3D Microfabricated Bioreactors

C.-G. Xia and Nicholas Fang*

* Department of Mechanical Science and Engineering
University of Illinois at Urbana-Champaign
1206 W. Green Urbana, IL 61801, nicfang@uiuc.edu

C. Sun, D.M. Wu and X. Zhang**
** Nano-scale Science and Engineering Center, 5130 Etcheverry Hall,
University of California, Berkeley, California 94720-1740, USA

ABSTRACT

We present here an innovative three dimensional microfabrication technology coupled with mass transport simulation to enable the design and fabrication of advanced microbioreactors. The core of our microfabrication technology is a high-resolution projection micro stereo lithography (PμSL) using a spatial light modulator as the dynamic mask. This unique technology provides a parallel fabrication of highly complex 3D microstructures. In this work, a set of poly(ethylene glycol) bioreactors are demonstrated with PμSL technology. Supported by the results of our numerical study, the precisely controlled channel density (>150/mm^2) in the polymer matrix and improved transport of nutrient and oxygen through advection and diffusion represent the key advantages of the microfabricated bioreactors to the traditional foam and hollow fiber based bioreactors. Development of this 3D microfabricated bioreactors are expected to have direct impact on applications such as analyte controlled and modulated drug and protein delivery, drug targeting, tissue engineering, and micro- or nano-devices.

Keywords: 3D microfabrication, bioreactor, transport, modeling

1 INTRODUCTION

Bioreactors[1,2] provide a gateway to investigate the physiological and pathological processes of living cells and tissue, by artificially control the local environmental conditions and stimuli during cell production and growth. Not only it can be used for large scale cell culture, the bioreactors also offer a unique opportunity to understand the fundamental mechanism of cell behaviors in a 3D environment. It is a rapidly growing field with potential impact on the application such as analyte controlled and modulated drug and protein delivery, drug targeting, tissue engineering, and micro/nano-devices.

One of the obstacles of culturing functioning tissues using bioreactors is to obtain a substantial biomass. Most cell cultures produce flat, one-cell-thick specimens that offer limited insight into how cells work together. With increased cell density, the metabolism during cell growth cycle will eventually exhaust the supply of nutrient and oxygen from the environment and the embedded cells suffer from the lack of nutrient, creating a bottleneck for the growth of 3D tissues. Studies [4, 5] confirm that the cells in the tissue are poorly cultured when they are further than ~400μm from the external nutrient source. In order to enhance the mass transport, several designs of bioreactor have been proposed, such as spinner-flask bioreactors by stirring the medium; rotating wall vessel bioreactor invented by NASA to suspend the cells in a medium; hollow fiber bioreactor delivering nutrient through permeable fibers or in the opposite way by culturing cells inside the fiber, and direct perfusion bioreactor using porous scaffold to transport nutrients to the cell in the pores. However, the state-of-the-art bioreactors share some common drawbacks: most of the bioreactors are designed for culturing a few types of cells or cell groups, and after the cells are cultured they have to be harvested and collected, losing the integrity of the tissue. In addition they are not compatible to fast throughput tissue assays to study the impact of local environment on a small volume of cells. Although there are some studies on the micro-bioreactor, for example, the micro-encapsulation immobilizing cells in a micro compartment, the nature of this method limited geometries of the compartment to very simple cases [6].

In this work, we introduce a novel three dimensional microfabrication technology, the Projection Micro-Stereolithography (PμSL) [7], coupled with mass transport simulation to the design and fabrication of micro bioreactors. The micro fabricated bioreactor dramatically enhances the mass transport by advection through microcapillary channels. This microfabrication method brings several unique advantages to the advanced microbioreactor research and development: first, the capability of PμSL to build truly 3D sophisticated microstructures with very fine spatial resolution at micron scale; second, a significantly shortened design cycle enabled by high fabrication speed (1000 layers in a couple

of hours)[7]; finally, the choice of biocompatible and biodegradable polymers offers flexibility on fabricating implantable bioreactors for different tissue culture[8,9].

