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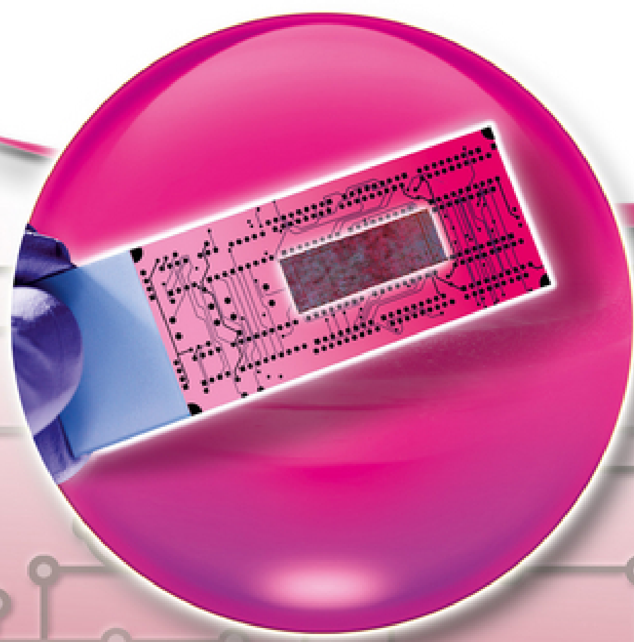

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Preface

Two of the fastest growing industries of the last 40 years have been microelectronics and biotechnology. Both have been propelled by advancing technologies for the manipulation of materials, whether semiconductors and metals, or DNA and cells. The ability to make smaller and smaller features on integrated circuits has powered the revolution in information technologies and personal computing. In parallel, advances in molecular biology and understanding of cellular metabolism have made it possible to create medicines designed to target specific pathways in a disease with increasing precision.

The coupling of these fields appears naturally synergistic in hindsight. The first years of small technologies for biology were marked with development – new methods for making structures, new ways to control flows of fluids, verification of the compatibilities of plastics and living cells, and so on. These small, often unrelated, technological advances and the accompanying proof-of-concept studies have now given way to a robust and active transitional period. Over the past 10 years, examples of micro- and nanotechnologies have begun to emerge that are capable of augmenting and enhancing our understanding of human diseases and facilitating the preclinical and clinical development of interventions to improve health. The present volume explores these two themes through a series of chapters that explain and highlight cutting-edge examples of where small systems are enabling new biology. The emphasis is not directly on the "making" of the technologies, but rather the emerging capabilities of the technologies to enhance biological discovery and drug development.

The first part of this volume focuses on microsystems for single-cell analysis. Comprising five chapters, this section considers the advancing capabilities for single-cell analysis enabled by microtechnologies. In some ways, small capillary systems and subnanoliter volumes are natural realms of biology – blood vessels are small conduits for cells and cells are comparably sized in volume. The ability to make defined, similarly sized structures to isolate individual cells, and to manipulate their environments, has been an essential objective of the earliest examples of microfluidic systems for cell biology. What has emerged in the last few years in biomedical research, however, has been an increasing realization that heterogeneities among populations of cells hold many insights that have been previously overlooked as a result of averaged measurements of populations.

For example, bulk measurements of the average composition of a population of cells could not resolve whether or not the population comprised two distinct types, or a single population with an average of traits. Single-cell resolution in measurements is required. Depending on the scarcity of some cells (e.g., circulating tumor cells) or variety of subpopulations of cells represented, it is also important to take many independent measurements as well to sample the population sufficiently. It is in these areas that microtechnologies have provided new solutions that are now rapidly maturing.

To assess heterogeneities among cells, it is useful to have tools and techniques to isolate cells of interest from a range of biological samples. Blood and fluids are common sources for many diagnostics because they are minimally invasive to collect, but for studying human diseases, tissue from affected areas such as a tumor or inflamed colon can be equally important to study. In Chapter 1, Fu and colleagues examine the range of biological samples from which cells are commonly isolated and the emerging technologies for isolating cells from these materials. With isolated cells in hand, there are a range of different types of measurements that can be made to resolve differences, including genomic, transcriptomic, epigenetic, proteomic, and secretomic measures. Microtools for single-cell analysis are well-suited for enabling these types of measurements with high-throughput. Chapter 2 by Sims and colleagues examines the current state-of-the-art in single-cell genomics and transcriptomics enabled by a combination of microtechnologies and next-generation sequencing. Chapter 3 by Fan and colleagues looks at how microtools can facilitate single-cell measures of immunophenotypes, including surface-expressed markers, intracellular signals, and secreted proteins.

