Optofluidic platform to investigate cell community behavior in microenvironments

Rudra Vaswata Roy Choudhury, Anil Prabhakar, and Sunil Laxman

Abstract— **An important aspect of cell biology is to study the effect of micro-environmental conditions on cell growth. This is usually achieved by culturing cells in macro bioreactors, followed by either cell growth quantification through measurement of optical density (OD) or imaging cells over a period of time using conventional microscopes to look at morphological characteristics of the cell. The presented work proposes an optofluidic platform to study nutrient responses in cell communities. A microfluidic cell culture device has been designed which generates nutrient gradients across multiple chambers where colonies of cells could be grown and maintained. The project further demonstrates a portable cellphone based microscopy platform to which the microfluidic device is augmented and the system acts as an integrated lab-on-chip platform for live cell imaging. The performance of the optofluidic platform has been evaluated by growing cells in the microfluidic chambers, characterizing nutrient mixing in the device and subsequent imaging of cells using the designed portable microscope.**

I. Introduction

Microfluidic technology is creating powerful engineering tools for cell biologists to manipulate and control cellular micro-environment, leading to the investigation of new questions and discoveries. Through miniaturization of macrosystems and taking advantage of the possibility of parallel processing, some microfluidic chips enable high throughput biological experiments. Microfluidic technology enables studies of cell behaviour from single to multi-cellular organism level with precise application of experimental conditions that could not be achieved using macroscopic systems [1]. The versatility of these devices enable the simulation of in vivo cellular micro-environments, for e.g. nutrient stress. The micro-environment of the cell is defined by chemical and mechanical parameters. One of the earliest applications of microfluidics in cell biology was to control the cell medium [2]. The study of cellular response to nutrient gradients requires fine control of local concentration since cells can respond to chemical gradients localized to a region as small as 2% of their diameter [3]. The poor spatio-temporal resolution of macroscopic gradient generators led to an interest in development of micro-scale gradient generator systems. These have been successfully used to study stem cell growth and differentiation [4], yeast gene expression under different nutrient gradients, etc [5].

Optical microscopy has demonstrated a pivotal role in biology and medicine with tremendous advancement being made in improving the resolution of images, penetration depth, etc [6]. However, although these techniques provide high-end capacities in probing biomedical problems, they are often unable to satisfy the need for high throughput observation due to their bulk. In the current scenario, microfluidic based lab-on-chip platforms are widely used to study real-time cell behaviour, for which using conventional bench-top microscopes is not practical. Recently, Khademhosseini *et. al*. [6], Prakash et. al. [7] have developed prototypes of a portable microscope. However, their applications are limited to a more general usage, rather than carrying out standard biological experiments.

The motivation of the presented research work is to design and develop an optofluidic platform to study nutrient responses in cell communities using portable microscopy. A microfluidic gradient generator is designed that controls the micro-environment for yeast cells and enables to study affect of key nutrient conditions on colony formation of yeast. In addition to this, the research proposes the development of a cellphone based microscopic platform that could image biological samples at a suitable magnification and resolution, thereby providing a cost-effective and portable tool for biomedical applications. The project further discusses the integration of the designed microfluidic device into the cellphone microscopy platform to develop a lab-on-chip system for live cell imaging.

II. Design of a Microfluidic Device to Study Cell Growth

A. Design of the device geometry

The microfluidic device has two inlets for nutrient inflow, and five cylindrical chambers or wells, with depth 'd_w' which lead to five outlets. The channels of the device, with width 'x' and depth 'd_c' follow a branching pattern such that different mixing conditions could be introduced and a concentration gradient of nutrient is generated across the five chambers. Fluid flow in a microfluidic channel is predominantly laminar, owing to low Reynold's number. To overcome this and enhance mixing between fluids, spiral meandering have been introduced along the channel path, before it leads to the chamber. The size of the device geometry has been decided based on the dimension of a typical glass slide used in biological laboratories. This ensures that along with the proposed technology, the device

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can also be integrated to any other biological test platform (e.g. standard microscopes).

