Microfuidics in Biological Systems

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***Abstract*— We have developed a novel, portable, gravity-fed, microfluidics-based platform suitable for optical interrogation of long-term organotypic cell culture. This system is designed to provide convenient control of cell maintenance, nutrients, and experimental reagent delivery to tissue-like cell densities housed in a transparent, low-volume microenvironment.**

Microfluidics, bioreactors, lorn-term cell culture, PDMS

I. INTRODUCTION

Over the past few years, there has been an increased interest in microfluidic and BioMicroElectroMechanical (BioMEMS) devices that have passive and active features on the same dimensional order as cells and cellular sub-features. Microfluidic technology and devices applied to cell culture offer important advantages over standard culture techniques. There is significant reduction in size and in reagent use; cells can potentially be cultured at higher densities with more realistic cell-to-fluid volume ratios that do not dilute paracrine and autocrine factors; media flows are laminar and the flow rates can be easily adjusted in real-time for proper delivery of media, proteins, or assay reagents; and various spatial and temporal chemical gradients can be established within the microfluidic cell culture devices. There have been several approaches used in the development of microfluidics-based cell culture bioreactors that address issues of long-term culture, the majority of which concentrate on interrogation of cells growing in a monolayer for up to 12 days. (1-4)

Our group is focused on developing microfluidics-based bioreactors for three-dimensional long-term culture of large populations of cells or small portions of excised tissue in a controlled microenvironment (5;6). Microfluidic bioreactors constrain culture chamber dimensions to the micrometer scale, limiting fluid interaction to several microliter volumes and cell numbers in the thousands, which allows for physiologically relevant fluidic nutrient and chemical exchange. The bioreactor designs utilize polydimethylsiloxane (PDMS), an optically transparent, gas-permeable biologically inert polymer, fabricated using photolithography and replica molding techniques (7) to accurately reproduce micrometer scale features that are compatible with conventional detection methodologies. These devices are designed for "closed-loop" automated data collection and maintenance in mind.

II. DISCUSSION.

The developed Thick-Tissue Bioreactor (TTB) is a compact, stand-alone, gravity-fed microfluidic system that 1) can be easily sterilized, assembled under sterile conditions, and transported between culture incubator and the imaging station; 2) maintains cell viability for long-term cell culture, with the future goal of long-term maintenance of tissue (*i.e.*, tumor) biopsy samples; and 3) allows convenient visualization of the response of cells and tissues to drug agent challenges. Its suitability to maintain long-term organotypic culture and regulate drug delivery, was validated using the long-term culture and morphogenic program of breast epithelial cell lines, MCF-10A and their invasive and tumorigenic variants (MCF-AT1 and MCF-CA1d), grown in 3D gelled tissue matrix. The basic reactor cartridge consists of four individual cell culture compartments each supplied by eight 100 μm tall and 100 μm wide microfluidic channels (Fig. 1).

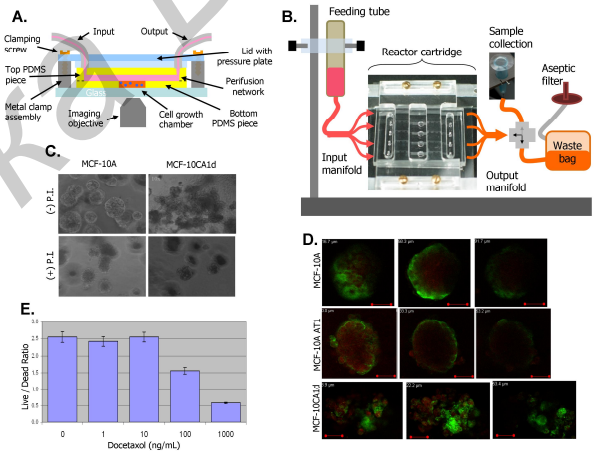

Figure 1. Thick Tissue Bioreactor (TTB). A) Block-diagram the clamped TTB indicating major components; B) A photograph of the fully assembled TTB with the gravity-driven feeding system, passively regulated flow through the input/output manifold; C) Matrix Proteinase Inhibitor (PI) treatments of organotypic culture of MCF-10A cell variants (+) shows a reduction in mammosphere size as compared to control (-); D) Selected confocal slices of MCF cell variants cultured within TTB for 21 days; E) Dose response of MCF10-CA1d cells to anti-mitotic chemo-therapy drug Docetaxel in TTB.

