PALL Life Sciences

Application Note

USTR 3010

The Effect of Membrane Selection and Operating Parameters on Sterile Filtration of Hyaluronic Acid

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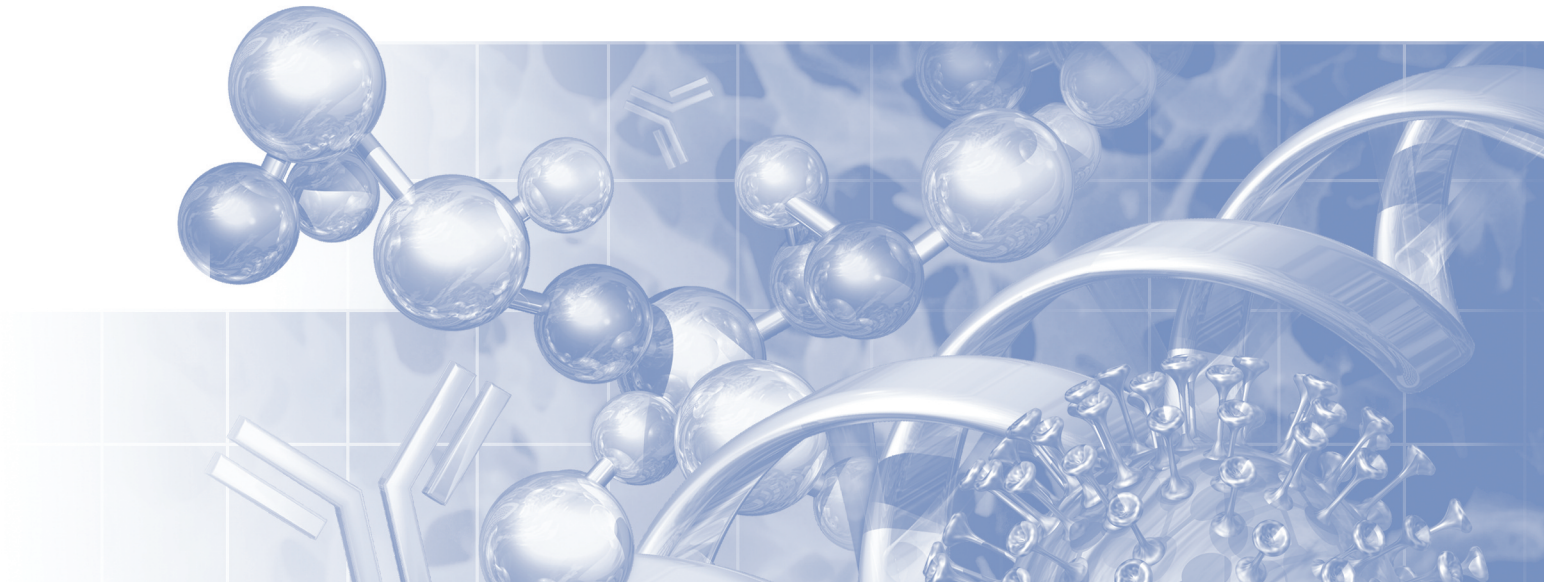


*Filtration. Separation. Solution.*SM

Background

Hyaluronic Acid (HA) is a naturally-occurring substance in the human body which has been identified as having properties that make it attractive for the healthcare industry. Manufactured on a large scale globally, it is commonly formulated in arthritis, skincare, ophthalmic and surgical treatments[1]. Emerging applications for HA also exist in drug delivery[2], regenerative medicine[3] and stem cell biology[4].

Since HA-containing treatments are often administered by injection or use a method of delivery that depends on them being free from microbial contamination, sterilizing filtration may be used during their manufacture to render them safe for use without loss of efficacy.

Although frequently performed, sterile filtration of HA-based formulations with microbially-rated membrane filters is regarded as a challenge, mainly due to the limitations of filter throughput performance: filters that are often selected by the end-user have typically been developed for formulations of lower viscosity with a particulate profile that tends not to cause premature plugging of the filter membrane - properties quite different to those of HA-based solutions.

This application note shows how the selection of an appropriate sterilizing grade filter can result in efficient filtration of a HA-based solution; it confirms the effectiveness of such a filter under microbial challenge conditions with *Brevundimonas diminuta* and makes some observations on the filterability of HA-based solutions under different operating conditions.

