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Letter

Would 20 nm Filtered Fetal Bovine Serum-Supplemented Media Support Growth of CHO and HEK-293 Cells?

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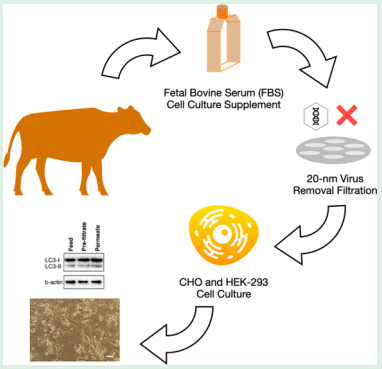
ABSTRACT: Virus safety of fetal bovine serum (FBS) is a critical issue for cell culture and clinical applications of cell therapies. The size exclusion filtration of FBS-supplemented cell culture media through small-size virus retentive filter paper is presented to investigate its effect on cell culture. A substantial proportion of proteins (ca. 45%) was removed by nanofiltration, yet important transport proteins (albumin, fetuins, macroglobulins, transferrin) were unaffected. The cell viability of Chinese hamster ovary (CHO) and human embryonic kidney 293 (HEK-293) cells that were grown in media supplemented with nanofiltered FBS was surprisingly high, despite the observed protein losses. Protein depletion following nanofiltration resulted in detectable levels of autophagy markers.



KEYWORDS: *nanocellulose, mille-feuille filter, cell culture, cell therapies, autophagy, cell media supplements*

Serum is historically the most widely used supplement in cell culture. Serum supports cell growth by providing the necessary hormones, transport proteins, growth factors, dissociation factors, attachment factors, and protease inhibitors.[1,2] However, the continued use of serum in cell culture features many drawbacks too. In particular, the composition of serum is poorly defined, and it is prone to significant batch-to-batch variation.[1,3,4] Further, serum may harbor a wide array of contaminants, such as bacteria, mycoplasma, viruses, endotoxins, and prions.[5−8]

The most commonly used type of serum in cell culture is fetal bovine serum (FBS) due to its strong growth-promoting capacity and relatively low immunoglobulin levels.[9] It has recently been reported that nearly 80% of the late clinical stage cell therapies based on mesenchymal stem cells use FBS.[10,11] In a recent report that compared several serum-free and xeno-free FBS alternatives to different FBS brands, the latter supported the growth of the human endothelial and neuronal cell lines better than its engineered alternatives.[12] To avoid the risk of bovine spongiform encephalitis (BSE), almost 90% of GMP-compliant FBS suitable for manufacturing of biologics is currently supplied by three countries, i.e. USA, Australia, and New Zealand. From a long-term perspective, the volumes of FBS production in these countries cannot satisfy the growing global demands of the biopharmaceutical industry.[9] In this context, it is worth noting that cases of fraudulent FBS diluted with adult bovine serum albumin (BSA) reaching the market have been reported in the past, and there were many suspicious mismatches over the years between the actual volumes of FBS produced in certain countries and the volumes sold under their label globally.[13]

In an attempt to avoid the many drawbacks associated with the use of FBS, serum-free and xeno-free supplements have been developed for cell culture.[11] While chemically defined media reduce the batch-to-batch variability of cell culture, the removal of albumin and other bulk serum proteins narrows the spectrum of cells whose growth they support. Even small-size supplements such as amino acids, e.g. tyrosine, cysteine, hydroxyproline, and various lipids, can be difficult to replace using synthetic methods.[14,7] Furthermore, serum-free cell media are not entirely risk-free from potential contaminants as they still may contain a number of animal-derived proteins.[11] Thus, despite the progress in the development of serum-free and chemically defined cell culture media, the issues of biosafety continue to be critical, and the demand for FBS for cell therapies and other cell culture remains very high.

