ULTRA-RAPID SAMPLE PRECONCENTRATION UNDER SLANT FIELD USING HIGH-ASPECT-RATIO NANOPOROUS MEMBRANES

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ABSTRACT

This paper describes a novel approach to fabricate high-aspect-ratio membranes in microfluidic devices using direct laser scanning. We have demonstrated rapid sample preconcentrations in 5 sec and shown lower fM sensitivity after a 5 min preconcentraiton. The presented device can be used for continuous sample preparation, injection, preconcentration, and biochemical binding/reaction applications.

KEYWORDS: Preconcentration, Continouos photopolymerization, Electrophoresis

INTRODUCTION

Continuous-flow (CF) biosample preconcentration (preparation) techniques are highly desired because they allow a non-stop sample injection/collection and real-time monitoring. Compared to batch procedures with limited throughput, CF preconcentraiton/separation can provide faster sample processing and do not require a precise and well-timed injection. As a result, CF techniques can be integrated into μTAS devices with minimum interferences between each other.[1] We present a novel approach for rapid and high throughput sample preconcentration using a high-aspect-ratio membrane fabricated *in-situ* with a novel continuous photopolymerization process. Rapid preconcentrations were made possible by continuously cross-linking selective-exclusion polyacrylamide membranes across a 1 mm wide microchannel using a shaped laser beam. A 40 μm wide membrane leads from one side of the sample loading channel into the entrance of the separation channel on the other side at 60^{o} angle as shown in **Figure 1**. Proteins can then be collected rapidly (within 5 sec) at the edge of the membrane due to the slant against the field and can then be transferred into the separation channel seamlessly in a two-step process shown in **Figure 2,** both images used Alexa Fluor 488 labeled 0.44 nM ovalbumin sample and have been superimposed with a bright field image. While membrane-based microfluidic devices have been proven a viable platform to perform multi-step analyses, the preconcentration step, however, has a limited accumulation rate governed by the maximum voltage that can be applied before joule heating or exclusion threshold is exceeded. In this contribution, we have demonstrated that high-aspect-ratio size exclusion membranes can increase the stacking rate and sensitivity (10 fM) while limiting the applied electric field.

EXPERIMENTAL

The microfluidic device was made from quartz wafers and etched to a thickness of 25 μm before bonding. The width is 1 mm for the sample loading/preconcentration channel and 70 μm for the separation channel. The preconcentration membrane was polymerized by a shaped UV laser beam (15 mW) on a transi-

tional stage at a speed of 40 μm/sec. The loading side was then casted with 3.5% polyacrylamide gels to eliminate electroosmosis flows. In this device, the full stacking advantage can only be realized when samples are perfectly pinched to the dimensions of the analysis channel and the ideal net gain in stacking/preconcentration efficiency is equal to the ratio of channel cross sectional areas (in this case 14-fold). The >10-fold improvement over the standard membrane configuration demonstrates that focusing was nearly ideal. The 60^{o} angle effectively guides the electrophoretic transport of excluded species to a focal point at the entrance of the separation channel. Compared with the diagnostic assay we reported in 2007,[2, 3] this device has ~30 times longer preconcentration membrane and provides at least 10-fold faster preconcentration speed, **Figure 3**. The signal enhancement was plotted in **Figure 4**.

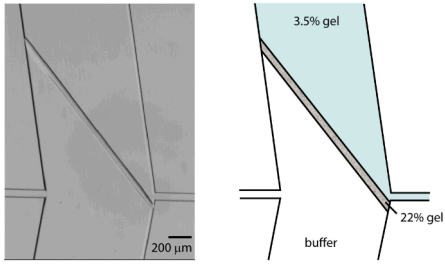

Figure 1: (left) Bright filed image of the device with laser patterned high-aspect-ratio polyacrylamide membrane (22% polymer solution with 6% cross linker), (right) schematic drawing of the device showing the boundaries between 3.5% gel, 22% gel and the buffer