All four chambers within the TTB can be perfused individually or connected to a single gravity fed system via input and output manifolds. The output of the feeding tube is directly connected to the input manifold assembly containing 100 μm wide and 50 μm tall microfluidic channels that split the input flow into four even streams for each of the reactors within the cartridge. A second output manifold (on the waste side of the reactor) is used as in-series fluid flow resistance to passively regulate the flow rate, to recombine the outflow from four reactors, and to direct effluent into a waste collector or, alternatively, to sample extraction systems. The fluid level in the feeding tube was typically checked

daily and replenished every other day to be maintained at 15 cm above the bioreactor surface. This provided targeted culture media flow of 1 mL a day (~700 nL/min)

One of the most fundamental issues in development of the reactors for long term cultures is the consistent nutrient delivery and waste removal. In the simplest approximation the microfluidic devices filled with quiescent or flowing liquids could be controlled either by hydrostatic pressure or external syringe pumps and valves (8-10). The most intricate devices use banks of pneumatically controlled on-chip valves and pumps to control complex bioreactors and microchannel networks (11-15). However, most previously proposed technologies are bulky or complex to fabricate, require external actuation and power source maintenance, or are not compatible with tall (greater than 20 µm) microfluidic channels, and severely limit the compatibility of the bioreactors they are designed to control, with respect to long-term culture and the real-time microscopy.

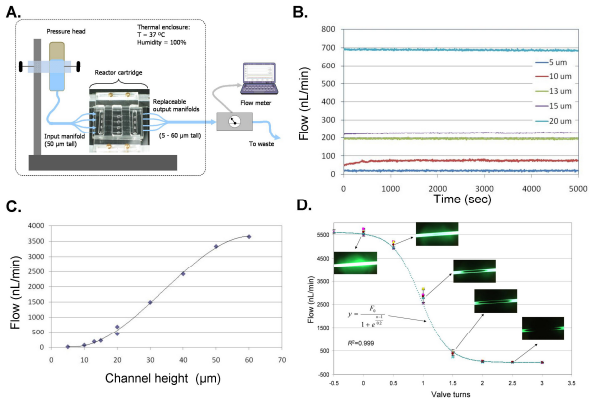

Figure 2. Passive regulation of the flow through TTB by the output manifold heights or a TURN valve. A) A block-diagram of the experimental set-up. B) Typical flow rates through the TTB with the output manifolds of varying channel heights. C) Flow rate as a function of output manifold channel height. Each point is a 20-minute average (error bars are covered by the dots). D) Performance of a single TURN valve closing a single 100 µm by 40 µm microfluidic channel at 0.5 psi of driving pressure. Insets are the fluorescent micrographs illustrating collapse of the micro-channel as function of valve closure. F_0 is the initial flow with valve fully opened.

In order to minimize cost, system's fabrication and operational complexity we have chosen liquid delivery system based on gravity-induced flow and passive flow regulation based on fluidic resistance of the supply microfluidic network. Initially we have tested TURN valve (16) positioned between the media supply reservoir and the TTB cartridge as a metering device. This valve is simple to fabricate and operate, does not require external power connections, and is capable of reversibly closing a variety of microfluidic channels. It showed excellent performance for starting and stopping flows but regulation of the intermediate flows proved to be challenging, mostly because the fluidic conductance, G, of a rectangular microfluidic channel with aspect ratio near 1 is proportional to the 4th power of its dimensions:

$$G = \frac{1}{R} = \frac{4ba^3}{3\mu L}\left[1 - \frac{192a}{\pi^5 b}\sum_{i=1,3,5\ldots}^{\infty}\frac{\tanh(i\pi b/2a)}{i^5}\right]$$

, where a and b are channel half width and height, μ is media viscosity, and L is the channel length. Thus minute deviations in the amount of channel collapse resulted in large variations in the measured flow. As an alternative to individual channel TURN valve-adjusted flow regulators, we investigated use of pre-fabricated microfluidic flow resistance manifolds (Fig. 2). Flow rates as a function of the waste manifold channel height (*i.e.*, flow resistance) were measured with the NanoFlow flow meter (IDEX Health & Science). We found that by selecting a manifold with an appropriate channel height, we could produce a sufficient resistance to flow that, in combination with the fixed height of the supply reservoir, could be used to passively limit the flow through the bioreactor (Fig. 2C). This technique has proved to be quite adequate and more economical in terms of fabrication time and complexity than the TURN valve based method. We found that 20 µm channel heights of the output manifold, while maintaining the same 100 µm width, provided the total target media flow rate of 1 mL/day.

III. Conclusions

We have developed a portable thick-tissue bioreactor (TTB) capable of maintaining sterile microenvironment and sustaining cell growth, maturation, and organ formation during 3 week-long cell culture. The performance of the TTB was validated with the MCF-10A cell line and its invasive and tumorigenic variants that were capable of developing into hollow lumen spheroids when cultured in 3D matrix over the course of 21 days. Use of passive only elements to control culture media delivery significantly reduced cost and complexity of the system, allowed it to be portable and compatible with regular and fluorescent microscopy.

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