COMBINING SOFT LITHOGRAPHY AND PHOTO-POLYMERIZATION FOR ENGINEERED MICRO-ENVIRONMENT

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ABSTRACT

Chemical and physical interactions are commonplace in a natural microbial community. However, it is technically challenging to study natural microbial communities in situ. Engineering artificial environments can offer insights into how natural interactions work both qualitatively and quantitatively. In this paper, we present a method for engineering microenvironments by combining soft-lithography and photo-polymerization of hydrogels. This method presents the opportunity to physical isolate micro-organisms while allowing chemical communication between two or more populations.

INTRODUCTION

Interactions of all sorts are ubiquitous in nearly all natural microbial communities including those in the oceans, soil, and human microbiome. Unfortunately there is a dearth of systems that can facilitate controlled experimentation on natural or synthetic microbial communities. Advances in microfluidics provide enabling technologies for this area including polydimethylsiloxane (PDMS) microfluidics, droplet microfluidics, photo-polymerized hydrogels, protein hydrogels, and combinations thereof[1]. Here, we present a method combining photo-polymerized hydrogels with PDMS microfluidics through replica molding. This method enables us to physically isolate bacteria in nanoliter scale chambers while facilitating chemical interactions.

METHOD

The fabrication method, as shown in Figure 1, involves replica molding of a hydrogel from soft-lithography fabricated PDMS molds. The process begins with PDMS soft lithography using SU-8 to create master molds. Then, the PDMS mold is used to pattern a UV-light cured hydrogel [poly(2-hydroxyethyl methacrylate-co-ethylene dimethacrylate), HEMA-EDMA]. After the hydrogel is solidified, the PDMS mold can be peeled off.

HEMA-EDMA was chosen because modulating its precursor composition can alter average pore size[2], and thus permeability to various compounds. The average pore size achieved ranges from hundreds of nanometers to a few micrometers. Scanning electron microscope (SEM) is used to qualitatively characterize the average pore size, while dual chamber diffusion experiments are conducted to quantify the diffusivity of various molecules.

A micro-chamber array device was built with nanoporous HEMA-EDMA with the working principle as shown in Figure 2. The device can be used to culture multiple microorganisms, with different organisms occupying different chambers. In such devices, because the pore size of the nanoporous HEMA-EDMA is smaller than the size of the microorganism, the microorganism will be physically isolated in the chambers. However, the porous structure of the chamber walls facilitates chemical communication between microorganisms in different chambers. The overall porosity and the distance between chambers will limit the speed of communication between microorganisms.

Figure 1. Schematic illustration of the fabrication method. 1) Standard photolithography of SU-8. 2) Resulting SU-8 master mold. 3) Casting PDMS to the SU-8 master mold. 4) Resulting PDMS mold. 5) PDMS mold is used to pattern HEMA-EDMA hydrogel. 6) Fabricated NMML as micro-well arrays of HEMA-EDMA on a glass slide.

Figure 2. Working principle of the HEMA-EDMA micro-well arrays. Individual micro-organisms can be statistically seeded into individual wells or homogeneous populations can be placed in individual wells. The walls of the wells are nanoporous, small enough to physically isolate cells, while still allowing inter-species chemical communication by diffusion.

RESULTS

The HEMA-EDMA in Fig. 3a shows SEM images of microporous HEMA-EDMA while Fig. 3b displays nanoporous

HEMA-EDMA. The SEM images indicate that pore size of the microporous HEMA-EDMA is larger than 1 μm , while that of the nanoporous HEMA-EDMA is less than 200 nm. Two representative molecules, glycine (molecular weight 75 Da) and bovine serum albumin (BSA, molecular weight ~ 66 kDa), are used in the dual chamber diffusion cell to characterize diffusivity across the HEMA-EDMA hydrogel. For nanoporous HEMA-EDMA, glycine diffusivity is $3.47 \times 10^{-10} \text{ m}^2/\text{s}$, while BSA $\sim 0 \text{ m}^2/\text{s}$. For microporous HEMA-EDMA, glycine diffusivity is $6.03 \times 10^{-10} \text{ m}^2/\text{s}$, and BSA $1.42 \times 10^{-10} \text{ m}^2/\text{s}$ [3]. This indicates that diffusion of large molecules can be modulated depending upon the application of interest.

Figure 3. Scanning electron microscope (SEM) images of a microporous (a) and a nanoporous (b) HEMA-EDMA membrane. Scale bar in (a) is 5 μm and in (b) is 200 nm.

As a demonstration of the method, we've create HEMA-EDMA micro-well arrays to culture multiple bacterial strains in physical isolation while maintaining chemical communication, as shown in Fig. 4. The dimensions of each individual well can be as small as 100 μm x 100 μm with 25 μm thick walls, enabling fabrication of roughly 10^5 culture chambers on a standard 50 x 75 mm² glass slide.

In this particular study, a pair of quorum sensing (QS) strains constructed from *Escherichia coli* K12 are statistically seeded into the micro-well arrays such that initially there is on average one cell per well. The sender strain is transformed to constitutively express red fluorescent protein (RFP) and encode QS molecules (N-Acyl homoserine lactone, AHL) at high local cell concentration. The receiver strain is transformed to express green fluorescent proteins (GFP) upon receiving the AHL

Figure 4. Optical microscope image of the fabricated nanoporous HEMA-EDMA micro-well arrays. The scale bar in the image is 400 μm .

Figure 5. Co-culture of a quorum sensing pair of *E. coli*. The green fluorescence is detected because quorum sensing molecules released from the red sender cells diffuse into the wells containing receiver cells.

compound. Fig. 5 is a composite image of red fluorescence and green fluorescence. It demonstrates that QS molecules diffuse from the red cells to the neighboring wells. When the neighboring wells contain receiver cells, GFP is expressed and green fluorescence is detected.

CONCLUSIONS

In conclusion, we have presented a method of replica molding to create a microfluidic system to cultivate cells and study chemical communication. The replica molding combines PDMS soft lithography and HEMA-EDMA photopolymerization in a simple and scalable process. Using HEMA-EDMA micro-well arrays, we demonstrate that bacterial species can be physically isolated during growth while QS communication can be retained. We envision that this method can be used to engineer complex microenvironments to quantitatively study microbial interactions for both fundamental and industrial purposes. Examples include the study of synthetic

microbial communities and the search for previously uncultivated microbes from natural habitats including the soil and the gut microbiome.

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