Using A New, Fast Flow, Low Protein Binding Membrane for Sterile Filtration

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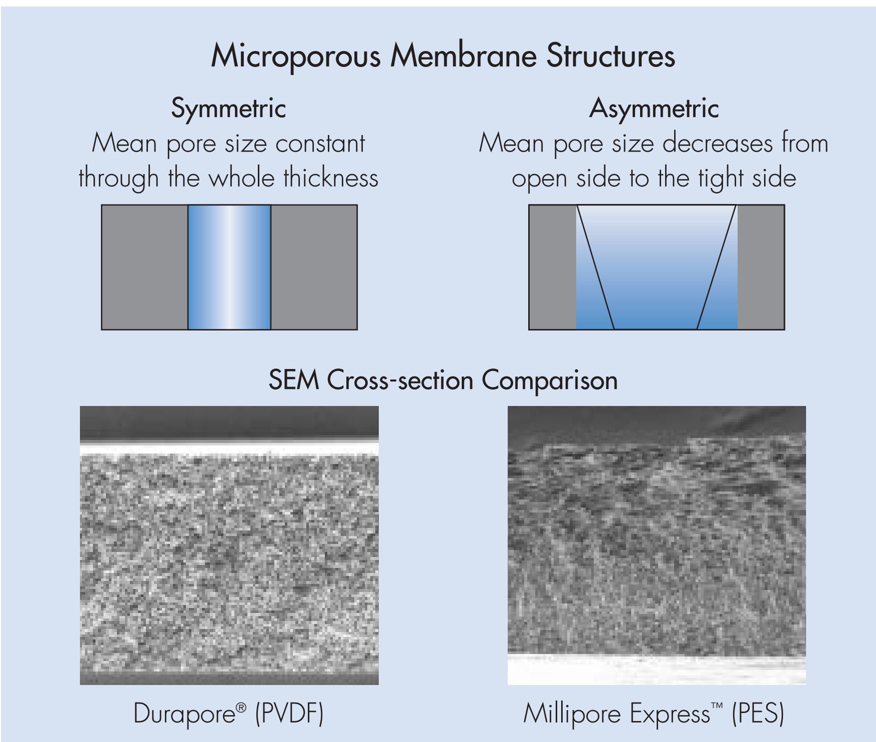

Figure 1. An SEM cross-sectional comparison of a traditional microporous filter (left), with a uniform pore structure from top to bottom, to a new fast-flow microporous membrane (right) capable of higher throughput because of the asymmetric pores. The new PES membrane is more open at the top, where filtration starts.

A variety of sterilization methods exist within the scientific laboratory. These methods include autoclaving, chemical sterilization and membrane filtration. If working within a biological system however, it is important to remember that many cell culture media components are heat sensitive. It is also important to remember that in addition to concerns about reactivity, chemical methods may leave unwanted by-products. Filtration is the method of choice for the sterilization of heat-sensitive tissue culture media and expensive protein additives. To improve on existing filter types, a new membrane was developed for speed, filtration capacity (throughput) and protein recovery.

Fast and Efficient Filtration

The key to this innovative membrane is in the asymmetry of its microporous structure. The Millipore Express polyethersulfone (PES) membrane used in this study is rated as a 0.22 µm filter. The SEM cross-section (Figure 1, right panel) of this membrane shows that it is more open at the top, where filtration starts, than at the bottom. The mean pore size decreases from the open side to the tight side. This membrane's unique architecture extends the useful life of the filter and promotes greater throughput while the 0.22 µm pores maintain bacterial retention. The open side acts as a built-in pre-filter catching large particles before they have a chance to clog the bacterially retentive tight side. In contrast, traditional membranes have a constant pore size throughout their whole thickness. The benefits of this new membrane design are also offered in a 0.1 µm Express (PES) filter.

