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Microfluidics Cell Culture With Sensing and SqueezeFluidics

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Abstract— **Many biological studies, drug screening methods, and cellular therapies require culture and manipulation of living cells outside of their natural environment in the body. The gap between the cellular microenvironment *in vivo* and *in vitro*, however, poses challenges for obtaining physiologically relevant responses from cells used in basic biological studies or drug screens and for drawing out the maximum functional potential from cells used therapeutically. One of the reasons for this gap is because the fluidic environment of mammalian cells *in vivo* is microscale and dynamic whereas typical *in vitro* cultures are macroscopic and static. This presentation will give an overview of efforts in our laboratory to develop microfluidic systems that enable control of both the chemical and fluid mechanical environment of cells. The technologies and methods close the physiology gap to provide biological information otherwise unobtainable and to enhance cellular performance in therapeutic applications. A key technological need is to integrate sensors to refine device design and operating parameters as well as to monitor real time cellular responses. Specific biomedical topics that will be discussed include, microfluidic models of small airway injuries with pressure sensing and microfluidic liver/cancer cell culture with oxygen sensing. Additionally, microfluidic circuitry with hardware embedded flow control systems that automatically perform sophisticated fluid manipulations powered only by hand squeezing of the device will be presented. This type of capability may be useful for point-of-care diagnostic devices in resource poor environments.**

I. Introduction

There is increasing focus on unconventional interdisciplinary research opportunities to address biomedical challenges. One fruitful area for cross-fertilization is at the interface of biology and microtechnology and sensing. Advances in the microelectronics industry have produced a variety of methods and technologies capable of fabricating and manipulating structures at the size scale of individual biomolecules and cells. There has also been an unprecedented expansion of knowledge and capabilities in the field of biology; sequencing of the human genome and production of human embryonic stem cells to just name a few key discoveries. Many of these biological advances are at the cellular and molecular levels. This paper will present several examples of biomedical research where microtechnology and biology is combined with different sensing schemes to characterize in-device cellular microenvironments. Finally, some results will be presented on basic microfluidic control technology where sophisticated flow control is powered by hand squeezing.

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II. Microfluidic Small Airway Models

A. Meniscus Occlusion of Small Airways

The lung's small airways can close due to the formation of a liquid plug bridge, airway wall collapse or a combination of both. This closure may occur in diseases such as chronic obstructive pulmonary disease (COPD) and respiratory distress syndrome (RDS). The propagation of a formed plug can produce high pressure, high shear stress, and large gradients of each, which may damage the cells lining the airway walls. Previously, we have reported a microfluidic model of small airways where this airway closure by meniscus occlusion has been reproduced in engineered small airways lined with primary human small airway cells [1]. We observed significant airway cell damage and attributed this to steep pressure gradients generated as estimated computationally from fluid mechanics models assuming rigid airway walls. What would be useful are experimental measures to confirm the types of pressures generated inside these microfluidic airway models.

B. Measuring Pressure by Flexible Wall Deformation

To enable measurement of pressures created in the wall by propagating liquid plugs as well as to better mimic the flexible nature of small airways, we fabricated a microchannel with a very thin flexible PDMS membrane with compliance similar to that of distal human airways. As liquid plugs propagated along a flexible microchannel, the local wall deformation was observed in the plug core region. The amount of deformation correlates with pressures generated. The maximum wall deformation increased with plug speed and slightly increased with plug length. The pressure drop across the plug was also measured and observed to increase with plug speed. In addition to experimental findings, needs, challenges and opportunities for microfluidic pressure sensors will be discussed.

III. Microfluidic Oxygen Gradients by Cellular Respiration

A. Physiological Oxygen Gradients

Limited oxygen supply from the bloodstream and active respiration by cells lead to production of oxygen gradients in

the body. In some tissues these oxygen gradients are known to be critical for their function. For example, hepatocytes express different enzymes along the length of liver sinusoids at least in part due to an oxygen gradient. This oxygen level-regulated metabolic zonation is critical for normal function and can modulate liver disease under pathologic conditions. In tumors, there is often a hypoxic core where cell phenotypes can be altered.

B. Microfluidic Cell Culture and Oxygen Gradients

We have previously described the use of poly(dimethylsiloxane) (PDMS)-based microfluidic cell culture systems to give rise to gradients in dissolved oxygen concentrations [2,3]. We also found that in PDMS devices, there was a limit to how hypoxic an environment can be generated by cell respiration alone due to the high oxygen permeability of PDMS. We have recently developed microfluidic perfusion systems fabricated from alternative materials that are less gas permeable [4]. Use of this new device enables generation of stable oxygen gradients with the hypoxic end of the gradient being less than 1% oxygen. This device is convenient and physiologically relevant, solely relying on cell respiration to generate gradients under otherwise ambient oxygen concentration (21%) conditions. Oxygen gradients were measured using an oxygen sensitive dye, ruthenium tris(2,2'-dipyridyl) dichloride hexahydrate (RTDP). The experimental results are consistent with a mathematical model that predicts the effect of flow rate, cell number, and oxygen flux through the device material on dissolved oxygen concentration gradients within the microchannels. Results will also be presented on the effect of oxygen concentration-sensitive drugs on cells. Additionally, challenges and opportunities for microfluidic oxygen sensors will be discussed.

IV. Hand Powered Microfluidics

A. Flow Control Technologies

Along with need for sensors, a critical need for development of inexpensive, practical, point-of-care diagnostics is sample preparation systems that can perform complex processing, yet is robust, power-efficient, and very user-friendly. Currently, CD-based centrifugal force-driven microfluidics, pneumatic-controls, and piezoelectric or other electromechanical actuation can enable sophisticated flow control required but are bulky, require electrical power which may not always be available, and are relatively expensive. There have been a variety of microfluidic systems that are very robust, power-efficient, and user-friendly such capillary force-driven and gravity-driven microfluidic systems. The level of flow control exerted by these passive flow systems, however, is limited.

B. Fluid Flow Powered Control Mechanisms

What would be useful is a microfluidic control system that would enable sophisticated flow control without any electrical connections or input, using only ubiquitously available power sources, such as hand squeezing. I will describe microfluidic substrate architectures and scalable fabrication procedures to construct interactive networks of self-regulating flow components. Appropriately configured networks perform self-regulating operations such as timed valve opening, sequential flow switching, and oscillatory flow switching. The standardized architecture, scalable fabrication procedures, flexibility of fluidic circuit design, and use of simple single phase Newtonian fluids open up new possibilities for sophisticated self-controlled microfluidic systems.

V. Conclusion

The microsystems described here provide examples of the integration of microfluidics, cell culture, and sensing to address needs in biology, medicine, and point-of-care diagnosis. From a microfluidics perspective, there are three types of needs and opportunities represented by these examples: (i) Finding good applications: Existing microfluidic technology when combined with sensing capabilities and a biomedical need can lead to interesting projects. (ii) Developing better materials: Widespread biological microfluidic applications require more materials options to provide biocompatibility, actuator-of-interest compatibility, and manufacturability. (iii) Better microfluidic control technology: Although the ability to fabrication microchannel structures is well developed, microfluidic control circuitry concepts are still very primitive compared to microelectronic circuitry.

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