B. Simulation Model

To design the microfluidic device, initial simulations on the device geometry were performed in COMSOL Multiphysics 5.0. The initial concentrations of two inflows at the two inlets are set as 10 mol/m^3 and 20 mol/m^3 respectively. The inflow liquids, flowing at 1ml/hr, represent nutrient and water, with the diffusion coefficient set to 7 x 10^{-10} m^2/s , which is the approximate diffusion rate of glucose in water. Glucose is a standard nutrient media that is used to study growth behaviour of yeast. The density and viscosity of the nutrient fluid is considered as 1110 kg/m3 and 0.0015 Pa.s while that of water is 1000 kg/m3 and 0.0010 Pa.s. We allow normal flow, and suppress backflow at the output. At the walls of the channel, we define 'No Slip' condition. At the outlets, we set the boundary condition as 'pressure' and use the value of 101325 Pa, the atmospheric pressure. Since the concentration for inflow is defined over a range, we set the inflow boundary conditions as 'concentration constraint'. Once all the boundary conditions are set, a 'physics-controlled' mesh is implemented on the geometry, with the element size set to 'fine'. Subsequently, all the chambers are probed at midway of the depth followed by which a time-dependent study is carried out to study the variation in concentration of the fluids as a function of time.

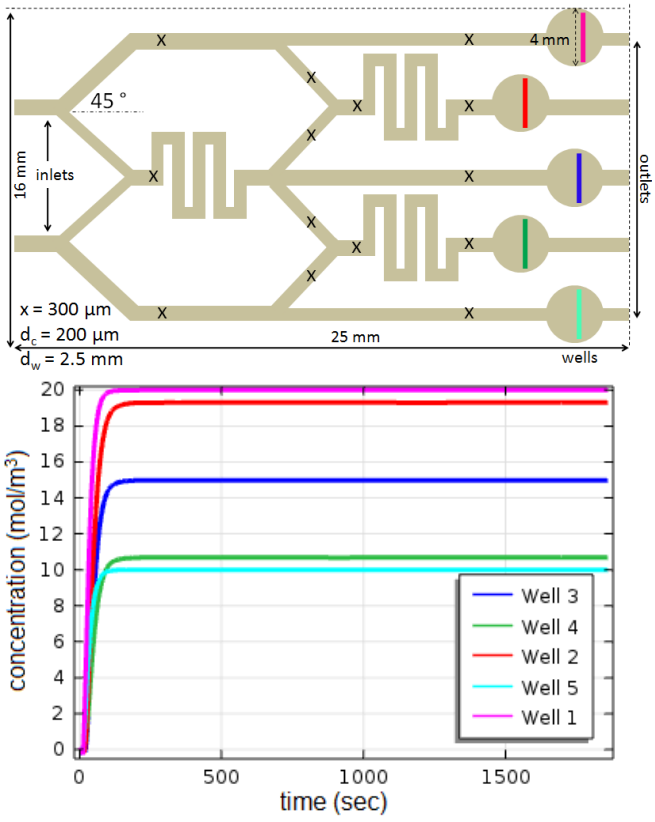

Figure 1. Simulation of device geometry showing gradient of concentration formed across the chambers

C. Prototype microfluidic device

The prototype microfluidic device has been fabricated using PMMA substrate, as shown in figure 2. The fabrication process was carried out at Achira Labs Pvt. Ltd., Bangalore, India. PMMA has excellent optical properties with low light absorptivity, and is less expensive than PDMS thereby making it suitable for fabricating prototype devices for imaging applications. Using the designed prototype, we carry out experiments on mixing and cell growth.