2 FABRICATION OF MICROBIOREACTOR

We reported the principle of projection micro-stereolithography in reference [7] and it is highlighted in Figure 1. The process starts from generating 3D structure in Computer Aid Design (CAD) software, then slice the structure into a sequence of bitmap images according to the desired spatial resolution on the direction perpendicular to the slicing planes. Each image is defining a polymer layer to be solidified in later fabrication process. During one fabrication cycle, one image is read in and displayed through spatial light modulator (SLM). The modulated light pattern is then delivered by the light path composed of beam splitter and 45^o mirror to the reduction lens. The reduced image is focused on the photo curable liquid surface. The whole layer (usually 2- 20 microns thick) is polymerized simultaneously. After one layer is solidified, the polymerized part is immersed deep into the liquid surface to allow a new fresh thin liquid layer atop. A new fabrication cycle starts. By repeating the cycles, a 3D microstructure is formed from the stack of layers.

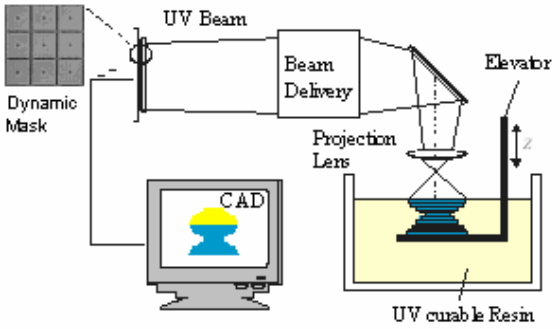

Figure 1: The 3D micro bioreactor is fabricated with a layer-by-layer photo-polymerization[7] of the biocompatible monomer, according to the slicing of the 3D computerized model.

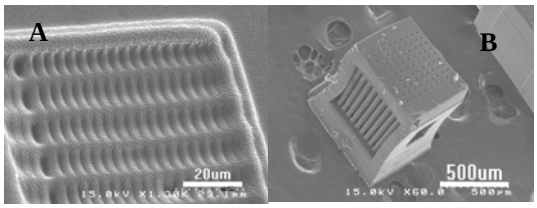

Figure 2. High resolution 3D microfabrication enabled by PμSL

To fabricate the micro bioreactors, the biocompatible monomer used in this work is a water-soluble poly (ethylene glycol) (PEG) diacrylate (molecular weight 575, from Sigma-Aldrich, with viscosity 57cP at 25^{o}C). Several 3D structures are fabricated. Fig.2A shows an array of 2μm parallel lines with 2μm spacing (on denser direction), demonstrating the spatial resolution of this system. Fig.2.B is a 9 by 9 micro tube array with 10μm inner radius, 20μm outer radius, 80μm spacing and 800μm long (aspect ratio >20, effective channel density >150/mm^2).

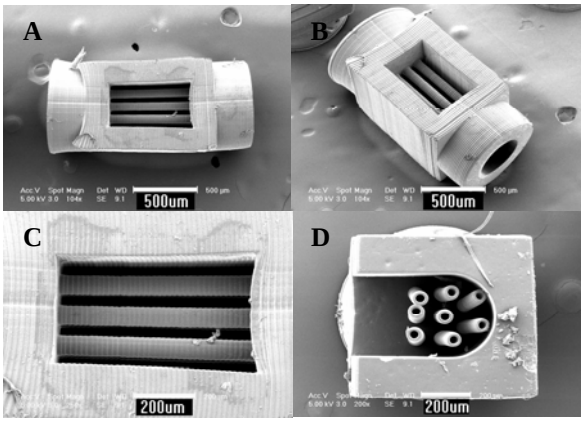

Figure 3. The scanning electron microscopy (SEM) of microfabricated bioreactor from different angles and cross-section.

We further designed and fabricated the micro bioreactor as shown in Fig. 3. The simulation results showed that for 800μm PEG (MW575) tube with 20μm inner radius and 40μm outer radius, the maximum distance between the nearest points of two adjacent parallel tubes should not be greater than 96μm. Two rings are connected to the bioreactor chamber, which are filled with parallel micro tubes. The external nutrient supply will be connected to the two rings, which has 400μm inner diameter. Fig.3D shows the cross-section view of the microbioreactor. The designed distance between two adjacent tubes is 90μm. Since the volume of the reactor is only 0.2μL, it allows culturing about 2,000 cells at the level of 10^7cells/cm^3.

3 MICROBIOREACTOR DESIGN

During tissue culture, the nutrients are delivered to the cells in the bioreactor via a series of capillary channels. It is very important than all the cells in the tissue are well fostered to reach high cell density. In normal tissue, almost no cell is farther than 100um away from vessels, because the nutrients are depleted at that distance. It means for certain cell density, the capillary network has to be dense enough to balance the consumption during cell growth. Similarly, in our microbioreactor design, we have the same requirement of channel density. The relative position of adjacent tubes defines the micro tube density. In the following, we will present the numerical study on the mass transport by a PEG micro tube which will eventually guide our bioreactor design.

