

MICROCHIP-BASED DIALYSIS OF PROTEIN SAMPLES USING PHOTOPATTERNED NANOPOROUS MEMBRANES

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ABSTRACT

A novel method for flexibly implementing sample dialysis on microchips is introduced. Nanoporous polymer membranes (5-20 μm thickness) with up to 1000:1 aspect ratio are fabricated *in-situ* on silica microchips via photopatterned phase-separation polymerization. These membranes enable selective diffusion of molecules smaller than a cutoff specified by the membrane pore size. Diffusion measurements on-chip indicate a molecular weight cutoff near 6 kD for typical membranes; further, this molecular weight cutoff can be engineered by varying the constitution of the solvent in the polymerized solution. The dialysis membranes reported here are hydrophilic and show essentially no protein adhesion.

KEYWORDS

phase-separated polymer, filtration, dialysis, microdialysis, sample preparation, polymer monolith

INTRODUCTION

Complex samples (*e.g.*, cell extract) often require extensive cleanup before introduction to analysis channels in a miniaturized device. These processing steps are often performed off-chip using large volumes of sample and other reagents, and hence often add substantially to the total analysis time and cost. Dialysis, or size-based separation of species via selective diffusion through a semipermeable membrane, is a widely used technique for cleanup of biological samples [1,2]. We have developed a technique for fabricating thin (5-20 μm) polymer dialysis membranes within the channels of microchips. Controlled placement of the dialysis membrane(s) is accomplished using UV photoinitiated polymerization with a shaped laser beam. Laser-photopatterning of dialysis membranes is rapid and inexpensive, and the ability to place multiple membranes of varying molecular weight cutoff will increase the functionality of integrated microfluidic devices. Here we demonstrate *in-situ* fabrication of nanoporous membranes with molecular weight cutoff near 6 kD, and demonstrate mass exchange across a 1cm-long membrane in counterflow configuration.

IN-SITU PHOTOPATTERNING OF POLYMER MEMBRANES

Thin (5–20 μm) porous polymer membranes are fabricated *in-situ* in glass microchannels by shaping and focusing the 355 nm output of a frequency-tripled Nd:YAG laser into a 5-15 μm sheet. We use this laser sheet to locally excite azo photoinitiators and generate

phase-separation polymerization [3-5] in the irradiated region (Figure 1). The membrane consists of a zwitterionic methacrylate (2-methacryloyloxyethyl dimethyl 3-sulfopropyl ammonium) cross-linked with methylene bisacrylamide (Figure 2). The membrane is covalently attached to the silica surface by preceding polymerization with acid-catalyzed reaction of acrylate-functionalized organosilanes at the silica surface. The polymer membrane is precipitated from a water/2-methoxy-ethanol solution whose solvent properties dictate the membrane pore size.

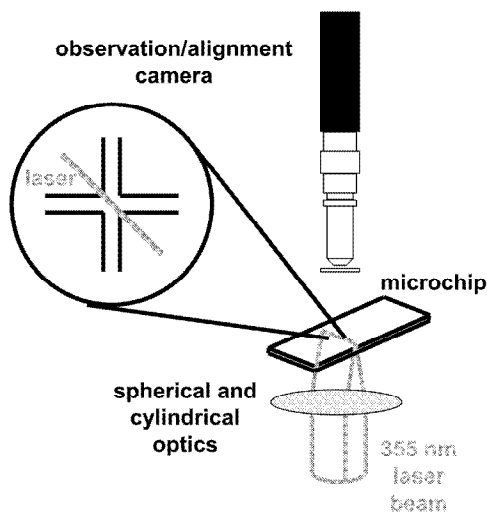


Figure 1. Laser-polymerization setup.

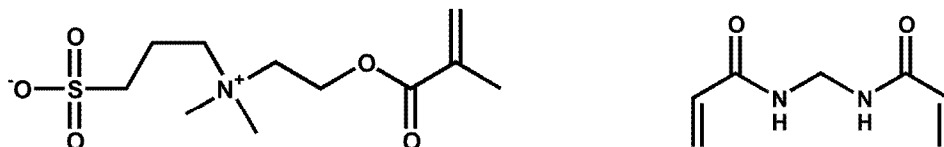


Figure 2. Polymer components of dialysis membrane. Left: Monomer (2-methacryloyloxyethyl dimethyl 3-sulfopropyl ammonium). Right: Cross-linker (methylene bisacrylamide). Zwitterionic monomer leads to a hydrophilic polymer, facilitating aqueous polymerization and minimizing protein adhesion.