D. Experimental quantification of mixing

We use spectrophotometric analysis to evaluate the performance of the device and quantify mixing. A sample mixing test was performed between yeast peptone dextrose (YPD), which is an essential nutrient media for growing yeast, and water. To impart characteristic colour to the two fluids, we add orange dye to YPD and green to water. The SP200i syringe pump was used to flow the samples at a flowrate of 1 ml/hr. Medical grade silicon tubings were used to connect up the microfluidic device to the syringe pump. After few minutes of fluid flow, considering equilibrium condition, 2 µl of samples was collected from each chamber and subsequently analysed using a Nanodrop 2000 spectrophotometer.

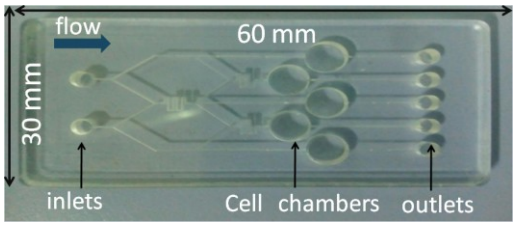

Figure 2. Prototype microfluidic device fabricated using PMMA substrate

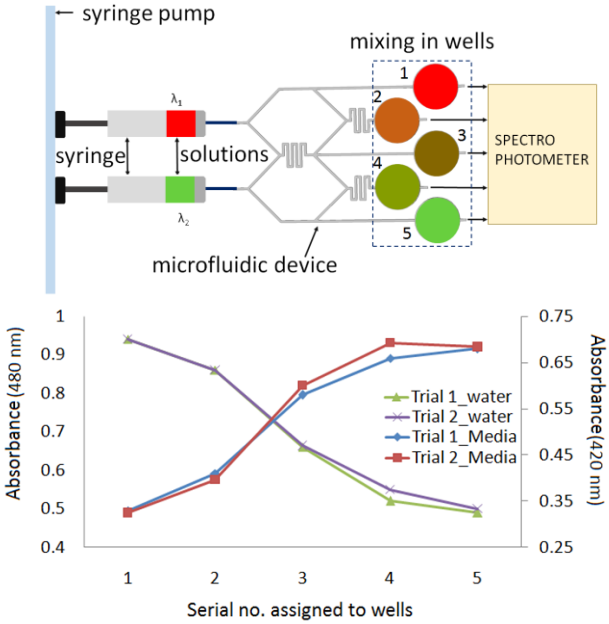

Figure 3. Quantification of mixing between YPD and water showing gradient of absorbance for different concentrations generated

Data collected from spectrophotometer for the mixed solution in each chamber was plotted to show the variation of absorbance for each component fluid in the five chambers. According to the Beer-Lambert law, we know that the absorbance is a direct measure of the concentration of the solution. Hence, the absorbance data collected from the spectrophotometer provides information on the concentration gradient of the fluid across the five chambers.

E. Cell-colony growth in device chamber

For growing yeast, 2% Agarose solution is made by adding 400 mg of Agarose powder to 20 ml of double distilled water, in a conical flask. Subsequently, the flask was heated inside an oven at a temperature of 50 °C for 90 seconds, when the solution just begins to boil. The heated flask is taken out and the solution is poured smoothly on a petri plate to form the gel layer. Following this, a novel technique is implemented to extract small layer of the gel and transfer to the microfluidic chambers, in which the cells are grown.

F. Studying yeast growth in response to minimal media

The effect of varying concentration of minimal media (SD) on colony growth of yeast has been studied. Here, minimal media acts as the chief nutrient which is diluted by 1X PBS buffer, to generate a gradient of the nutrient.

