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Use of preconditioning to control membrane fouling and enhance performance during ultrafiltration of plasmid DNA

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ABSTRACT

Several recent studies have demonstrated that small pore size ultrafiltration membranes can be used for purification of supercoiled plasmid DNA for therapeutic applications, but the performance of these membrane systems is severely limited by membrane fouling. The objective of this work was to examine the potential of pre-conditioning, in this case accomplished by pre-elongating the DNA by passage through a region with large pore size, to minimize fouling and enhance DNA separations. Data were obtained using both asymmetric hollow fiber membranes, with flow in either the normal or reverse orientation, and with composite membrane structures made by placing a larger pore size flat sheet microfiltration membrane in series with an ultrafiltration membrane. In all cases, flow through the larger pore size region pre-stretched the plasmid, leading to an increase in plasmid transmission and a significant reduction in fouling. This pre-conditioning also provided a significant increase in selectivity for separation of the linear and supercoiled isoforms. These results clearly demonstrate the potential for dramatically increasing the performance of membrane systems for plasmid DNA separations by controlling the pore morphology to pre-stretch the DNA before passing through the narrow pores of an ultrafiltration membrane.

1. Introduction

There is growing interest in the use of plasmid DNA for gene therapy and DNA-based vaccines [1,2]. Membrane processes are highly attractive for plasmid DNA purification since they avoid the problems of low binding capacities and slow diffusional mass transfer that limit the performance of many chromatographic processes. Membranes can be used for sterile filtration [3], DNA concentration by ultrafiltration [4], and DNA isoform separation [5,6]. Isoform separations are possible using small pore size ultrafiltration membranes in which the DNA elongates in the flow field above the membrane pores; the different transmissions of the supercoiled, linear, and open-circular isoforms are due to differences in the elongational flexibility of the DNA [5].

One of the challenges in DNA ultrafiltration is membrane fouling [7–9]. Borujeni and Zydney [7] showed that membrane fouling increased dramatically at high feed concentrations, with the extent of plasmid transmission decreasing rapidly during the ultrafiltration process due to the partial blockage of the membrane pores by individual plasmids that became trapped at the pore entrance. The rate of fouling was greater for the larger plasmids due to the greater probability of a plasmid getting trapped at the pore entrance. This trapping phenomenon was likely due to the incomplete extension of the plasmid in the converging flow field into the membrane pores and/or the increased likelihood of "knot" formation in the larger plasmids [10].

Plasmid "trapping" has also been observed in micro-/nano-fluidic systems designed for DNA separations, manipulations, and sequencing [11]. A variety of approaches have been used to facilitate DNA extension by "pre-conditioning" the DNA in nano-fluidic systems, e.g., by applying shear forces, passing the DNA through a gel matrix or an array of nano-obstacles [12], or using a conically-shaped constriction to gradually elongate the DNA [13]. For example, Cao et al. [12] used a gradient array of nanostructures with decreasing spacing to enhance the extension of long DNA molecules; DNA that passed through the nanostructured array was able to enter narrow slit-shaped nanopores under conditions in which the DNA was trapped at the pore entrance in the absence of "pre-conditioning" by the nanostructured array.

The objective of this study was to examine the effect of "pre-conditioning" on the transmission and fouling behavior of plasmid DNA during ultrafiltration. Data were obtained with asymmetric hollow fiber ultrafiltration membranes, oriented with the flow through either the skin or the substructure first, and with composite

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membranes formed by placing a microfiltration membrane immediately on top of a small pore ultrafiltration membrane. The results were analyzed using available models for polymer elongation, with the data used to identify conditions that could provide enhanced separation of the plasmid DNA isoforms. The results clearly demonstrate the potential of controlling the membrane pore morphology to pre-condition the DNA, reducing fouling and enhancing the performance of membrane systems for DNA purification.

2. Materials and methods

2.1. Materials

Ultrafiltration experiments were performed with hollow fiber polysulfone (PS) membranes obtained from GE Healthcare (Niskayuna, NY) with nominal molecular weight cutoffs of 50 kDa (UFP-50-C-03 M) and 500 kDa (UFP-500-C-03 M). These membranes are highly asymmetric, with the tight "skin" on the inner surface of the fiber lumen. Each module contained 30 hollow fibers with 5×10^{-4} m (0.5 mm) ID and 0.3 m length, giving 0.014 m^2 of membrane area. The membranes were initially flushed with buffer for a minimum of 30 min to remove any storage or wetting agents. Modules were stored at 4 °C between experiments.

A limited number of experiments were conducted with Ultracel composite regenerated cellulose membranes (EMD Millipore, Billerica, MA) with nominal molecular weight cutoff of 100 kDa. These membranes were used in a composite (sandwich) structure with a Durapore hydrophilic polyvinylidene fluoride (PVDF) membrane with pore size of 0.22 μm (EMD Millipore). Membrane discs (25 mm in diameter) were cut from large flat sheets using a specially designed cutting device. All flat sheet membranes were initially soaked in isopropyl alcohol and then flushed with water to remove residual storage agents and to insure thorough wetting of the pore structure.

