DEVELOPMENT OF NOVEL MEMBRANE STRUCTURES FOR ENHANCED PURIFICATION OF PLASMID DNA USING SMALL PORSE SIZE ULTRAFILTRATION MEMBRANES

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Several recent studies have demonstrated that small pore size ultrafiltration membranes can be used for purification of supercoiled plasmid DNA for use in both gene therapy applications and as DNA-based vaccines. Plasmid transmission during ultrafiltration is governed by the elongation of the large DNA molecules in the converging flow-field entering the membrane pores. However, the performance of these membrane systems is severely limited by membrane fouling, and the selectivity for the separation of the DNA isoforms is often low. The objective of this work was to examine the potential of using "pre-conditioning", in this case accomplished by pre-elongating the DNA by passage through a region with larger pore size, to minimize fouling and enhance DNA separations.

Stock solutions of 3.0 and 16.9 kbp (kilo-base pair) supercoiled plasmids were prepared by Aldevron; solutions of the linear and open-circular isoforms were generated by enzymatic digestion of the supercoiled DNA. Ultrafiltration experiments were performed using both asymmetric hollow fiber polyethersulfone ultrafiltration membranes, with flow in either the normal or reverse orientation, and with composite (layered) 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 caused an increase in plasmid transmission and a significant reduction in fouling by pre-stretching (or pre-conditioning) the DNA. This facilitated transmission of the large DNA through the small pores of the ultrafiltration membrane while simultaneously minimizing DNA trapping at the pore entrance. Results for the critical filtrate flux for DNA transmission were in good qualitative agreement with available theories for the flow-induced elongation of flexible polymer chains.

The pre-conditioning also provided a significant increase in selectivity for separation of the linear and supercoiled isoforms, in this case by using conditions where only the linear isoform was elongated sufficiently to pass through the membrane pores. The effectiveness of the pre-conditioning was a function of the pore size and morphology of the top layer in the composite membrane structure. The best performance was obtained using a pre-conditioning layer that had a pore size that was approximately the same as the radius of gyration of the plasmid. The use of larger pores in the pre-conditioning layer provided insufficient pre-stretching of the DNA, while the use of smaller pores led to DNA trapping at the entrance to the pre-conditioning layer. These results clearly demonstrate the potential for dramatically increasing the performance of membrane systems for plasmid DNA separations by controlling the membrane pore morphology.