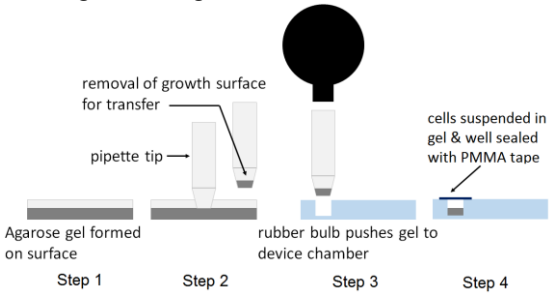

Figure 4. Schematic illustrating the procedure of cell growth in microfluidic chambers

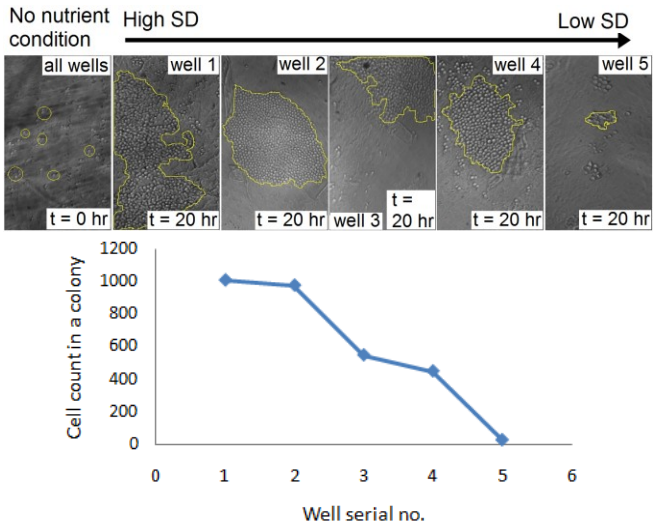

Figure 5. Yeast colony growth for varied concentration of minimal media

Initially all the wells are imaged under no media conditions when the cells are randomly scattered in the chamber. Subsequently, media is flown into the device followed by incubation, and the chambers are again imaged after a period of 20 hours. Imaging illustrates growth behaviour of yeast micro-colonies, with the colony size decreasing with a reduction in the nutrient concentration.

III. DEVELOPMENT OF THE CELLPHONE MICROSCOPE

The cellphone microscope has been designed using cost-effective and easily available optoelectronic components, thereby making the tool simple and convenient for use to a wide range of users. A low-cost white LED (3V, 20mA) is used as the light source to illuminate the sample from the bottom. To create uniform illumination. a diffuser sheet is mounted on top of the LED which cuts off the bright spot of the LED. A miniature XY translation stage is used that holds the sample in a sample holder, and thereby allows the user to manually scan across the sample, as required for imaging. A miniaturized Z translation stage has also been used to hold the cellphone and manually focus sample (cells) as needed. The cellphone camera, with the built-in CMOS sensor, acts as the primary eyepiece of the microscope. However, the lens of the phone camera doesn't have sufficient magnification or resolution required to image cells and other microbial organisms. Thus, an external ball lens is coupled to the cell-phone camera lens which then acts similar to the objective lens of the microscope. The microscope is powered using an on-board battery pack which makes the microscope a stand-alone platform. The hardware configuration of the microscope is illustrated in figure 6. The parametric equations interrelating the focal length (EFL), back focal length (BFL) or working distance (W_d), diameter (D), refractive index (n) and magnification (M) of the ball lens are[7]

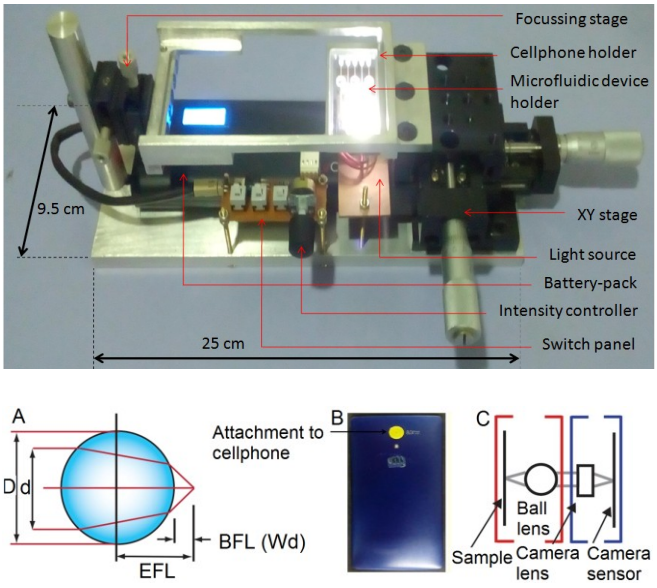

Figure 6. Hardware configuration of the cellphone microscope (top) and modification of the camera optics using a ball lens