A benefit of microtechnologies for single-cell analysis is the ability to manipulate and control the environments of cells, and to build defined biological systems for study. In Chapter 4, Varadarajan *et al.* examine a powerful approach to learning about the interactions among cells in biological systems using discrete cocultures of cells combined in microfluidic or microstructured systems. The ability to define and track the nature of the interactions among cells, such as T cells and tumor cells, with such precision makes it possible to resolve dynamic functional heterogeneities among cells that cannot be determined by single-cell measurements following bulk cocultures – the nature and number of interactions is obscured by the measurement itself in that case. Chapter 5 by Lam and colleagues examines another level of modeling in which microfluidic systems are used to construct model systems of microvasculature for understanding disease. These systems allow single-cell resolution by imaging, but also recapitulate key features of the biological system they mimic.

The second part of this volume focuses on tiny technologies for modulating biological systems. Here, the five chapters examine micro- and nanotechnologies that have emerged to allow for manipulating and modifying biological systems, whether at the scale of individual cells, tissues, or even whole organisms. These technologies provide new tools with great potential for both preclinical and clinical development of interventions in a range of diseases from cancer to neurological diseases. Chapter 6 by Robinson and colleagues considers the advances in

nanotechnologies that provide direct electronic interfaces to neurons. The ability to interface with neurological systems with nanowires can reduce mechanical damage, and enhance signal transfers. This exciting area could provide new approaches to prosthetic control and advanced bioelectronic systems. Chapter 7 by Jensen and colleagues examines some of the challenges of delivering materials inside of cells. For drug screening and biological manipulations of cell-based therapies such as stem cells, the ability to deliver chemicals or other compounds into a cell remains a challenge. Microfluidic and nanotechnologies are providing solutions to overcome the barrier of the cell wall.

Chapters 8 – 10 examine the utility of microfluidic systems for preclinical studies from single cells up to whole organisms. Kenis and colleagues describe advances in microfluidic technologies for testing small molecule drugs on bacteria and single-cell organisms. The ability to rapidly test for variations in susceptibility in small volumes could enable new approaches to screening libraries of drugs and identifying underlying causes of resistance in rare outliers. In Chapter 9, Wood *et al.* consider the advances in microfluidic systems and microfabricated structures that allow for creating a range of preclinical models for tissues. In one example, model structures of liver tissue that recapitulate the spatial and temporal organization provide a useful tool for toxicology measurements. Finally, in Chapter 10, Ben-Yakar and colleagues describe advances in microfluidic technologies that allow for high-throughput screening and analysis of whole organisms – in particular, the worm *Caenorhabditis elegans.* This model organism has been commonly used in developmental biology and genetic screening. The ability to precisely trap and observe individual animals, and modulate their environment through chemical, optical, and thermal means, has opened up new avenues for organism biology.

Taken together, it is noteworthy that the authors of the current volume represent a highly interdisciplinary group with a strong foundation in engineering, from chemical engineering to electrical engineering to materials science. The intersection of ideas from biology with these disciplines has spurred on innovations that are beginning to reach commercialization in meaningful and impactful ways. Several of the basic technologies described in this volume are reaching the market to advance discovery and development of biopharmaceuticals in a number of ways. Nonetheless, the chapters also highlight the ongoing challenges and opportunities where the field still requires new insights and developments. It is hoped that this volume provides a strong foundation for understanding how micro- and nanotechnologies for use in biomedical research have evolved from concepts to working platforms, and encourages further research and development in these areas to accelerate our understanding of human disease and means for interventions.

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Part I
Microsystems for Single-Cell Analysis

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1
Types of Clinical Samples and Cellular Enrichment Strategies

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1.1
Introduction

The study of cells within the native tissue or at a single-cell level falls under the broad field of cellular pathology. Rudolf Virchow, widely regarded as the father of modern pathology, espoused the principle of examining cells as a method of obtaining information on the patient's well-being [1]. Although technology has evolved to allow clinicians and researchers to adopt better ways of examining cells from the tissue level all the way to the subcellular level, the underlying principle has remained unchanged throughout the years. There are many different types of biological samples regularly handled in the clinic, and they are mostly solid or liquid in nature. It is important to note that not all clinical samples will contain cells. Examples of solid clinical samples include tissues obtained through a biopsy or surgical excision, while liquid clinical samples include blood or urine. Examining cells from such clinical samples can fall under two independent but not mutually exclusive categories: visual examination of cellular morphology under the microscope or analyzing the molecular makeup of the cell. With advances in molecular biology, it is now possible to sequence the genome and study the gene expression at the single-cell level [2]. Although such high sensitivity permits the analysis of rare single cells, it is critical that specimen preparation is clean and free of contamination to ensure specificity of analysis.

