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Quantitative evaluation of the depletion efficiency of nanofractures generated by nanoparticle-assisted junction gap breakdown for protein concentration

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ABSTRACT

Sample preconcentration is crucial to the accuracy of biochemical or clinic detection efforts, particularly for samples with extremely low protein concentrations. Overlapped electrical double layers in nanofluidic channels can generate concentration polarization through the application of an electric field. This induces nonlinear electro-kinetic flow and results in the exclusion-enrichment effect: the rapid accumulation of proteins in front of the induced ionic depletion zone. This study created nanofractures to preconcentrate proteins via the exclusion-enrichment effect, in which protein samples are driven by electro-osmotic flow to accumulate at a specific location. A preconcentration chip was fabricated by standard soft lithography using a polydimethylsiloxane replica. Nanofractures were formed by nanoparticle-assisted electrical breakdown. This study also developed a simple method of quantitatively evaluating the depletion efficiency of nanofractures, whereby proteins are stacked in the area at which the microchannels intersect by balancing the depletion force with the driving force produced by electro-osmotic flow (EOF). The proteins leak into the left reservoir when the driving voltage of EOF is higher than that necessary for stacking; i.e., the depletion force is less than the driving force of EOF. Increasing the depletion force works to repel the proteins toward the lower corner of the measurement region, close to the intersection of the microchannels. An increase in depletion force expands the area without proteins and the relative size of this area can be measured using grayscale and binary images to evaluate the depletion efficiency. The proposed deposition of gold nanoparticles at the junction gap between microchannels greatly reduces the electrical breakdown voltage required for the formation of nanofractures.

1. Introduction

Sample pre-concentration is essential to the accuracy of biochemical or clinic detection results, particularly for samples with extremely low solute concentrations. Several techniques have been developed for the pre-concentration of proteins [1], including field-amplified sample stacking (FASS) [2], isotachophoresis (ITP) [3], isoelectric focusing (IEF) [4], temperature gradient focusing (TGF) [5], nanofilters [6], and nanoporous membrane/nanochannel techniques [7–10]. Unfortunately, these procedures can be very complicated. FASS, ITP, and IEF require at least two kinds of buffer solution; TGF requires precise temperature control because the electrophoretic velocity of the analyte changes as a function of temperature. To compensate for these difficulties, [6] proposed filtering analytes through a nanoporous membrane or nanochannel and [11] employed the exclusion-enrichment effect in nanopores or nanochannels for the concentration of analytes. The overlapping of electrical double-layers in the nanofluidic channel enables ion-permselectivity, in which counterions may pass through the nanochannel but co-ions are excluded. Under an electric field, concentration polarization leads to ionic depletion on the anodic side of the nanochannel. As a result, ions are enriched at one end and depleted at the other end of the nanochannel. This process is termed ion-enrichment and ion-depletion (IEID) [11]. Inducing nonlinear electrokinetic flow causes the rapid accumulation of proteins in front of the induced ionic depletion zone, the so-called exclusion-enrichment effect. The major benefit of this method is its use of simple buffer systems [12]. Several methods have been developed for the fabrication of nanochannels/nanopores [13]. Wang et al. [7] proposed a microdevice with nanofluidic channels fabricated using standard photolithography and etching techniques to generate an extended space charge region which electrokinetically collects and traps proteins at concentration factors as

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high as 10^6–10^8 folds. Porous membrane techniques offer the advantage of being able to incorporate commercially available membranes onto microchips. Moreover, polydimethylsiloxane (PDMS) microdevices integrated with polycarbonate track etched (PCTE) membranes with 10-nm nanopores [6] have been shown to produce an accumulation factor of 10^5–10^6. Previously, a highly ion-conductive charge-selective polymer, poly-AMPS (2-acrylamido-2-methyl-1-propanesulfonic acid), was applied to a preconcentration system for microfluidic samples [14] and achieved a concentration factor of 10^3 in tetramethylrhodamine isothiocyanate (TRITC)-tagged bovine albumin after only 20 min. Nafion resin is a highly porous ion-selective material, which has been widely integrated with PDMS/glass-based microfluidic chips. In one study, the preconcentration of a multiplexed proteomic sample achieved a concentration factor of 10^4 in only 5 min [8]. Kim and Han [9] extended their previous work [8] by developing a simple concentration method which integrated polymeric nanoporous junctions within a PDMS microchip. The PDMS gap created by mechanical cutting was infiltrated with a Nafion polymer solution that subsequently self-sealed. In this study by Kim and Han, the preconcentration of β-phycoerythrin proteins in large channels (dimensions: 1000 μm (width) × 100 μm (depth)) achieved a concentration factor of 10^4. Moreover, a massive array of 128 parallel nanofluidic concentration microdevices with Nafion nanoporous junctions for high-throughput biomolecule detection has greatly increased the dynamic range of immunoassays [15]. Unfortunately, nanoporous membranes are not easily embedded into microchips without the leakage of liquid. Photopolymerization can help to overcome this problem; however, this approach requires a complex optical setup and careful operation [16]. Nonetheless, a simpler technique using the junction-gap electrical breakdown between two PDMS microchannels has also been developed for the fabrication of nanochannels or nanofractures [17]. This method applies high voltages to form nanogaps between the PDMS microchannels. Applying direct-current (DC) voltage of 1000 V between microchannels 40 μm in width produces a corresponding electric field of 25 V/μm, which is slightly greater than the dielectric strength of PDMS (21 V/μm). This results in a nanogap with a depth of approximately 80 nm. Using this technique, Lee et al. [17], achieved a concentration factor of 10^4 within 1 h. Kim et al. [18] used the same technique to spontaneously form nanochannels beneath a PDMS layer reversibly bonded to a glass substrate. The resulting microchip with chevron-shaped microchannels in a mirror-image orientation achieved a concentration factor of between 10^3 and 10^6 in 30 min. However, the reversible bonding between PDMS and glass substrate is less robust than the permanent bonding obtained using oxygen-plasma treatment. [16] presented a microchip with two printed V-shaped microchannels in a mirror-image orientation, separated by a 100-μm gap. Nanofractures were then formed by electrical breakdown under a high electric field, resulting in concentration factors of between 10^3 and 10^5 for proteins.