MATERIALS CHARACTERIZATION

Molecular weight cutoff was determined on-chip using microscope observation of molecular diffusion of free dye (Rhodamine560), and FITC-labeled proteins with different molecular weights: monomeric insulin (5.7 kD), lactalbumin (14 kD), bovine serum albumin (66 kD), and anti-biotin (150 kD) when placed on one side of photopatterned membranes fabricated at four-port intersections. The precise nanoporous structure of these thin membranes is best evaluated *in-situ* (rather than with SEM or BET on bulk samples) since monomer transport during localized polymerization can lead to nonequi-

librium phase separation. Figure 3 shows sample results for a typical membrane: Rhodamine 560 dye diffuses through the membrane while insulin experiences barely measurable diffusion through the membrane, and lactalbumin shows no measurable diffusion. This indicates that the molecular weight cutoff for this membrane is near that of insulin (5.7kD). The larger species (>14 kD) show no diffusion and for brevity are not shown. Preliminary results show that control of molecular weight cutoff is achievable by precisely engineering the constitution of the solvents in the polymerization solution—pore sizes can be modified over two orders of magnitude through solvent changes.

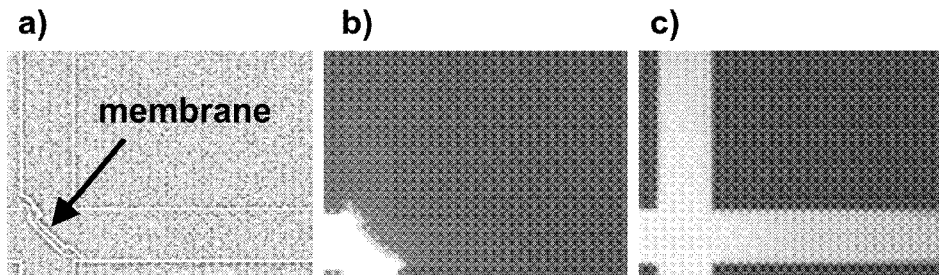


Figure 3. Determination of membrane molecular weight cutoffs with selective diffusion of fluorescent tracers. a): ~10 mm membrane patterned at 4-channel intersection. (b) when FITC-lactalbumin (14 kD) is injected at lower left, no diffusion across the membrane is observed over a 20-minute period. (c) when rhodamine 560 is injected at lower left, it immediately and quickly diffuses through the membrane after several seconds. Repeated tests with several species allowed molecular weight of this membrane to be estimated at 6 kD. The flow in the cleanup channels (upper-right) was quiescent to allow for diffusion-dominated transport.

COUNTERFLOW MASS EXCHANGE BETWEEN SAMPLE LIQUID AND PERFUSION LIQUID

A pilot, 1cm dialysis membrane was fabricated piecewise along a series of 60 support posts placed at ~150 μm spacing. The support posts facilitate rapid fabrication and maximize the pressure drop the membrane can withstand (typically 0.4-1.4 bar). Pressure-driven flow of sample liquid (colored) and counterflow of perfusion liquid (clear) was visualized in transmission (Figure 4). The effective transport over this length shows clear potential for application for on-chip processing of complex samples.

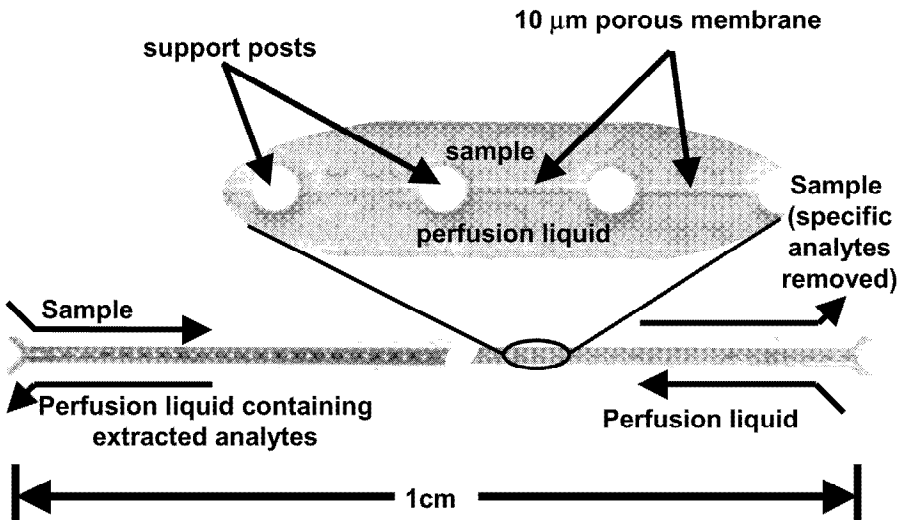


Figure 4. Dialysis membrane (1cm length) used in counterflow configuration to transfer low-molecular weight dye from sample liquid (dye+water) to perfusion liquid (water). Sample is injected at upper left, perfusion liquid is injected at bottom right. Inset: blowup showing $\sim 10 \mu\text{m}$ thin membrane fabricated between $50 \mu\text{m}$ posts.

CONCLUSIONS AND FUTURE WORK

Because the technique described allows for rapid photopatterning of dialysis membranes with programmable molecular weight cutoff, we expect this technique to allow microchip-based cleanup of (and extraction from) complex samples. In future work, we expect to integrate on-chip sample dialysis with electrokinetic analysis techniques.

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