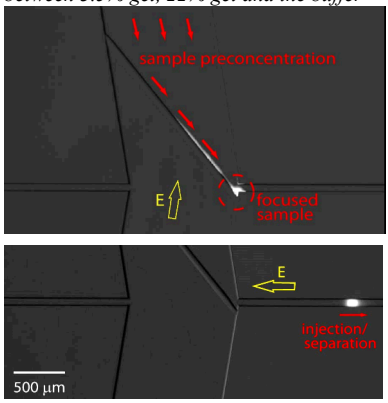

Figure 2. Device operation illustrated by fluorescent images: (top) the preconcentration step, 20 V/cm field was applied across the membrane, samples are focused at the corner due to the field effect; (bottom) the separation step, field was applied across the separation channel

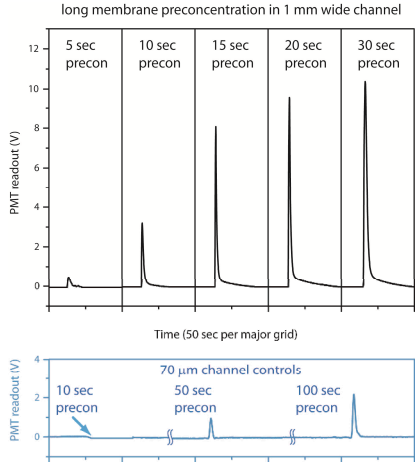

Figure 3. Ultra-rapid 4.4 pM ovalbumin sample preconcentration and control experiments: (top) Electrophoregram of ovalbumin protein plugs with various preconcentration time from 5 to 30 seconds, the same intensity level achieved by 5 sec long gel preconcentration requires ~50 sec in the control experiment; (bottom) Control experiments using 70 μm wide devices with a double-T injector, no peaks were detected if preconcentrated for only 10 seconds (both detection points located 5 mm below the injection point with a field strength around 25 V/cm)

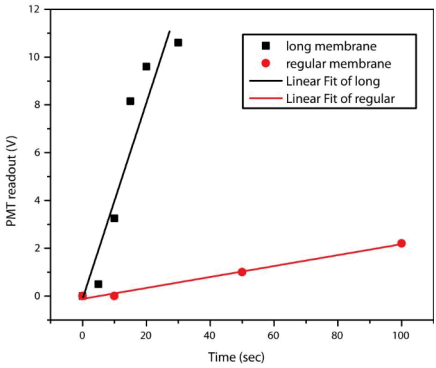

Figure 4. Plots from the high-aspect-ratio membrane device (black) shows 10 times preconcentration speed enhancement compared with previously reported device with a 70μm preconcentration membrane [2](red)

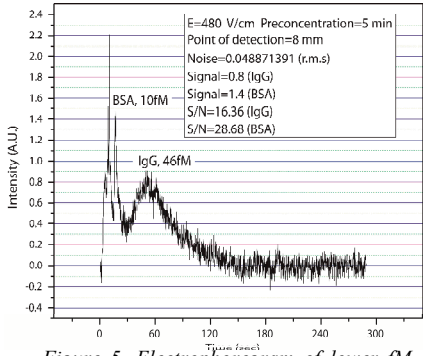

Figure 5. Electrophoregram of lower fM BSA and goat antibody at 480 V/cm after preconcentration for 5 min under 15 V/cm slant field

The gain in preconcentration rate enables the device to address most preconcentration needs for pg/ml biotoxin within 30 seconds. Such agileness is critical since a rapid identification is the key for effective disease/infection treatments and spread controls.[4]

RESULTS AND DISCUSSION

The high-aspect-ratio polyacrylamide membrane provides perfect fluid isolation and its aspect ratio (1:50) is among the highest reported. Since its continuous polymerization relies on the transactional stage rather than complex optics, there is no practical limit on the span of the membrane. We will also present recent studies on desalting efficiency with respect to gel charge polarities. With its simplicity and seamless interfacing, the high-aspect-ratio nanoporous membrane enables rapid (<5 sec) preconcentration and can be applied to biosample pre-treatment, purification, desalting, mixing, and biochemical binding/reaction applications.

CONCLUSIONS

The continuous memberane patterning technique presented in this work can be applied to different continuous flow sample preparation procedures. Because the filtration property of acrylamide gels can be tailored by researchers, having these high-aspect-ratio membranes as part of a microfluidic conduit can significantly improve the throughput and functionality of the various microfluidic assays and can lead to breakthroughs in various areas including: continuous biosample fractionation, encapsulation, desalting, and dialysis.

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