In the light of continued usage, the virus safety of FBS is one of the most critical issues in cell culture. Several suppliers report that their FBS product is triple filtered through 0.1 μm filters, mainly targeting mycoplasma and other larger microbial contaminants.[15] To produce defined FBS, 40 nm retentive

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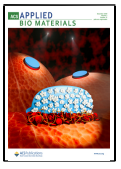

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filtration has been adapted. The vendors may additionally process FBS by gamma-radiation (≥25 kGy) or heat treatment (56 °C for 30 min).[15] Gamma-irradiation is currently the most commonly used method for viral inactivation of raw materials for cell culture but it is not a point-of-use method and is associated with large capital investments.[8] Furthermore, highly resistant parvoviruses, picarnoviruses, caliciviruses, and polyomaviruses may require higher than normal doses of radiation to avoid contamination.[7,16]

Other validated virus clearance approaches are also acceptable according to EMA, given that they provide adequate consistency and effectiveness, while maintaining biological serum performance. Virus removal filtration is a point-of-use and robust method commonly used in bioprocessing to physically remove viruses. The use of 50 nm large-size virus retentive triple-layer hydrophilic PVDF (polyvinylidene difluoride)-based DV50 filters to filter 10% FBS in Dulbecco's modified eagle's medium (DMEM), has been reported, featuring robust clearance of large-size viruses but poor clearance of small-size 25 nm viral particles.[17] In the recent years, more advanced barrier virus removal filters for upstream bioprocessing have been introduced on the market, e.g. Viresolve Barrier Filters.[18] The use of 20 nm virus-retentive barrier filters to process FBS has hitherto not been reported to the best of our knowledge. The use of 20 nm virus-retentive barrier filters in upstream bioprocessing is generally limited by the high costs associated with nanofiltration. Thus, more affordable nanofilters are highly demanded for upstream bioprocessing.[8] In the case of serum filtration, it is extremely challenging to filter such a highly complex protein mixture through 20 nm parvovirus-retentive filters, since these filters rapidly foul during the processing.[19] To avoid fouling, it is common to introduce multiple prefiltration steps to exclude coarse aggregates.[20] The drawback of the latter approach is that every prefiltration step may lead to unwanted product losses due to nonspecific adsorption of media components. Currently, FBS is not processed through 20 nm virus retentive filtration, and, overall, there is currently poor understanding of how the cell culture would be affected by the nanofiltered FBS as its proteomic composition will inevitably change during processing. The aim of this article is to investigate the effect of 20 nm filtration of FBS on the cell culture performance. To achieve this goal, we used a nanocellulose-based virus removal filter paper which was developed at Uppsala University previously.[21−23]

To evaluate the nanocellulose-based filter's applicability for serum-supplied cell culture applications, FBS (1:20, v/v) solution was filtered with these filter papers. The nanocellulose-based filter paper is a nonwoven virus removal size-exclusion filter[21,23−25] that was previously shown useful for upstream bioprocessing of basal and chemically defined media supplemented with insulin-transferrin-selenite (ITS).[19,26] Considering the high complexity of FBS, the prefiltration with 11-μm thick filter paper was performed prior to the filtration with 33-μm thick filter paper as described previously for the nanofiltration of plasma-derived prothrombin complex concentrate[25] and human serum albumin.[27]

Figure 1 shows the flux data for prefiltration with 11-μm thick filter paper at 1 bar overhead pressure and subsequent filtration with 33-μm thick paper at 3 bar pressure. Significant decline in the flux during the prefiltration step is caused by filter fouling, driven by large Mw proteins and protein aggregates, as will be shown in dynamic light scattering

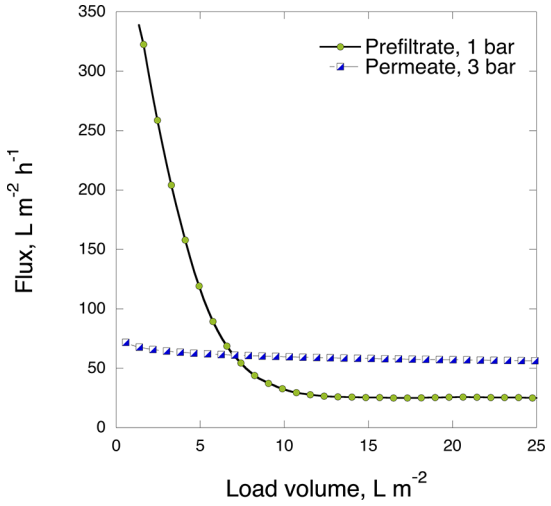


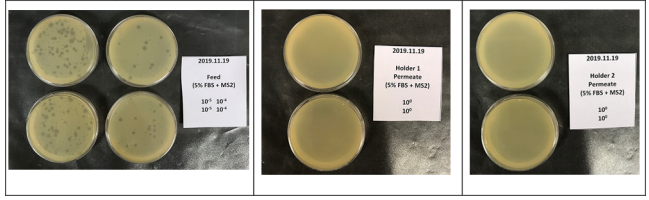
Figure 1. Observed fluxes for prefiltration with 11-μm thick and filtration with 33-μm thick filter papers at 1 and 3 bar, respectively.

