A DIFFUSION-DEFINED PHOTOPOLYMERIZATION PROCESS FOR POLYACRYLAMIDE GRADIENT GELS FOR ON-CHIP PROTEIN SIZING

C.T. Lo[1,3], D.J. Throckmorton[2], A.K. Singh[2], and A.E. Herr[2,4]

[1]*Yale University, USA*

[2]*Sandia National Laboratories, USA*

[3]*now at National Institute of Standards and Technology, USA*

[4]*now at University of California, Berkeley, USA*

ABSTRACT

We report on a new technique to generate linear and non-linear pore-size polyacrylamide gradient gels in a microfluidic format. Diffusion of low viscosity polymer precursor solutions and a two-step photopatterning process were used to define the decreasing pore-sizes in polyacrylamide gels. We characterize the gradient gels and assess the performance for on-chip protein sizing. Baseline resolution of six proteins was demonstrated in 4 s using linear gradient gels.

KEYWORDS: photopatterning, gradient gel, fabrication, electrophoresis

INTRODUCTION

For decades, gradient "slab gels" have proven effective for high resolution protein sizing [1,2]. We present here development and characterization of on-chip polyacrylamide gradient gels for protein and DNA analysis. Using an *in situ* photopolymerization process to cross-link a diffusion-defined acrylamide concentration gradient, we readily tune the slope of the resulting polyacrylamide pore-size gradient. In this work, we detail: i) a novel fabrication method that combines multi-step photolithography and well-controlled molecular diffusion and ii) characterization of the gradient gels for two canonical cases: a short and a long diffusion interval, relative to the diffusivity of the acrylamide monomer. The on-chip gradient gels are relevant to further extending the power of bioanalytical systems, particularly for translational instruments.

Pressure-driven channel-filling methods adapted from the macroscale (e.g., syringe pumps) are fraught with stringent fabrication demands when adapted to microdevices [3]. Difficulty in controlling precursor flow rates and filling times also leads to low reproducibility in concentration profiles [4]. Here we build on an elegant approach for generating gradient gels that has been reported for capillaries and is based on diffusion processes [5]. To define the gradient, we employ a "diffusion interval" between serial introductions of two polyacrylamide precursor solutions. Original to our work, we utilize two photopatterning steps to cross-link the diffusion-defined acrylamide concentration profile which results in the pore-size gradient gel [6]. We demonstrate use of the diffusion-based method for fabrication of decreasing pore-size gels, analogous to standard gradient slab gels.

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EXPERIMENTAL

Figure 1 outlines the two-step process for fabricating gradient polyacrylamide gels in standard quartz microfluidic chips. The protocol includes: i) fabrication of a small pore-size polyacrylamide gel plug at the outlet of the separation channel *via* photomasking and UV exposure (Figure 1A), ii) pressure-filling of a second different composition acrylamide precursor solution into the loading channels (Figure 1B) and iii) subsequent defined "diffusion interval" followed by iv) a 15-min flood UV exposure step (Figure 1D). Electrophoresis is then performed using standard protocols.

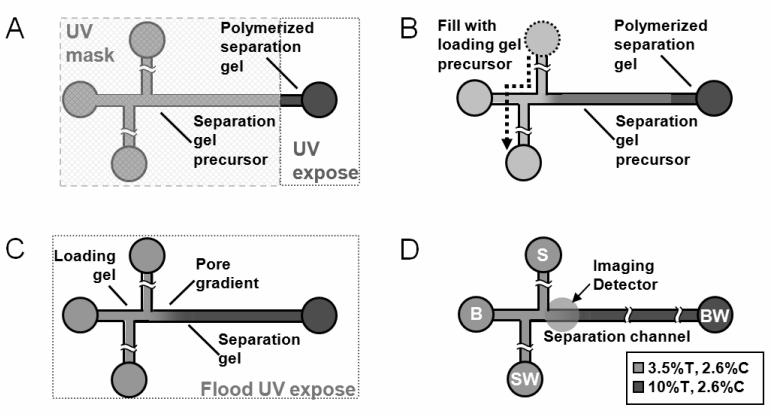

Figure 1. Photopatterning defines gradient in sieving gel pore-size. Sample (S), sample waste (SW), buffer (B), and buffer waste (BW).