The micro tubes are arranged in a hexagonal array as shown in Figure 4a. The simulation is based on one micro

tube supported by thick polymer structures at both end. Figure 4b and 4c show the geometries of the model and the reduced simulation domain according to the symmetry of the model. In the numerical study we further take the following assumptions:

- The nutrient species diffuse at a speed nearly one order faster in tissue than in the bulk polymer, so we can neglect the diffusion through the thick polymer support structure.
- The nutrient concentration inside the tube only changes along the axial direction. Advection transport dominates in the tube.
- Diffusion transport dominates within the tube wall and tissue, no advection in these regions. This can be regarded as "worst-case scenario" design. The actual advection effect will only increase the mass transport, ensuring a better condition for tissue culture.
- The final mass transport distance is determined at the steady state when the number and density of cells reach the maxima. So in the simulation we assumed that the mass transport is steady.

Based on above assumptions, the mass transport phenomenon in this model can be described by elliptic equations:

$$D_{pi}\nabla^2 c_i = 0 \qquad r_0 \le r \le r_0 + W \qquad (1)$$

$$D_{ti}\nabla^2 c_i - R_i = 0 \qquad r_0 + W \le r \le L \qquad (2)$$

Here D_{pi}, D_{ti} are the diffusion coefficients for metabolite species i in polymer and in tissue respectively, they are assumed to be constant. c_i and R_i are the concentration and consumption rate of species i, and r_0, W, L are the inner radius, wall thickness of micro tube and width of simulation domain, respectively. We keep D_{pi} in equation (1) to differentiate the two regions and later to impose the mass conservation condition at the interface. In the case of steady state, the process of cells consuming metabolites is often described by Michaelis-Menten kinetics [10, 11]:

$$R_i = \frac{V_{\max} c_i}{K_M + c_i} \qquad (3)$$

Where $V_{\max}$ is the maximal uptake rate and K_M is the metabolite concentration when the uptake rate is half of the maximum. In Michaelis-Menten kinetics, the consumption behavior follows first order kinetics at low concentration. That means the consumption rate is proportional to the concentration. But there is a threshold of concentration that cells start to starve. As a rule of thumb, condition $c_i = K_M$ is often used. We adopt this condition to determine the bound of mass transport distance. As the concentration of the metabolite increases, the consumption behavior will become zero order kinetics gradually. At certain point, the cell is saturated and the intake of metabolites reaches a plateau.

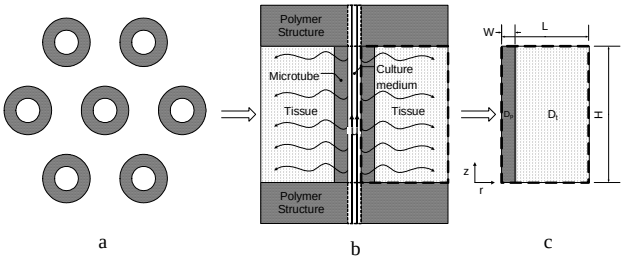

Figure 4 a, the tubes are arranged in a hexagonal way and the structure is repeated in 2D manner; b, one micro tube with polymer structure is isolated from the hexagonal structure as in a; c, the simulation domain in cylindrical coordinates.

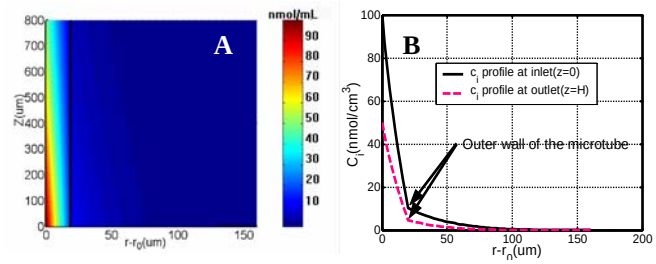

Figure 5 A, Simulated oxygen concentration distribution over the whole simulation domain. The black line indicates the outer wall of the tube. B, the concentration profiles at the inlet side and outlet side.

