A rapid filtration method for harvesting mammalian cell culture grown in Cellbag bioreactors

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A rapid filtration method for harvesting mammalian cell culture grown in Cellbag™ bioreactors

Disposable bioreactors have gained widespread acceptance in cell culture applications because they provide a flexible resource for multiproduct facilities and speed the production of biomolecules. We used ULTA™ Prime GF glass fiber and ULTA Prime CG bioburden reduction or ULTA Pure HC sterilizing-grade normal flow filters to harvest Cellbag contents using a simple, turn-key approach that can be easily coupled directly to the bioreactor chamber at the time of harvest.

Introduction

Disposable bioreactors offer a number of advantages for scientists including the reduction of preparation time, elimination of cleaning and sterilization time, and ease of use (1). WAVE Bioreactor™ is the most popular disposable bioreactor with over 2000 units in operation worldwide. And for many applications, WAVE systems have become essential components in the cGMP production of human therapeutics (2).

Recent advances in filter integration, aseptic connectology and disposable sensing allow for fully disposable operation of the bioreactor; however, harvest and clarification operations remain largely dependent on centrifugation, cross flow filtration and depth filtration (3)—all techniques that are yet to be widely adapted to single-use implementation. The use of these techniques to harvest disposable bioreactors can result in process bottlenecks (4), especially in settings where large numbers of relatively small bioreactors are in use (e.g., clinical and research laboratories). In these cases, the advantages of ease-of-use, disposability, and turn-key processing often outweigh the need for optimized, moleculespecific process development.

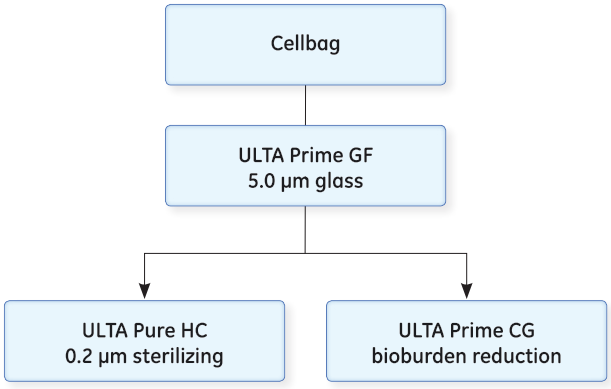

Fig 1. Process outline for the harvest of mammalian culture from Cellbag bioreactors.

Experiments and results

Cell harvest operations that employ normal flow filtration are generally designed to use serially coupled filters of decreasing pore size. Most commonly, a depth- or pre-filter is coupled to a sterilizing-grade membrane in order to produce material that can be immediately purified using chromatography or ultrafiltration operations.

Depth filters are available in a variety of formats and different material compositions. Historically, mammalian cell culture operations rely on depth filters composed of a cellulose matrix blended with inorganic filter aids (e.g., diatomaceous earth or perlite); however, these products are not suitable for single-use applications due to their inherently high levels of extractables—that must be removed via a pre-use flush of 50 to 100 L/m^2 of water (5)—and the limited availability of encapsulated formats.

In this study, we focused on glass-fiber based depth filters over traditional depth-filter technologies for the following reasons:

1. Glass fiber has a high void volume and well-defined filter matrix that provides longevity and efficient cell removal.
2. Glass fiber filters can be preflushed and dried at the point of manufacture thereby eliminating any need for pre-use flushing by the end-user.
3. Glass fiber is compatible with common sterilization techniques such as autoclaving and gamma-irradiation.

Sterilizing-grade membranes are generally coupled to depth filters in order to facilitate additional removal of cell debris and particles or fines that may be shed by the depth filter. In this study, we tested two membrane-based options for this secondary clarification step—ULTA Prime CG and ULTA Pure HC. ULTA Pure HC is a sterilizing-grade (0.2 µm) filter that incorporates an onboard 0.6 µm polyethersulfone prefilter. ULTA Prime CG is a bioburden reduction filter (validated for 5 LRV of *B. diminuta*) that incorporates an onboard 0.6 µm polyester prefilter. Providing the option of sterilizing-grade versus bioburden reduction enables the end-user to choose between lower cost (ULTA Prime CG) and maximum quality assurance (ULTA Pure HC).

Filter sizing and selection

Eight pilot scale experiments were performed on three cell lines at varying levels of viability and cell density (Fig 2). All eight cultures were grown in WAVE Cellbag bioreactors of sizes varying from 5 to 10 liters. All the experiments were performed with capsule filters under constant flow conditions. We chose flow rates that allowed complete processing of the Cellbag contents in one hour.