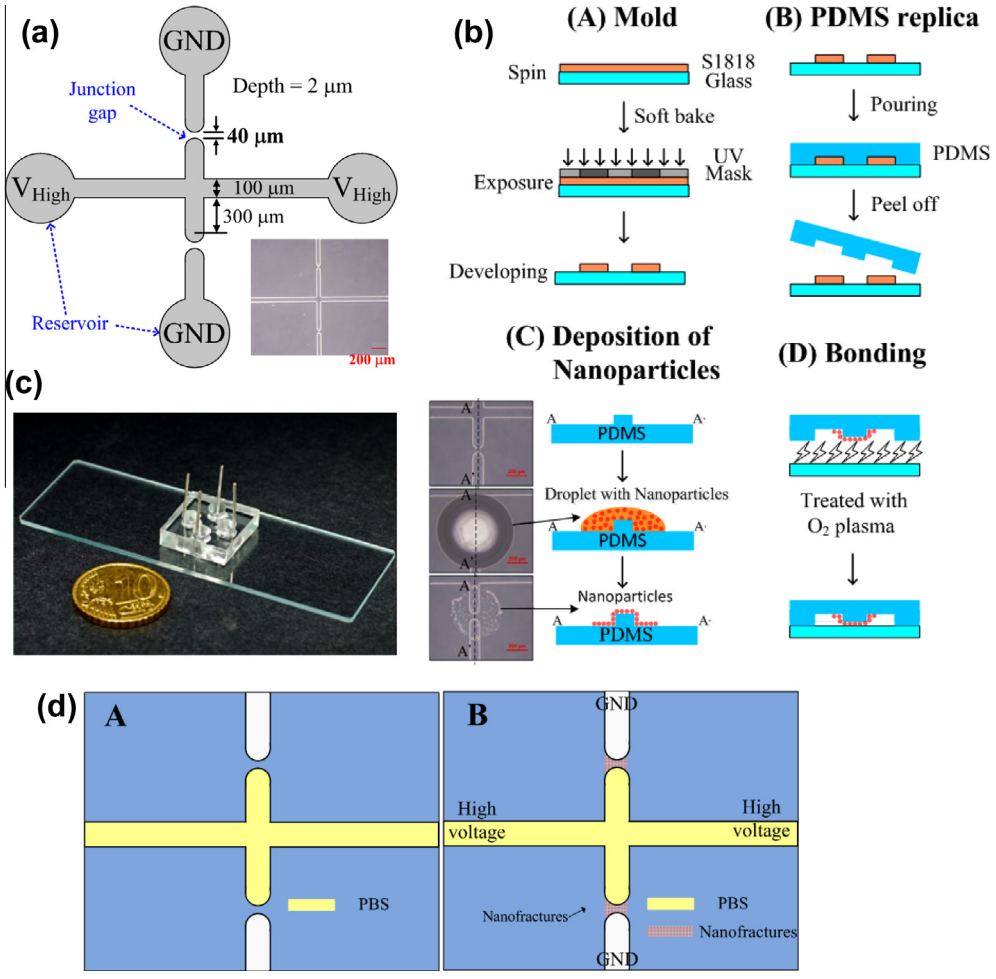

Fig. 1. (a) Layout and dimensions of the preconcentration chip (optical microscopy image in inset); (b) schematic diagrams of fabrication processes and nanoparticle deposition to assist electrical breakdown of junction gaps; (c) image of fabricated chip with inserted electrodes; and (d) illustration of nanofracture formation for protein preconcentration.