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

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ABSTRACT

We describe a novel approach to fabricate high-aspect-ratio membranes in microchannels by direct laser scanning, and demonstrate >10-fold improvement in sample preconcentration speed by achieving lower fM detection of proteins within 5 minutes. The integrated device can be used for continuous sample preparation, injection, preconcentration, and biochemical binding/reaction applications.

KEYWORDS: Preconcentration, Continuous photopolymerization, Electrophoresis

INTRODUCTION

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. The device addresses the need for rapid detection of low abundance proteins including trace toxins and cytokines. 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 relatively wide (1-2 mm) sample loading channel to the entrance of the narrow (0.08 mm) 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 then seamlessly transferred into the narrow separation channel in a two-step process shown in Figure 2. While membrane-based microfluidic devices have been proven a viable platform to perform multi-step analyses, the preconcentration step 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 translational stage at a speed between 5 to 40 μm/sec. The thickness of the membrane can be controlled based on the scanning speed. The remaining polymer solution was then washed away by buffer solution and filled with 3.5% polymer on one end before a flood exposure to eliminate electro osmosis flows.

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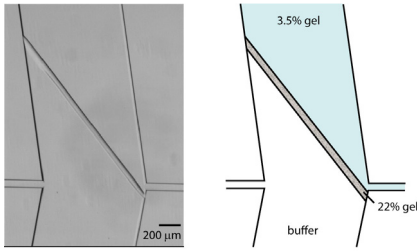

Figure 1. (left) Bright field image of the device with laser patterned high-aspect-ratio polyacrylamide membrane (22% polymer solution with 6% cross linker), (right) schematic drawing of the device showing the boundaries between 3.5% gel, 22% gel and the buffer solution

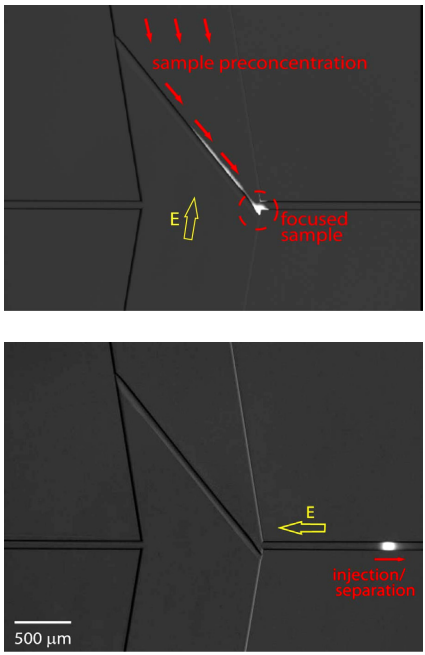

Figure 2. Device operation illustrated by fluorescent images: (top) the preconcentration step, 20 V/cm field was applied across the membrane, samples are focused at the corner due to the field effect; (bottom) the separation step, field was applied across the separation channel (both images used Alexa Fluor 488 labeled 0.44 nM ovalbumin sample and have been superimposed with a bright field image)

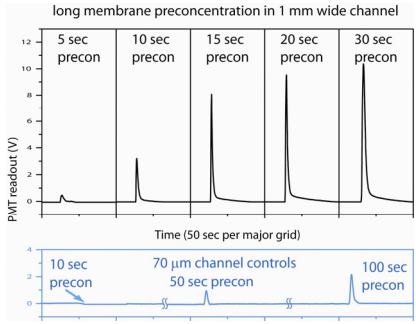

Figure 3. Ultra-rapid 4.4 pM ovalbumin sample preconcentration and control experiments: (top) Electrophoregram of ovalbumin protein elution with various preconcentration time, the same intensity level achieved by 5 sec long gel preconcentration requires ~50 sec in the control experiment; (bottom) Control experiments using 70 µm wide devices with a double-T injector

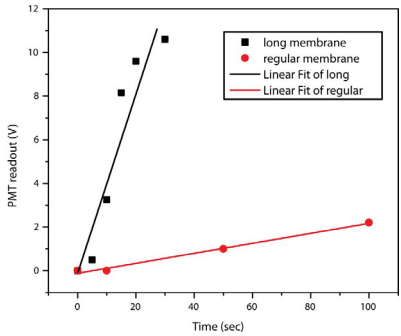

Figure 4. Plots from the high-aspect-ratio membrane device (black) shows 10 times preconcentration speed enhancement compared with previously reported 1:1 precon: separation channel configuration [1] (red)

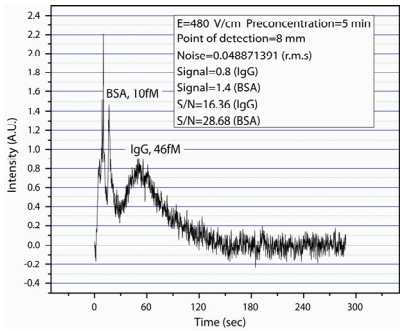

Figure 5. Electrophoregram of lower fM BSA and goat antibody separated at 480 V/cm after preconcentration for 5 min under 15 V/cm slant field

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 translational stage rather than complex optics, there is no practical limit on the span of the membrane. In this device, the full stacking advantage can only be realized when samples are 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 previous 1:1 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, [1, 2] this device has ~30 times longer preconcentration membrane and provides at least 10-fold faster preconcentration speed with <10 fM sensitivity within 5 minutes, Figure 3-5. The gain in preconcentration rate enables the device to address most preconcentration needs for pg/ml analyte within 30 seconds. Such agileness is critical since sensitive and rapid identification are key for rapid diagnostics at point of incident/outbreak .[3] These membranes have also been successfully implanted on desalting devices with high efficiency.

CONCLUSIONS

With the simplicity and seamless interfacing of high-aspect-ratio nanoporous membrane we successfully address needs for faster integrated preconcentration. The continuous membrane patterning technique presented in this work has broader implications for membrane-based applications and continuous flow sample processing and analysis. The filtration properties of polymer monoliths can be tailored a number of ways by researchers (size/charge/field/pH selectivity) for diverse utility. High-aspect-ratio membranes can significantly improve throughput and functionality thereby impacting microfluidic techniques and may also lead to novel breakthroughs. Applications include biosample pre-treatment, purification, desalting, mixing, and biochemical binding/reaction applications.

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