Equations (1, 2) combined with Equation (3) turns out to be a nonlinear system. It is solved using 3 points central difference scheme in the domain and 5 point bias difference on the boundaries. Newton iteration was implemented. In the current design, we only simulated the oxygen transport which is a typical factor limiting critical biomass [1]. The typical diffusion coefficient of oxygen in tissue at 37^0C is from [1], the inlet oxygen level is set to be arterial oxygen levels. The ultimate cell density level is set at10^7cells/cm^3. The flow rate in the channels is set at 1.5mm/s. The diffusion coefficient of oxygen in polymerized PEG is conservatively estimated from [2].Other parameters are set as follows: L=160mm, H=800mm, W=20mm, r_0=20mm, D_{pi}=2X10^{-6}cm^2/s, D_{ti}=2X10^{-5}cm^2/s, C_0=100nmol/cm^3, V_M=4nmol/cm^3/s, K_M=0.6nmol/cm^3.

Due to the consumption outside the tube, the oxygen concentration in the tube gradually decreases from inlet to outlet. For this reason, the oxygen diffusion distance decreases in the same direction, setting up the spacing of micro tubes in the design of microbioreactor. With above simulation parameters, the diffusion distances truncated according to the hypoxic condition at inlet of the tube is 74 μm, and 48 μm at outlet, which are consistent with in normal tissue that almost no cell is farther than 100μm from vessels. It is evident from Fig.5B that the concentration of oxygen decreases dramatically (~90%) through the micro tube wall. This is due to the much smaller diffusion coefficient of oxygen in PEG. Our study indicates two ways to increase the oxygen concentration reaching the tissue under certain perfusion concentration: one is to reduce the thickness of the wall, but this will also reduce the

mechanical capability of the tube. There is a critical strength for the tube to sustain the mass and stress from surrounding cells. On the other hand, we can increase the permeability of the polymer by selecting an appropriate polymer with higher diffusivity or increasing the porosity of selected polymer. However, higher diffusivity of polymer cannot completely solve the problem of mass transport without a significant advection rate. Our argument is based on the fact that for a tube of certain length, too high diffusivity of the polymer tube will cause too much "upstream" consumption and the oxygen concentration in the tube will decrease quickly. This will eventually decrease the mass transport distance at "downstream". However the distance between parallel micro tubes in micro bioreactor is determined by the mass transport distance at downstream outlet. Hence there is an optimal diffusion coefficient for the polymer selection. Fig. 6 shows the effects, the blue line is the mass transport distance at the inlet and red line for outlet. As the diffusivity of the tube increase, the mass transport length increase monotonously at the inlet, but the transport length at the outlet increases first, then at a certain value (~4×10^{-6}cm^2/s) it starts to decrease, this is the optimal diffusion coefficient for the this specific tube geometry.

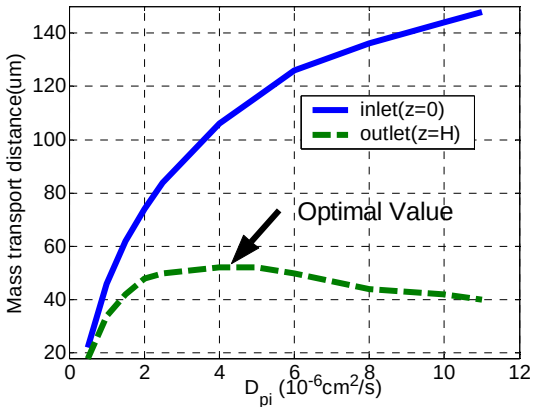

Figure 6. Mass transport distance at the inlet and outlet of the micro tube as a function of the diffusivity of the polymer.

4 CONCLUSION

Projection Micro-Stereolithography (PuSL) promises rapid design and manufacturing of advanced microbioreactors by offering a unique opportunity to understand the fundamental mechanism of cell behaviors in a 3D environment. By integrating high density microcapillary channels within the microbioreactors, the mass transport can be enhanced by advection to withstand the increasing demand of oxygen and nutrient during cell growth. Simulation based on oxygen diffusion model showed that the bottleneck of effective transport is the diffusivity of the polymer material of the capillary. The oxygen concentration dramatically decreases after diffusing through the wall of the capillary. For certain size of capillary, there exists an optimal diffusion coefficient of the capillary polymer, which will maximize the mass transport distance of the whole capillary. Micro fabricated bioreactor with a volume of 0.2μL is shown. We are currently pursuing suspension cell culture in PEG microbioreactor and experimentally validating the effect of mass transport.

5 ACKNOWLEDGEMENT

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