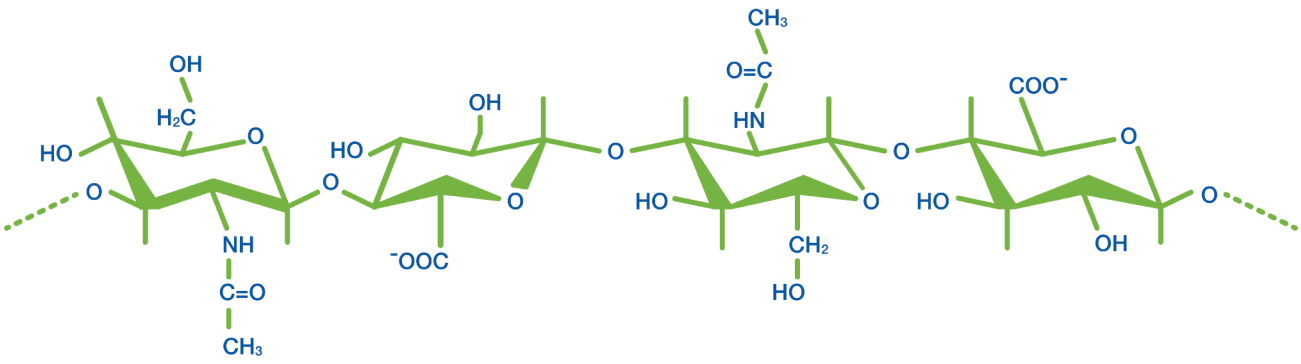
Properties of HA

Also commonly known as hyaluronan, HA is a heteropolysaccharide and more precisely, a glycosaminoglycan (GAG), alternating and repeating two sugar units, D-glucuronic acid and N-acetyl-D-glucosamine to form a linear chain[5] (Figure 1). The number of sugar units is variable. It can exist in salt form (sodium hyaluronate for example).

HA is highly hygroscopic and, in aqueous solution electrostatic repulsion occurring between negative carboxylate functional groups, causes this molecule to exhibit viscoelasticity, moisture holding and lubricating properties, which increases water viscosity and creates a gel. The solution viscosity is a complex function and depends on its molecular weight (MW), concentration, temperature, pH, conductivity, physical environment (some proteins) and manufacturing processes (source, provider, formulation method) to name a few. Furthermore, these parameters can also have an impact on the HA molecule conformation because of intra-molecular hydrogen bonding between the acetamido and carboxyl groups, and also extramolecular interactions.

Polymers of HA can range in size from 5,000 to 20,000,000 Da *in vivo*. The average molecular weight in human synovial fluid is 3–4 million Da, and hyaluronan purified from human umbilical cord is 3,140,000 Da.[6]

Figure 1
Structure of HA



Glucuronic acid

N-acetylglucoseamine

HA Production

There are three major methods of HA production, which in order of commercial development are: chicken (or rooster) comb extraction, *Streptococcus* fermentation, and *Bacillus subtilis* fermentation[7]. Chicken comb extraction remains widely-used, with an increasing trend towards fermentation methods. The number and type of unit operations required to deliver a pure, sterile-filtered product varies with each process type, and whilst in all cases the final output may be challenging to filter when in solution, HA derived from chicken comb and from *Streptococcus* equi are known to be more challenging to sterile filter, in comparison to that derived from *Bacillus subtilis*.

Sterile Filtration of HA

A typical sterile filtration step involves filtering a HA solution through a membrane filter in a direct flow mode. The filtration can be conducted either in a constant pressure or constant flux mode. In the former, the pressure across the filter is kept constant. Then, depending on the fouling nature of the fluid, the flux of the fluid will decrease with time, as more and more fluid volume is filtered. Eventually the flux across the filter will decrease to a point where it becomes impractical to continue operation and at that point the filter is deemed to be exhausted. Conversely, in a constant flux filter operation, the fluid is pumped through the filter under a constant flux, defined as fluid flow rate per unit membrane area, and the pressure differential across the filter will increase as volume is passed through the filter element. At some point the pressure differential across the filter reaches a value high enough that makes it difficult to continue with typical fluid transfer systems such as pumps which are commonly used in the biopharmaceutical industry. In either case, constant pressure or constant flux, these respective endpoints are commonly defined as the throughput of the filter, which is defined as the amount of fluid volume per unit membrane area that can be processed through a given filter element before the filter's life is exhausted.

Filterability Testing of HA with Commercially Available Filter Membranes

Pall has performed constant pressure filterability testing with a HA solution produced by fermentation of *Streptococcus equi* with a range of commercially available sterilizing grade filter membranes rated at 0.2 µm and retentive for *Brevundimonas diminuta* at a challenge level of 10^7 colony forming units (cfu) per cm^2 membrane as per ATSM 838-05.

The HA had an approximate molecular weight of 1.8 MDa and was prepared with phosphate buffered saline (PBS) solution at a 0.2% (wt/vol) concentration. The fluid was stirred for about 16 hours in order to solubilize the HA sufficiently for filtration, and the fluid had a final viscosity of approximately 20 cP. Table 1 summarizes the test conditions and Table 2 summarizes the filters used.