$$EFL = nD/4(n-1) \quad (1)$$

$$BFL = EFL - \frac{D}{2} \quad (2)$$

$$M = \frac{250}{EFL} \quad (3)$$

The value of '250' in the equation for magnification comes from the fact that 250 mm is the smallest distance that human eye can focus. While carrying out the experiments on yeast growth, as explained in figure 5, it has been figured out that a microscopic objective of 40X (equivalent to a total magnification of 400X) is required to image yeast cells at suitable magnification. Based on this technical requirement, we scan through the list of commercially available ball lenses and finally select a ball lens of N-BK7 material (n = 1.517) with diameter of 1 mm (Edmund Optics: #43-708). The specified lens provides an effective focal length of 0.73 mm, magnification of ~340X, an effective field of view (FOV) of 300 µm x 300 µm and a resolution of 1.4 µm, which is calculated from the lens equations [7]. In addition to the magnification achieved by the ball lens, we also take advantage of the digital magnification of the cellphone camera, which varies in the range of 0 to 4X for standard smartphone. The working distance of the lens system is 200 µm. However, this can pose as a limitation while imaging samples that are mounted at a greater distance from the ball lens, for e.g. cells in microfluidic chabers. Hence to increase the working distance of the microscope and image samples positioned at a greater distance, an achromat lens is used between the sample and the ball lens. This achromat creates a virtual image of the sample, at a position as per the focal length, which is subsequently imaged by the ball lens. Imaging of samples using the cellphone microscope has been illustrated in figure 7. Different types of biological samples have been imaged to validate the performance quality of the designed microscopy platform.

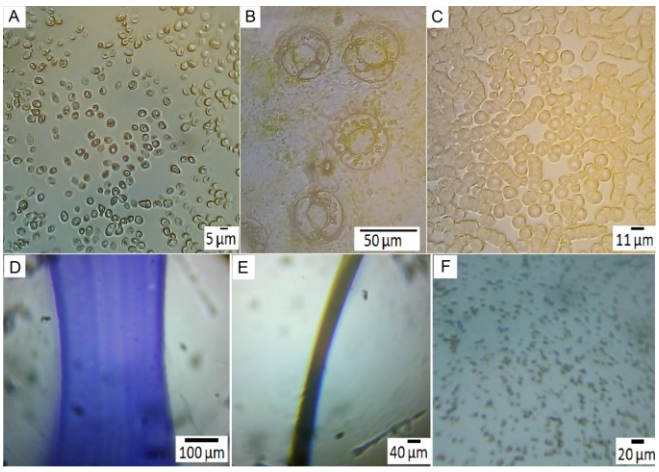

Figure 7. Imaging of biological samples using the designed microscope showing (A) yeast cells on slide, (B) plant stomata cells, (C) blood cells, (D) grided slide, (E) human hair sample and (F) cells grown inside the chambers of the microfluidic device

SUMMARY

We have designed and prototyped a lab-on-chip platform to carry out high throughput experiments on the effect of nutrient gradients on cell growth. The designed microfluidic device provides mixing between fluids and the device has been characterized to show the generation of concentration gradient across the chambers. A novel method has been proposed to grow cell colonies in the microfluidic chambers. The method has been experimentally verified by growing colonies of yeast. The project also demonstrates the cellphone based portable microscopy platform that has been validated by imaging different biological samples. The microfluidic device augmented with a cellphone microscope acts as a standalone platform to study and investigate cell behaviour in microenvironments.

CODE OF ETHICS

Biosafety and ethical approval for use of model organisms has been obtained by Dr. Sunil Laxman lab from the institutional biosafety committee.

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