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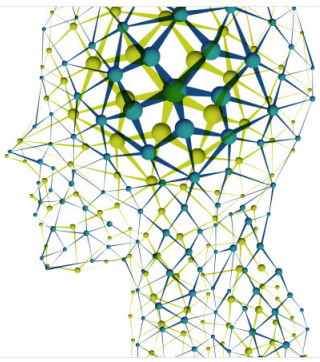

Preface to Special Topic: Microfluidics in cell biology and tissue engineering

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In this special issue of *Biomicrofluidics*, a wide variety of applications of microfluidics to tissue engineering and cell biology are presented. The articles illustrate the benefits of using microfluidics for controlling the cellular environment in a precise yet high rate manner using minimum reagents. The topic is very timely and takes a stab at portraying a glimpse of what is to come in this exciting and emerging field of research. © *2011 American Institute of Physics.*
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Microscale technologies have enabled a number of new methods of controlling the various aspects of the cellular microenvironment. In particular, advances in polymeric fabrication methodologies have led to the development of various microfluidics-based technologies with potential applications to tissue engineering and regenerative medicine. Over the past few years, numerous studies have explored the application of microfluidic systems for various biological applications.[1] For example, microfluidic systems have been used to regulate the surrounding soluble factor-cell interactions to study phenomena such as the presentation of gradients or controlled shear stress on cells.[2] In addition, microfluidic systems can be used to study the factors that regulate stem cell behavior for generating a renewable source of cells for various regenerative medicine applications.[3] Furthermore, it may be possible to use the ability to engineer microfabricated channels to recreate artificial blood vessel-like structures that can be used for generating vascularized tissues or disease models for drug screening.[4] In this issue, a series of papers is presented from a number of prominent groups that highlight the latest advances in this exciting area of research.

The papers in this special issue can be grouped into three parts. In the first part, three papers report the use of microbioreactors in regulating cell behavior. In the second part, a number of microfluidics-based tools for tissue engineering are presented. Finally, in the third part, five papers discuss the development of *in vitro* cellular microenvironments.

The engineering of three-dimensional (3D) tissue constructs is a complex process that involves biophysical and biochemical cues. An important aspect in creating such structures is to develop bioreactors that can be used to generate controlled conditions that can direct cells to form functional tissues. For cells to survive in large tissue constructs, a sufficient supply of oxygen and nutrients is required, which in the normal physiology is provided by the blood vessels. In an interesting study, Truslow and Tien[5] formulated a methodology to investigate the fraction of vascularization by using analytical expressions and confirmed their results with computational modeling. They mentioned that minimizing the fraction of vascular volume is beneficial and

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further noted that the device designers still need to consider maintaining oxygen concentrations above a set threshold, which is crucial for realizing healthy tissue constructs for the generation of spheroids, multilayered cells, and 3D structures. Accordingly, Sugiura *et al.*[6] used a finite element method to present a simulation study to evaluate oxygen and glucose supply to multilayered cells in a microwell array chip for perfusion culture as well as for static cell cultures. Their simulation results indicated that oxygen supply was a more crucial factor for cell culture than glucose supply for both cultures, and that the supply of oxygen through the oxygen permeable walls of the culture was more important than the supply of oxygen through the perfusion medium. Cell trapping in microfluidic platforms is one of the strategies used to generate 3D tissue constructs and, hence, it has been investigated by numerous researchers. Choudhury *et al.*[7] reviewed the physical and chemical methods for cell trapping to develop 3D cell cultures for tissue engineering. They specifically focused on various physical and chemical trapping methods for engineering 3D microtissue constructs in microfluidics systems that recapitulate the *in vivo* tissue microstructures and functions. Thus, it has become evident that advances in both the control in assembling large clusters of cells as well as the microscale assembly of cells are required to precisely engineer complex tissue structures.

The next part presents papers that discuss various tools for tissue engineering. The ability to pattern extracellular matrix proteins inside microfluidic chambers enables users to culture cells inside such cavities with ease. Despite the simplicity in fabricating conventional polymeric poly(dimethylsiloxane)-based chambers, it is difficult to selectively pattern within the chambers once a device is made. To address this problem, Hattori *et al.*[8] presented a method for protecting the patterned regions during the plasma treatment process before bonding. After patterning different molecules inside the chambers including fibronectin and collagen, they cultivated CHO-K1 cells to demonstrate their process. Spheroid culture is the preferable cell culture method to study certain cell types, including hepatocytes as this type of culture often preserves organ-specific functions. In their latest design, Fukuda and Nakazawa[9] reported a microwell array chip, which consists of 300-μm-diameter cylindrical wells that have 100-μm-diameter cell adhesive regions surrounded by nonadhesive regions. The chip allowed stable immobilization of hepatocyte spheroids in microwells, which can be used to evaluate drug metabolism with high efficiency. After seeding primary hepatocytes onto this chip, they demonstrated spontaneous formation of cell spheroids with uniform diameter on the cell adhesive regions. The miniaturized features of this chip helped reduce the number of cells that were required per experiment as well as the amount of reagents required for high-throughput screening assays for drug discovery and tissue engineering applications. Cell isolation and enrichment is often required for numerous applications in tissue engineering. However, the available techniques are limited in throughput and are not capable of viable off-chip collection. In an innovative approach, Hur *et al.*[10] utilized cell size as the sorting mechanism and presented a high-throughput strategy for cell isolation. Their approach employed a simple single-layer microfluidic device that used microscale vortices and isolated target cells from a heterogeneous suspension in a high-throughput manner (7.5 million cells/sec). The examination of the collected cells indicated that the proposed microfluidic process did not adversely affect cell viability and hence was a promising approach that could enable cell enrichment. In another study, Xu *et al.*[11] combined bioprinting technologies with the conventional hanging drop method to make embryoid bodies with uniform dimensions for directing the differentiation of embryonic stem cells. This simple approach could be further enhanced by encapsulation of cells inside biomaterials, which extends their lifetime and provides a natural environment to build higher order tissue structures. Lee *et al.*[12] used a coaxial flow microchip and created chitosan-alginate fibers using wet-spinning and then successfully encapsulated human hepatocellular carcinoma (HepG2) cells. They noted that chitosan-alginate fibers were better suited for cell encapsulation compared to pure alginate fibers and also HepG2 cells adhered better to chitosan-alginate fibers compared to alginate fibers. Their novel cell friendly fabrication technology has the potential to enable a broad range of applications in tissue engineering. Another important area is the ability to fabricate low-cost microfluidic devices that can be used as educational platforms. Nguyen *et al.*[13] described an innovative technology for rapid prototyping microfluidic chips. Their