Table 1
Test Conditions

HA Source	Molecular Weight	Concentration mg/mL	Temperature (ºC)	Operating Pressure	% Flow Decay End point	Challenge Fluid	Filter Format
S.equi	1.8 MDa	2	Ambient	30 psid	100% (Complete blockage of filter)	PBS	47 mm disc or small capsule

Table 2

Filters Tested, 47 mm Disc Format or small capsule

Filter Name	Membrane Construction
Supor® EX grade ECV	Asymmetric PES upstream/Symmetric PES downstream
Supor grade EKV	Asymmetric PES upstream/Symmetric PES downstream
Alt. vendor PES	Asymmetric PES upstream/Asymmetric PES downstream

Table 3 shows the membrane throughput performance of each of the filters tested, the effective filtration areas (EFA) of standard 10-inch filter devices incorporating each of the membranes tested and the calculated throughput per 10-inch filter element until blockage, based on assumed linear scale up.

Table 3

Throughput Performance of Filters Tested

Filter Tested	Supor EX Grade ECV (PES)	Supor Grade EKV (PES)	Alt. Vendor (PES)
Throughput in L/m²	357 ± 32	69 ± 4	10 ± 2
EFA 10-inch filter in m²	1.04	0.6	0.5
Throughput per 10-inch filter element in L	371	41	5

Figure 2 shows a throughput comparison of the sterilizing-grade filter membranes tested. Among the candidates tested, Supor EX grade ECV membrane filters provided a significant improvement in throughput over the other sterilizing-grade membranes. The tests were conducted in triplicate. Figure 3 shows the predicted throughput of corresponding 10 inch filter assemblies using each membrane, assuming linear scale up for all.

Figure 2

Filter Membrane Area Throughput Performance with 1.8 MDa HA

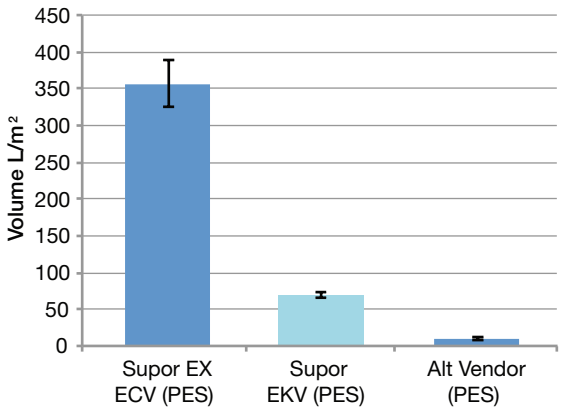
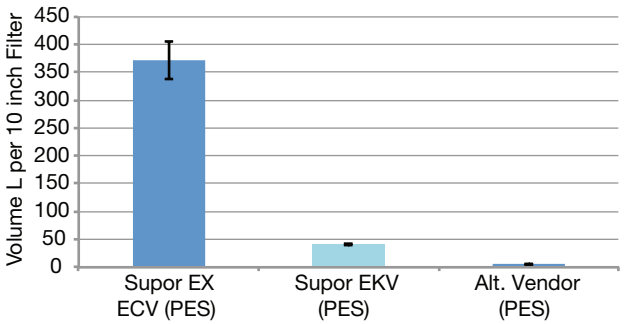


Figure 3

Predicted 10-inch filter cartridge throughput performance with 1.8 MDa Hylauronic Acid, assuming linear scale-up for all filters



The throughput performance of Supor EX grade ECV filters with HA solutions is attributable to a unique membrane layer combination incorporating a highly asymmetric upstream membrane layer. This high performance is augmented in a 10-inch filter device (Figure 3) owing to a laid over pleat membrane geometry and cartridge design which enables a high filtration area per 10-inch filter element, in excess of 1 m².

Furthermore, the investigation confirmed from the results of the viscosity measurements of HA taken before and after filtration, that Supor EX grade ECV membrane had no effect on the filtrate viscosity.

Bacterial Challenge Study with HA using Supor EX grade ECV filters

With the best-performing candidate from the filterability studies, bacterial challenge testing was performed. The objective of these tests were to demonstrate that Supor EX grade ECV filters could perform reliably under worst-case microbial challenge conditions with a HA solution, thus supporting the use of this filter in a HA solution filtration step where an end-user requires the filter to produce a sterile effluent.

2 mg/mL HA solution derived from *Streptococococcus equi* was prepared in PBS a solution containing *B. diminuta* at a level of greater than 10^7 cfu per cm^2 of 47 mm filter disc to be subject to bacterial challenge.

The results shown in Table 4 show that both Supor EX grade ECV filters produced sterile effluent with a *B.diminuta* challenge level of greater than 10^7 cfu per cm^2 membrane.