In this chapter, we first briefly discuss the types of clinical samples available, focusing primarily on the ones that contain cells (Section 1.2). In Section 1.3, we discuss about the conventional technology currently used for cell enrichment. In Section 1.4, we review some of the micro- and nanoscale microfluidic devices, their underlying principles, and how these devices are rapidly changing the ways we approach cellular enrichment from clinical samples.

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1.2
Types of Clinical Samples

Clinical samples are mainly distinguished into two types: solid or liquid. Solid samples include pieces of tissues harvested during biopsies or surgery and can be either fresh or fixed in a fixative. Liquid samples include bodily fluids such as blood or urine. Depending on the type of downstream processing required, different additives may be added to liquid samples. This section briefly describes each category and provides information on the types of cells typically found in each category.

1.2.1
Solid Clinical Samples

In a hospital setting, solid clinical samples are obtained for the primary purposes of either obtaining a clinical diagnosis or to preserve the patient's well-being. In diseases such as cancer, a biopsy is recommended if the clinician determines the patient is at risk of having cancer. The entire biopsy is processed and examined visually under a microscope by a pathologist for the presence of cancer. Depending on the type of cancer, different methods of obtaining biopsies may be conducted. In suspected cases of melanoma, which occurs on the epidermis, a biopsy is typically harvested from the part of the skin where the suspected melanoma is situated through the use of a surgical blade [3]. In other cancers, such as prostate cancer, where the tissue is not easily accessible, needle biopsies are performed. The prostate is first located using ultrasound and a biopsy is obtained transrectally through the use of a biopsy needle and gun. Once the tissue biopsy is harvested, it is placed in fixative and sent to a clinical laboratory for further processing and staining before being examined by a pathologist under the microscope for the presence of cancer [4, 5]. There are other diseases apart from cancer, such as hepatitis, myopathies, or lupus that may require tissue biopsies [6–9]. In hepatitis, a liver biopsy is performed to determine the extent of fibrosis that has occurred in the liver [10]. In myopathy, a muscle biopsy is required to determine the degree of muscle atrophy as well as to make a clinical diagnosis on the type of myopathy the patient might be suffering from [6, 8]. In systemic lupus erythematosus (SLE), a form of autoimmune disease, biopsies can be extracted from multiple tissue types such as skin or kidney to provide better information on whether the patient is suffering from SLE and to determine the severity of the disease [7].

Larger clinical samples can be obtained through surgical resection. Such situations occur when there is a need to remove part or whole organs to preserve the health and well-being of the patient. This is most commonly performed in cancers of the prostate, breast, or colon to name a few examples and is an invaluable resource for studying tumor heterogeneity [11–13]. In prostate cancer, if cancer was detected in the biopsy, a decision may be made by the clinician to perform radical prostatectomy. During this procedure, the entire prostate is removed from the prostate and surrounding lymph nodes may be resected. The harvested prostate

and lymph nodes are placed in fixative, stained, and sent to the pathologist for grading. This is an important step as the clinicians need to know whether the surgical margins are clear, indicating full resection of the tumor or whether the cancer had already invaded out of the prostate and into surrounding tissues such as the lymph nodes [14]. Therefore, it is apparent that the purpose of removing solid tissue from a patient is to achieve both clinical diagnosis as well as removing any diseased tissue. If fresh human tissue is desired for research purposes, it often involves coordinating with a pathologist, setting up an internal review board for complying with the ethical implications of using human research subjects as well as informing and obtaining patient consent to participate in the study [15].

In the research setting, fresh tissue samples can be readily obtained from animal sources. Although animal use still requires adherence to ethical treatment of the animals, tissue samples from animals are more abundant and easily accessible as they do not involve patients' clinical diagnoses and the number of animals can be increased easily through purchase. Furthermore, animal facilities can and are often situated closer to a research laboratory, allowing for shorter tissue-processing times.

