Chapter 1
Microfluidic Applications in Vascular Bioengineering

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ABSTRACT
Microfluidics is the manipulation and control of fluids in small scale, and has heralded a new age in science as evidenced by the rapid increase in the amount and quality of academic and industrial research output in this area in the recent times. Microfluidics has shown tremendous promise in both fundamental and applied research in the field of vascular bioengineering. In this review, we outline the basic principles of microfluidic flow and fabrication techniques, and describe the recent advances in the applications of microfluidic devices in diagnostic and prognostic vascular bioengineering. The field is still in its infancy and has a great potential for research and development as it matures to deliver commercially viable products. This review, focusing on the current status of microfluidic applications to diagnose and treat blood-related disorders, should be a valuable and opportune addition to the literature of interest to both academia and industry.

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INTRODUCTION
Blood, the vascular tissue, is vital for nutrient transport, immune-surveillance, hemostasis, and wound healing, all of which are critical in order to maintain normal physiology (Lichtman, et al., 2006; Rosenberg & Aird, 1999). Consequently, the pathophysiology of a large number of diseases can be traced to abnormalities in blood flow and function. Hence, assays for composition and function of blood are crucial in both diagnosis and treatment of diseases (Barone & Feuerstein, 1999; Danesh, Collins, Appleby, & Peto, 1998; VanWijk, VanBavel, Sturk, & Nieuwland, 2003).

Blood is a complex tissue comprised of both cellular and acellular components. The cellular components are composed of erythrocytes, leukocytes, and platelets, and the acellular components

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are comprised of lipids, lipoproteins, carbohydrates, and numerous proteins of widely differing structural and functional attributes. Blood-based assays often require isolating and identifying certain specific cell population or molecular entity as a marker for the presence and activity of diseases (Packard & Libby, 2008). For instance, the presence of antibody against varicella indicates the immunization status against chicken pox (Gilden, Cohrs, Mahalingam, & Nagel, 2009); or the presence and number of CD45+ lymphocyte population is a measure of the severity of myeloid leukemia (Countouriotis, Moore, & Sakamoto, 2002). Hence, sample preparation process, which may involve separating one or more components of the blood, is the first and important step for blood assays. Upon extraction of the components of interest, the next step is functional assay or molecular analysis to obtain the requisite clinical or scientific information. This is achieved by identifying specific proteins on cell surface such as cluster of differentiation (CD) markers or identifying free proteins or lipids present in the plasma by using detection systems such as optical or fluorescence read-outs. Thus, the entire process usually takes at least a few hundred microliters to a few tens of milliliters of sample volume. Furthermore, such a process involves separation of cells from plasma, and isolation and retrieval of specific population of cells or proteins for detection and analysis (Gatti, et al., 2009; Kraemer & Maurer, 1998). Unfortunately, these assays suffer from a number of disadvantages: large sample volumes, long assay time period or low throughput, lack of sensitivity, and often a need for a centralized test station. These disadvantages adversely impact diagnosis, treatment and patient recovery thus increasing healthcare costs, morbidity and even mortality rates. Recent progress in multiplexed, high-throughput screening (HTS) methods, particularly, microfluidics has greatly alleviated these problems, thus making blood-based assays rapid, reliable, informative, and inexpensive (Bange, Halsall, & Heineman, 2005; D’Orazio, 2003; Whitesides, 2006). The greatest advantage of microfluidic technology is miniaturization, which substantially cuts down reagent use, analysis time and assay costs, and minimizes or eliminates labor intensive steps (D’Orazio, 2003). Over the last decade, microfluidic technology has been bolstered by advances on several fronts: transfer of know-how and skills on microfabrication from microelectronic industry, liquid handling and dispensing capability, process control and automation, development of novel materials, and development of better analytical tools for detection (Verpoorte & De Rooij, 2003). As a result, microfluidics is poised to revolutionize the field of wet-lab science in just the same way microelectronics revolutionized the semi-conductor industry about a generation ago. Literature reports indicate that the number of patents issued for microfluidic devices have increased from less than 20 in 1998 to more than 350 in 2004 (Haber, 2006).

Of interest, microfluidics is particularly suited for vascular bioengineering applications because the in vitro flow system can be designed to accurately mimic the in vivo blood flow patterns or hemodynamics, thus making the microfluidic assays physiologically relevant. Microfluidic systems offer a number of advantages including miniaturization, laminar flow characteristics, large surface area to volume ratio, and decrease in reaction time. Microfluidic applications in vascular bioengineering have seen a leap in past few years as evidenced by the rapid increase in the amount and quality research output in this area. A ‘web of science’ search on ‘microfluidics and blood’ show that on an average less than two articles were published every year till 2003, but has risen steadily ever since with nearly 45 articles in 2008 alone.

In this review, we will highlight some of the important advances in microfluidics applications in vascular bioengineering. First, we will discuss the principles that govern flow in microfluidics. Next, we will detail the various fabrication techniques
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