To demonstrate protein sizing, a fluorescently labeled molecular weight ladder containing six species (from 20.1 kDa to 205 kDa) was analyzed on the gradient gels. Here we show results for two gel fabrication diffusion intervals: 0 min (? t_0) and 120 min (? t_{120}). The two intervals were used to fabricate 3.5%T to 10%T (large to small pore size) gradient gels. Proteins were prepared for sizing per manufacturer instructions. Epi-fluorescence imaging (IX-70, Olympus and CoolSnap HQ, Roper) with a 4× objective was employed to monitor protein migration along the separation axis. Image analysis was performed using ImageJ software (NIH, Bethesda, MD).

RESULTS AND DISCUSSION

Results of the gradient profiles, as well as the baseline resolution difference between the two generated gradient gels, are presented in Figure 2. As expected, longer diffusion intervals (? t_{120}) produce linear gradient gels. According to our analytical model of non-steady state diffusion (not discussed here), we anticipate that an overnight (15 h) diffusion interval would yield a linear polyacrylamide pore-size gradient over 0.66 cm in separation length. Given that our 0.3 cm separation length is sufficient to resolve protein species ranging from 20.1 to 116 kDa, we would expect the molecular weight range of resolvable species to extend with double the separation length.

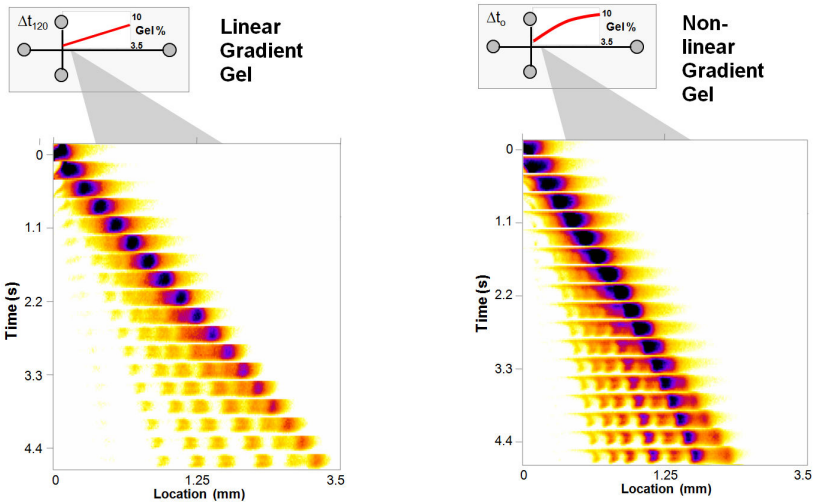

Figure 2. Protein mobility reflects (left) linearity and (right) non-linearity of gel pore-size gradient. Linear gradient fabricated with 120 min interval, 0 min interval was used for non-linear gradient. E = 298 V/cm.

CONCLUSIONS

We have successfully demonstrated protein sizing in short (0.3 cm) gradient gels. Such efficient electrophoretic analysis in ultra-short separation lengths has noteworthy implications: mainly that low applied electric potentials are needed to achieve the required electric field strength for protein separations. This property is especially important for translation of the microanalytical system into clinical settings. The fabrication methods reported here is amenable to adaptation to non-sizing protein assays, as well as integration with upstream sample preparation steps and subsequent downstream multi-dimensional assays.

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REFERENCES

[1] J. Margolis and K.C. Kenrick, Nature 214(5095), pp. 1334-6, (1967).
[2] D. Rodbard and A. Chrambach, Anal Biochem 40(1), pp. 95-134, (1971).
[3] H.S. Chen and H.T. Chang, J Chromatogr A 853(1-2), pp. 337-47, (1999).
[4] J Margolis and K.C. Kenrick, Anal Biochem 27(2), pp. 319-22, (1969).
[5] R. Ruchel, J Histochem Cytochem 24(7), pp. 773-91, (1976).
[6] C.T. Lo, D.J. Throckmorton, A.K. Singh and A.E. Herr, Lab Chip, DOI: 10.1039/ b804485f, (2008).