Other Observations Concerning Filterability of HA Solutions

Pall's Scientific and Laboratory Services (SLS) and applications R&D group has performed further test work to investigate the impact of different operating parameters on the filterability of HA solutions and the following observations have been made:

Impact of Molecular Weight (MW) of HA on Filterability[8]

MW has an impact on HA solution viscosity. For some types of HA, a non-linear relationship between MW and viscosity has been observed such that where all parameters other than HA MW are kept constant, a two-fold increase in MW may result in greater than two-fold increase in solution viscosity. In such circumstances, filter throughput is expected to be affected in relation to fluid viscosity, whereby the lower the viscosity, the better the throughput.

Impact of Concentration of HA on Filterability[8]

For types of HA solution tested by Pall, where all process parameters are kept constant apart from concentration of HA, it has been observed that as the concentration of a solution is decreased, the throughput performance of a given sterilizing-grade filter will tend to increase.

Table 4

Bacterial Challenge Test Results for Supor EX grade ECV membrane in Streptococococcus equi-derived HA

Filter Configuration	Bubble Point Integrity Test Results (psi)		Total Challenge (CFU/Filter)	Area Challenge (CFU/cm^2)	Total Recovery* (CFU)	Titer Reduction
	Pre-Challenge	Post-Challenge				
47 mm disc Supor EX grade ECV membrane	64.5	66.0	4.0×10^9	2.9×10^8	0	$>4.0 \times 10^9$
	65.3	66.0	4.0×10^9	2.9×10^8	0	$>4.0 \times 10^9$
	54.4	58.7	2.4×10^9	1.7×10^8	0	$>2.4 \times 10^9$
	58.0	56.6	2.4×10^9	1.7×10^8	0	$>2.4 \times 10^9$
	58.0	59.5	2.4×10^9	1.7×10^8	0	$>2.4 \times 10^9$

**All filters tested gave a sterile effluent. The results of the bacterial challenge tests are not intended to serve as a surrogate for any process-specific bacterial challenge validation studies, which if required must always be performed under the end-user's stated process conditions following an appropriate risk assessment.*

Impact of Temperature of HA Solution on Filterability[9]

For types of HA solution tested by Pall, where all process parameters are kept constant but temperature is adjusted, it has been demonstrated that the filterability of a HA solution tends to improve with increased temperature. This is due to the decrease in molecule rigidity with increasing temperature, which subsequently reduces the viscosity of the solution.

The extent to which temperature affects filterability of a HA solution will be more or less pronounced depending on other properties, for example, concentration and molecular weight.

Pall has performed studies throughput studies with HA at up to 60 °C and recommends that when product is tested at temperatures higher than 20 °C, the stability of the product at those temperatures must be evaluated.

Impact of Age of HA Solution on Filterability

The filterability of HA solution tested under process conditions where age of the solution is variable may not result in consistent filter performance due to a potential change in the solution over time. In one instance it was observed that when filterability of a HA solution was performed on a given solution immediately after and two days following preparation, with other process conditions consistent in each case, that the aged solution showed improved filterability. This observation confirms the importance of benchtop filterability testing of HA being fully representative of process conditions, to mitigate the risk of filter sizing problems at manufacturing process scale.

Impact of Performing Filtration under Constant Pressure or Constant Flow

Using constant pressure will typically offer better filtration results than using constant flow. Therefore, for the most efficient filtration performance working with a pressurized vessel may be preferred over the use of a pump. Also, performing filtration studies under a higher applied pressure generally leads to an increase in throughput. Again, it is important to ensure not to exceed the maximum recommended operating differential pressure for a selected filter.

Summary and Conclusions

HA is already used for range of biopharmaceutical and biomedical purposes with emerging applications in regenerative medicine, drug delivery and stem cell biology.

This application note reports on the evaluation of commercially available sterilizing-grade products for filtration of HA. With volumetric throughput prioritized as the main driver for process efficiency among end-users of sterilizing-grade filters, the studies have consistently shown Supor EX grade ECV filters to provide the highest throughput per unit membrane area and per 10-inch filtration device.

A bacterial challenge study with Supor EX grade ECV filters demonstrated that these filters were able to deliver a sterile effluent challenged with greater than 10^7 cfu *B.diminuta* per cm^2 filter membrane, suspended in a HA solution.

Both sets of test results indicate that Supor EX grade ECV filters can offer a highly efficient and economical solution for the sterile filtration of under worst-case bacterial challenge conditions.

It has been demonstrated that HA solution properties (concentration, molecular weight, fluid temperature and others) and also filtration conditions (constant pressure mode, pressure applied) can have a clear impact on filtration performance, independent of membrane selection. Adjustments and optimization of these process parameters can contribute to filtration improvement.

Please contact the author(s) or Pall's Scientific Laboratory Services global technical support group for more specific information in regard to any test work or observations reported in this document.

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