vessels. For symmetric bifurcations, an important consequence of this geometric rule is that the tangential shear stress at the wall remains constant throughout the vascular network. Based on Murray's law, multi-width and multi-depth 3D microchannels have been fabricated by a direct laser writing to generate biomimetic vasculatures as described in the previous section [25]. Emerson et al. generalized Murray's law to apply to the design of constant-depth microfluidic channels and manifolds found in lab-on-a-chip systems [40]. A comprehensive series of computational fluid dynamics simulations considering branching networks composed of square, rectangular, and trapezoidal cross-sections were performed. These biomimetic design principles can be applied to microfluidic devices fabricated using conventional batch processing techniques without difficult multi-exposure and alignment steps. Vascular geometry of the human pulmonary arterial and venous trees has also been modeled by Huang et al. [41]. The diameter-defined Strahler ordering model was used to assign branching orders, the connectivity matrix was used to describe the connection of blood vessels from one order to another, and a distinction between vessel segments and vessel elements was used to express the series parallel feature of the pulmonary vessels.

Weinberg and Kaazempur-Mofrad et al. used computational fluid dynamic approaches to develop models for microcirculation [22, 42-43]. The microfluidic network was modeled as a resistor network and solved iteratively as the resistance of each vessel was altered in response to its internal pressure. The deformation of the vessel in response to an internal pressure and the resistance of the deformed vessel were calculated by numerically solving the plane-strain and Navier-Stokes equations respectively. Models for predicting hematocrit distribution within an engineered vascular network were also established [22]; similar calculations were made for pressure drop, fluid velocity, and wall shear stress within a large family of designs for tissue engineered microcirculation. Three-dimensional microflu-

idic constructs with uniform wall shear stress throughout the network were designed based on mathematical modeling, which could achieve more uniform endothelial cell seeding, more confluent cell coverage on the wall, and better control over cell behavior for in vitro and in vivo studies [44].

Mathematical models have been used to predict the transport and metabolic phenomenon of the microfluidic scaffolds. In the work of Choi et al., diffusivities of small and large biomolecules from the microfluidic channels into the porous hydrogel scaffold have been modelled [36]. Their results indicated that the mass transfer within the microfluidic hydrogel scaffold was diffusive but not convective. They also did computational analysis for the diffusion reaction of reactive solute (calcein-AM) and the diffusion of non-reactive solute within the bulk of microfluidic scaffolds.

7.6 Future challenges:

With the rapid development of tissue engineering and microscale technologies, tremendous opportunities have been created for building vascularized tissue engineering constructs using microfluidic systems. It will continue to be one of the most important and challenging directions in the tissue engineering field. Numerous obstacles need to be overcome to build microfluidic systems for clinical applications in the future. These include: (1) how to achieve full endothelialization of the microfluidic systems with different geometries and materials; (2) how to precisely fabricate vascularized microfluidic systems with small vascularized structures that mimic the capillaries in a scalable manner; (3) how to enrich the complexity of the microfluidic tissue constructs by involving extracellular matrices, multiple cells types and controlling cell-matrix and cell-cell interactions; and finally (4) how to implant the vascularized tissue engineering constructs built ex vivo into the human body to replace lost tissue function without clogging of the blood vessels and activating the immune response.

7.7 Conclusions:

The merger of tissue engineering and microscale technologies opens new opportunities to build vascularized tissue engineering constructs. In this chapter, various applications of microfluidic systems in tissue engineering have been demonstrated to generate vascularized tissue constructs, which included 2D and 3D microfluidic systems (involving biodegradable and non-biodegradable polymers) and hydrogel-based microfluidic systems. Mathematical modeling has been shown as a powerful tool for optimizing the microfluidic system design. Future challenges for the microfluidic-based tissue engineering constructs have been also discussed for success in clinical applications.

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