Buffer solutions were prepared by 100× dilution of Tris–EDTA (TE) concentrate with DI water obtained from a Barnstead International Nanopure water purification system (Thermo Scientific, IL). The ionic strength was adjusted by addition of NaCl; the solution conductivity was measured using a Thermo Orion 150A plus conductivity meter. All solutions were prefiltered through 0.2 μm pore size Supor® 200 disc filters obtained from Pall Corporation (Port Washington, NY).

200×10^{-6} kg/m^3 (μg/mL) stock solutions of 3.0 and 16.9 kbp (kilo-base pair) supercoiled plasmids were prepared by Aldevron (Fargo, ND) and stored frozen at −20 °C. A small amount of the stock solution was thawed and diluted with TE buffer containing 150 mM NaCl immediately prior to use in the ultrafiltration experiment. The linear and open-circular isoforms were prepared in our laboratory by enzymatic digestion of the supercoiled isoform using restriction and nicking endonucleases (New England Biolabs, MA), respectively. For each digestion, 100 μg of the supercoiled isoform was mixed with 1 U/μg of the appropriate enzyme along with the recommended buffer concentrates. The mixtures were incubated at 37 °C for 3 h, with residual enzyme removed by the QIAQuick PCR purification kit (Qiagen, CA). Additional details are provided elsewhere [5].

2.2. Assays

Plasmid concentrations were determined using the Quant-iT PicoGreen dsDNA assay kit (Life Technologies, Carlsbad, CA). The PicoGreen solution was prepared by 10,000× dilution of the concentrated dye with DI water and the analytical TE buffer provided by the manufacturer. 70 μL of each sample and an equal amount of the PicoGreen reagent were loaded into separate wells of a 96-well microplate (VWR, Philadelphia, PA). The microplate was inserted into a TECAN GENiosFL microplate reader, mixed by orbital shaking for 10 min, and the fluorescence intensity at 530 nm measured after excitation at 485 nm. Actual concentrations were determined using calibration standards included on each microplate. The quality of the plasmid stock solutions and the effectiveness of the enzymatic digestions were examined by the agarose gel electrophoresis (AGE) following the procedures described by Borujeni and Zydney [7]. AGE was also used to estimate the relative concentrations of the different isoforms in separation experiments using mixtures of the linear and supercoiled isoforms.

2.3. Plasmid ultrafiltration

The membrane permeability (L_p) was evaluated from data for the filtrate flux (J_v) as a function of the transmembrane pressure (ΔP) as:

$$L_p = \frac{J_v}{\Delta P} \tag{1}$$

For the hollow fiber membranes, the buffer solution was pumped into the module (through the fiber lumens) using a Masterflex peristaltic pump at a constant feed flow rate of 100 mL/min, with the transmembrane pressure adjusted using a ball valve installed on the exit retentate line. The volumetric filtrate flow rate was measured by timed collection of the permeate using an AG104 Mettler-Toledo analytical balance.

Plasmid ultrafiltration experiments were conducted in both the normal (from lumen to shell) and reverse flow directions. During normal operation, the feed flow was introduced into the fiber lumen with permeate withdrawn through the shell. The module was oriented horizontally with the upper permeate port kept closed. During "reverse" operation, the feed flow was introduced into the shell, with filtration occurring through the membrane substructure and then the skin, with the filtrate collected from the lumen exit. In both cases, data were obtained in total recycle mode, with the retentate and permeate lines recycled back to the feed reservoir to maintain a uniform plasmid concentration. Filtrate samples were collected periodically throughout the ultrafiltration experiment to evaluate the plasmid concentrations.

Ultrafiltration experiments with flat sheet membranes were conducted in a 25 mm diameter stirred ultrafiltration cell (Millipore) as described by Latulippe and Zydney [5]. The membrane disk was placed at the bottom of the stirred cell, either alone or in a sandwich configuration with a 0.22 μm pore size Durapore membrane. The stirred cell was carefully sealed, with the permeability evaluated to verify that there was no damage to the membrane or problems with sealing the two membranes in the base of the cell. The stirred cell was then filled with the plasmid solution and air pressurized, with the transmembrane pressure set using a pressure regulator.

3. Results and discussion

3.1. Membrane orientation

Fig. 1 shows data for the observed sieving coefficient of the 16.9 kbp supercoiled plasmid during ultrafiltration of a 3 μg/mL solution through a 500 kDa hollow fiber membrane in both the forward (lumen-to-shell) and reverse (shell-to-lumen) orientations. Data were obtained at a feed flow rate of 1.7×10^{-6} m/s (100 mL/min) using a constant transmembrane pressure of 55 kPa (8 psi), yielding an initial filtrate flux of 45 μm/s (160 L/m^2/h) in both orientations, consistent with the very similar values of the