Animal models are typically used for the sake of studying the progression of normal development or developing treatment strategies against human diseases such as cancer, in a preclinical setting. In studying cancer treatments, the disease is first induced in animals, which can be done through the use of xenograft transplant of human cancer cells, the use of transgenic animals that bear a mutation that makes them susceptible to developing the cancer of interest, or through the use of carcinogens. Once the cancer is initiated, the animal may be treated with different types of drugs to test the efficacy of the drug in treating cancer. This is an important preclinical step as the efficacy and toxicity of the drug need to be demonstrated in animals before it can be possibly considered for future use in human subjects. At the end of the study, the animals are euthanized and various organs, tissues, or the tumor itself are harvested from the animal for downstream cellular enrichment and analysis [16].

1.2.1.1 Cellular Subtypes Found in Solid Clinical Samples

There are many different types of organs and tissues in the body each with different cells. Due to space constraints, we are unfortunately unable to cover everything. Instead, we provide a brief introduction to some of the different cell types typically encountered when working with solid tissue samples. The goal is to highlight the diversity of cellular subtypes found within any tissue sample. Specifically, we discuss the epithelium, a form of tissue that is highly abundant in the human body, as well as the underlying connective tissue.

The epithelium performs many functions, one of which is to act as a physical barrier between the environment and other tissue. At the same time, it is also responsible for selective transport of molecules such as oxygen (lung) into the blood, nutrients (intestine), or secretion of enzymes (salivary gland). It can exist as a single (simple epithelium) or multiple layers (stratified epithelium) of cells

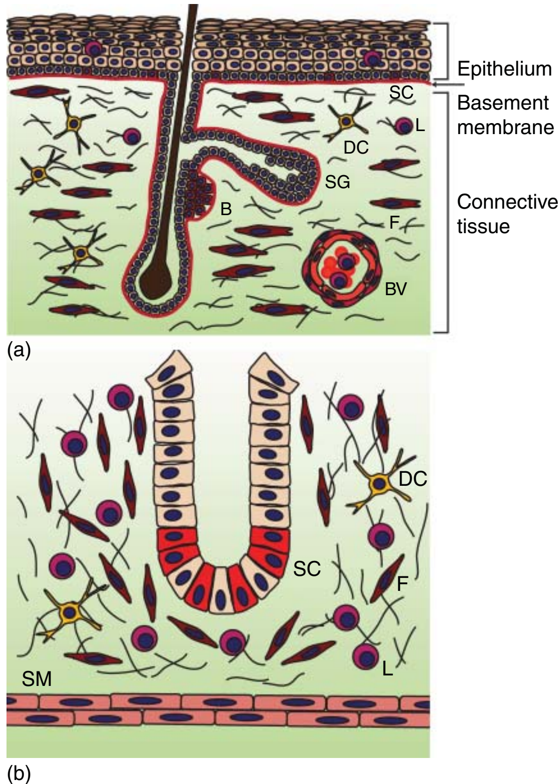

Figure 1.1 Schematic representation of stratified epithelium and underlying connective tissue (a) and simple intestinal epithelial crypt (b). Typical cells found in the epithelium and connective tissue include epithelial cells (E), dendritic cells (DC), lymphocytes (L), fibroblasts (F), and smooth muscle cells (SM). Stem cells (SC) can be found within the crypts of intestinal epithelium or within specialized compartments such as the bulge (B) in stratified epithelium. Additional structures such as blood vessels (BV) or sebaceous glands (SG) can be observed as well.

(Figure 1.1) [17]. Examples of single epithelium can be found in intestinal tissue, while stratified epithelium can be found in the epidermis. Immediately underneath the epithelium separated by a basement membrane lies the connective tissue. While the epithelium itself consists of either a single or a few layers of cells, it is often harvested together with the underlying connective tissue during biopsies or surgical resection. Therefore, most epithelial tissue samples are usually a mix of cell types of different origins (Figure 1.1). Some of the cell types observed in such samples are discussed in the remainder of this chapter. They include but are not limited to epithelial cells, fibroblasts, endothelial cells, stem cells, and immune cells. The connective tissue is also composed of a network of noncellular components, usually fibrous proteins such as collagen, proteoglycans, and glycoproteins. Growth factors and clotting factors can also be found bound within the connective tissue [18]. In diseases involving the epithelium such as cancer or pathogenic