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Protein Binding

Since many media additives are proteins, researchers must be confident that when these solutions are filtered, either to ensure sterility or to remove particulates, the proteins are not lost. For example, concentrations of growth factors needed to maintain special cell types must remain constant before and after filtration. The Express (PES) membrane and the Durapore (PVDF) membrane meet this rigorous need. Figure 2 compares the binding of ^{125}I-labeled IgG to a variety of polymeric membranes. A 13 mm membrane disk was incubated in a 1 mg/ml solution of ^{125}I-IgG, washed and counted in a gamma counter to determine the amount of protein nonspecifically bound to the filter. The graph (Figure 2) shows that not only does protein binding vary considerably among polymer types but that even membranes made from the same polymer, polyethersulfone (PES), have different protein binding characteristics. Other PES membranes nonspecifically bound over 6 times more protein than the Express (PES) membrane. However, the Durapore (PVDF) membrane bound the least protein among the samples tested. It is important to note that both the Express (PES) and the Durapore (PVDF) membranes have been surface-modified to render them both hydrophilic and low protein binding.

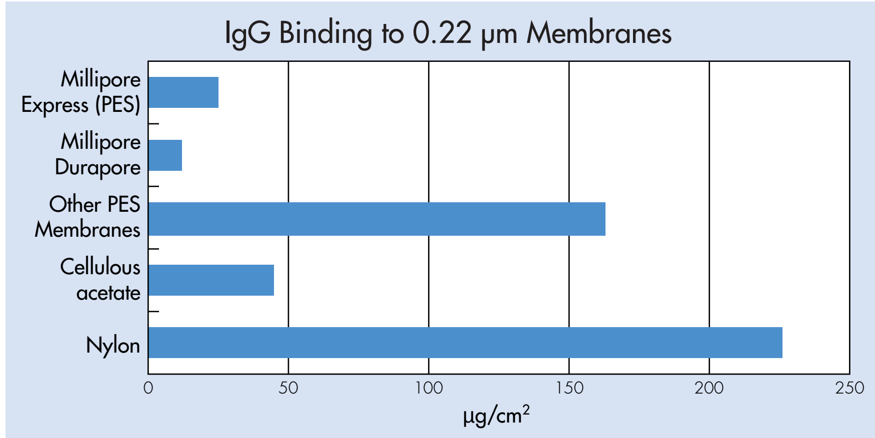

Figure 2. A comparative study was performed to assess nonspecific protein binding to 0.22 µm membranes. A 1 mg/mL solution of ^{125}I labeled IgG was offered to 13 mm membrane disks. After incubation, the amount of protein bound was determined. The other PES membranes nonspecifically bound over 6 times more protein than the Express (PES) membrane. Among all membranes tested, Durapore remains the lowest protein binding membrane available.

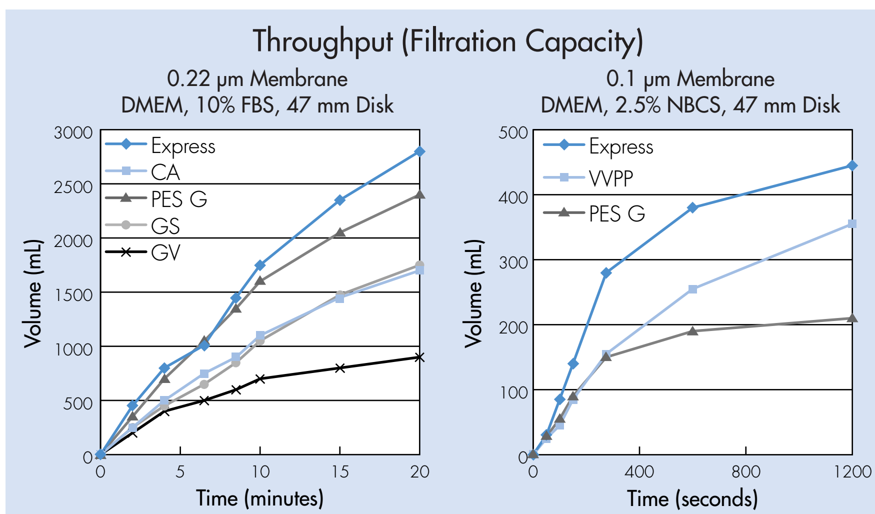

Figure 3. Several membrane types were compared for throughput or filtration capacity. 47 mm disks were challenged with either DMEM, 10% FBS (0.22 µm filters, left), or with DMEM, 2.5% NBCS (0.1 µm filters, right). In both cases, the Express (PES) filters exhibited the highest filtration capacity.