approach required minimum expertise and was meant to cultivate hands-on research experiences from middle school to college students. Despite its simplicity, their comprehensive design cycle included chip design, fabrication, and testing where the end goal was to address a real world problem. The versatility of microfluidics technology enabled the students to gain experience in fundamental science as well as the engineering design cycle in an interdisciplinary environment.

In the third part, five papers describe *in vitro* microenvironments for regulating cell behavior. Microfluidics technology provides a toolset for generation of biochemical gradients to stimulate the microenvironment in a well controlled manner. Sip *et al.*[14] reported a user-friendly stacked-flow system to achieve highly stable gradients of biomolecules over large areas with low shear forces. Breast cancer cells were cultured in the device and there was no effect of shear flow on migration. Furthermore, the scalability of their approach was demonstrated by creating an array of combinatorial gradients. During the formation of blood vessels, endothelial cells experience a wide range of flows in a spatially and temporally dependent manner. Even though numerous studies have focused on high flow rates, there have been relatively few studies conducted to understand the influence of low shear stress levels to endothelial cells. Henceforth, Park *et al.*[15] first described the need for extremely low shear flows [shear stress levels of 10^{-4}–10^{-2} dyn/cm^2] and then presented a methodology to generate these small flows. After realizing an extremely slow flow regime inside a microfluidic channel, they cultured endothelial cells and demonstrated that even extremely low flows enhance proliferation and nitric oxide secretion rates, yet did not induce actin filament reorganization. In another study, using a microfluidic chip, Nakao *et al.*[16] fabricated hepatic cords, a microscopic structure in liver tissue. They showed that the aligned hepatocytes gradually self-organized and formed bile canaliculi along the hepatic cordlike structure similar to *in vivo* conditions. Their approach resulted in better mimicry of the liver structure with potential use in drug screening and toxicity studies. Microfluidic devices provide numerous attractive features for improved control at the microscale, yet specific challenges remain to be addressed, which include the control of gradients of oxygen and other soluble factors and the presence of hemodynamic shear forces in small and confined channels. For instance, the flow associated with nutrient delivery also effects cells such as mesenchymal stem cells (MSCs), where shear is known to regulate signaling, proliferation, and expression. Inamdar *et al.*[17] presented a microfluidic device configuration that enabled independent tuning of multiple parameters in cell culture. They presented a relationship between oxygen transport and shear in a "membrane bilayer" microfluidic device, in which soluble factors were delivered to a cell population by means of a flow through a proximate channel separated from the culture compartment by a membrane. They also presented an analytical model that described the characteristics of this device and its ability to independently modulate oxygen delivery and hemodynamic shear imparted to cultured cells. This bilayer design provided a more uniform oxygen concentration profile than was possible in a single-channel system and enabled independent tuning of oxygen transport and shear parameters to meet requirements for MSCs and other cells known to be sensitive to hemodynamic shear stresses. The ability to create gradients using microfluidics is also enabling niche applications, including the study of disease progression. In their article, Seidi *et al.*[18] presented a miniaturized microfluidics-based high-throughput cell toxicity chip to study Parkinson's disease. After culturing PC12 neural cells inside a microfluidic channel, they created concentration gradients of 6-hydroxydopamine (6-OHDA) to investigate neural apoptosis. Their findings indicated that at low concentrations of 6-OHDA, the neuronal death in the microchannels was mainly induced by apoptosis, while at higher concentrations of 6-OHDA, neuronal death was mainly through necrosis. Their technology has numerous applications, including the generation of *in vitro* disease models for drug discovery applications.

As illustrated by the articles in this issue, microfluidic technology has emerged as a useful tool with widespread use in biomedical engineering. The enabling nature of this technology may result in numerous future advances in diagnostics, therapeutics, and basic biology studies. We hope that the articles in this special issue provide a stimulating platform for discussions and further investigations in this emerging and exciting field.

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