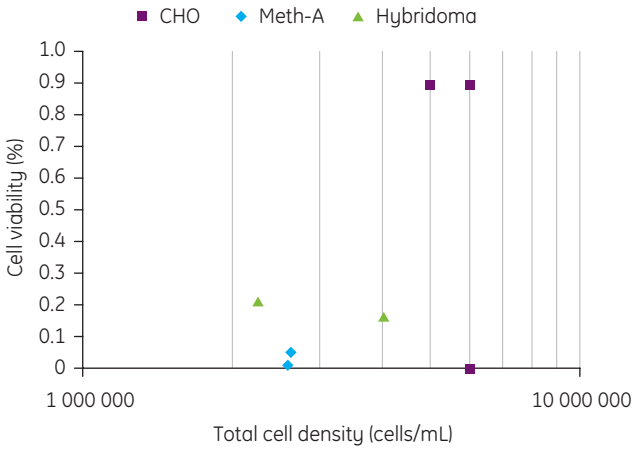

Fig 2. Cell culture characteristics. (Two experiments performed at the same culture conditions).

Primary (i.e., ULTA Prime GF) and secondary (ULTA Pure HC or ULTA Prime CG) filters were run in series with pressure transmitters located upstream of each filter. Filtrate volume and pressure data was collected as a function of time. Each experiment was continued until a total pressure drop of 1 bar was observed or until the feed vessel was exhausted. Capacity calculations were performed for both filters (Fig 3).

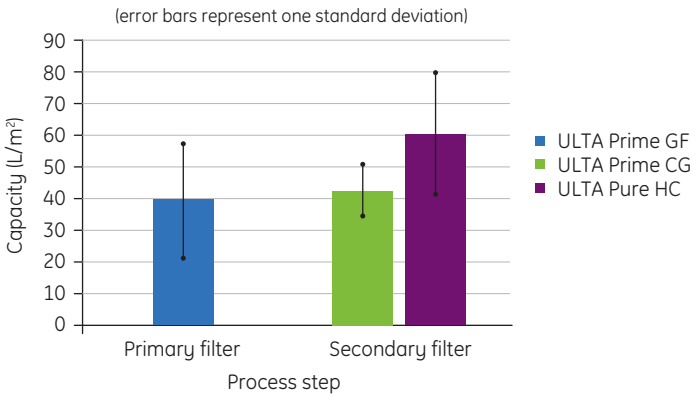

Fig 3. Capacity of primary and secondary filters.

Based on the experimental results, filter recommendations were developed for each Cellbag size up to the Cellbag 50 L (Table 1).

Table 1. Recommended filters for Cellbag harvest

Cellbag size	Culture volume (L)	ULTA Prime GF 5.0 µm	ULTA Prime CG or ULTA Pure HC
2 L	up to 1	2 in	2 in
10 L	1 to 5	5 in	6 in
20 L	5 to 10	10 in	5 in
50 L	10 to 25	20 in	10 in
100 L	25-50	2 × 20 in	30 in
200 L	50-100	3 × 30 in	2 × 30 in

Conclusions

ULTA Prime GF filters coupled to a sterilizing-grade ULTA Pure HC or bioburden reduction ULTA Prime CG filter is a highly efficient method for harvesting WAVE Cellbag bioreactors. This combination provides a rapid solution that can be implemented with very little preparation. Glass fiber and polyethersulfone membranes are inherently low in extractables and are validated for low levels of endotoxin and other contaminants. The methods presented in this work provide a reliable solution designed to work in the majority of mammalian cell culture applications with no upfront process development efforts.

References

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2. Disposable bioreactors become standard fare. *Genetic Engineering & Biotechnology News* **25**, No. 14 (2005).
3. Shukla, A. *et al.* Harvest and recovery of monoclonal antibodies from large-scale mammalian cell culture. *BioPharm International.* **21**, No. 5 (2008).
4. Tathagata, R. *et al.* Cell culture clarification by depth filtration. Biospectrum Asia. http://www.biospectrumasia.com/content/100908IND7060.asp (2008).
5. Application note: Using Millistak+® HC Filters for mammalian cell culture clarification. Millipore Corporation, AN1100EN00 (2003).

Ordering information

Product	Code number
ULTA Prime GF, 2 in	28-9084-27
ULTA Prime GF, 5 in	28-4136-61
ULTA Prime GF, 10 in	28-4136-64
ULTA Prime GF, 20 in	28-4136-68
ULTA Prime GF, 2 × 20 in	28-4136-68
ULTA Prime GF, 3 × 30 in	28-4136-72
ULTA Prime CG, 2 in	28-9085-17
ULTA Prime CG, 6 in	28-9085-23
ULTA Prime CG, 5 in	28-4137-08
ULTA Prime CG, 10 in	28-4137-12
ULTA Prime CG, 30 in	28-4137-18
ULTA Prime CG, 2 × 30 in	28-4137-18
ULTA Pure HC, 2 in	28-4002-31
ULTA Pure HC, 6 in	28-4002-37
ULTA Pure HC, 5 in	28-4004-27
ULTA Pure HC, 10 in	28-4004-28
ULTA Pure HC, 30 in	28-4004-30
ULTA Pure HC, 2 × 30 in	28-4004-30

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