Membrane Throughput or Filtration Capacity

Several membrane types were compared for their ability to filter a common tissue culture medium. The tissue culture medium used was Dulbecco's Modified Eagle's Medium (DMEM) with either 10% fetal bovine serum (FBS) or 2.5% newborn calf serum (NBCS). The Express (PES) 0.22 µm membrane filtered almost 3 liters of tissue culture medium with minimal decay in filtration rate (Figure 3, left). This is a direct benefit of the asymmetric architecture of this membrane. The increase in throughput is even more distinct using the 0.1 µm Express (PES) membrane. The asymmetric structure of the 0.1 µm Express (PES) membrane offered greater filtration capacity than both traditional symmetric membrane types tested (Figure 3, right).

Bacterial and Mycoplasmal Retention

Consistent, quantitative removal of bacteria is the most important requirement of a sterilizing grade microporous membrane filter. Challenging a membrane with *Brevundimonas diminuta* (ATCC 19146) according to HIMA (Health Industry Manufacturers Association) guidelines assesses bacterial retention. The *B. diminuta* challenge concentration is maintained at equal to or greater than 1 x 10^7 cells per cm^2 of membrane filtration area. Assessment of the retentive nature of a sterilizing membrane is expressed as the log reduction value (LRV). The LRV is a mathematical expression of the microbial retention efficiency of a sterilizing membrane filter. It is defined as the logarithm to the base ten of the ratio of microorganisms in the challenge to the number of organisms in the filtrate. In the case where the filtrate is sterile, the LRV is expressed as greater than the log10 concentration of the total microbial challenge. When tested according to HIMA guidelines, the Express (PES), 0.22 µm membrane and devices were 100% retentive (Figure 4, top panel) for *Brevundimonas diminuta*. In addition, the new Express (PES) 0.1 µm membrane, when tested for the retention of the *Mycoplasma*, *Acholeplasma laidlawii* gave a *Mycoplasma* LRV of 7 (Figure 4).

IgG Binding to 0.22 µm Membranes

Pseudomonas dimunita

Manufacturer	Device Type	% Retention Failure	Quantity Tested	Membrane Type
Millipore	Millex GP25	0%	25	PES
Brand G	25 mm syringe filter	0%	25	PES
Brand W	25 mm syringe filter	4%	25	PES
Brand S	32 mm syringe filter	0%	25	cellulose acetate
Brand S	32 mm syringe filter	0%	25	glass fiber cellulose acetate

Manufacturer	Membrane Type	# of Membrane Lots Tested	% Retention Failure	Quantity Tested
Millipore	PES	5	0%	9
Brand G	PES	1	0%	2

Mycoplasma Acholeplasma laidlawii

Manufacturer	Membrane Type	Sample Tested	Quantity Tested	% Retention Failure	LRV
Millipore	0.1 µm PES	Sterivac VP20	6	0%	7
Millipore	0.1 µm PES	47 mm membrane disk	9	0%	7

Figure 4. Both 0.22 µm and 0.1 µm Express™ (PES) membranes and devices were 100% bacterially retentive when tested according to HIMA guidelines. In addition, the 0.1 µm Express™ (PES) membrane and devices, when tested for Mycoplasma retention, had a log reduction value (LRV) of 7.

Conclusion

It is important to consider all important performance properties when choosing a microporous membrane for filtration of tissue culture media and media additives. These performance characteristics include flow time, filter capacity, protein binding, and bacterial retention. The Millipore Express (PES) family of membranes offers fast flow, high filter capacity and low protein binding while remaining bacterially retentive. The unique asymmetric structure of these membranes extends their filtration capacity and useful lifetime by allowing them to tolerate higher particle loads and protein concentrations. This structure also yields a filter with a faster flow rate. The process by which the surface of this PES membrane is rendered hydrophilic is responsible for its low protein binding character. The fast flow, low binding, high throughput and bacterial retentive properties of the Millipore Express (PES) Membrane family provides scientists with a reliable, cost effective and time saving membrane for sterile filtration.

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