(DLS) analysis below. It should however be noted that, following the prefiltration step, the flux the through the 33-μm thick filter paper at 3 bar pressure was improved and appeared much more stable compared to prefiltration.

Table 1 summarizes the results of the $\log_{10}$ virus reduction values (LRV) for the MS2 (27 nm) coliphage-spiked FBS

Table 1. $\log_{10}$ Virus Reduction Values for MS2 Phage after Filtration of FBS (1:20, v/v) with 33-μm Thick Filter Paper[a]

Run	Pressure, bar	Load volume, L/m^2	Feed titer, PFU/ml	Permeate titer, PFU/ml	LRV
1	3	28	6.1	< 0.7	> 5.4
2				< 0.7	> 5.4



[a]The inset shows hard agar plates from corresponding PFU assay analysis of virus titers in feed solution and permeate samples.

(1:20, v/v) filtration with 33-μm thick filter paper. The results showed an over 5-log virus titer reduction in permeate samples compare to the feed solution. The typical PFU assay images are presented as insets in Table 1.

Figure 2 shows particle size distributions in the feed, prefiltrate, and permeate samples. Particles with size over about 100 nm were removed from the solution by 11-μm thick prefilter, in permeate sample after 33-μm thick filter paper, particle size distribution was further narrowed toward about 30 nm particle size. In the permeate sample, low-intensity peaks appeared for particles over 100 nm, which may represent *de novo* generated aggregates and/or lipid vesicles. Further, high performance liquid chromatography (HPLC) analysis was performed (Figure 2B). Peak distribution for all analyzed samples were similar. However, intensities of the peaks were significantly decreased after prefiltration with 11-μm thick

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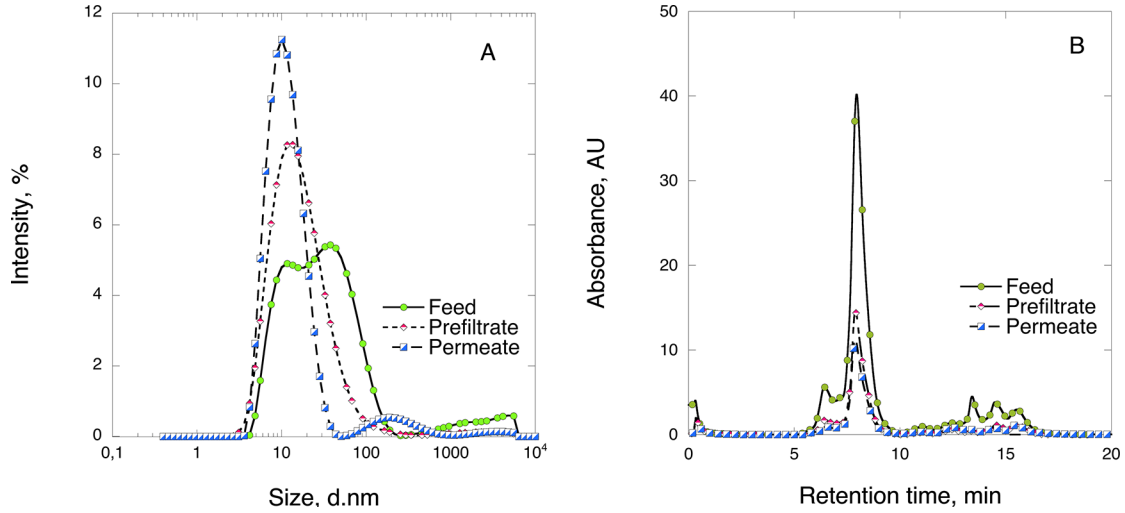

Figure 2. DLS (A) and HPLC (B) profiles for 5% FBS feed, prefiltrate, and permeate samples.

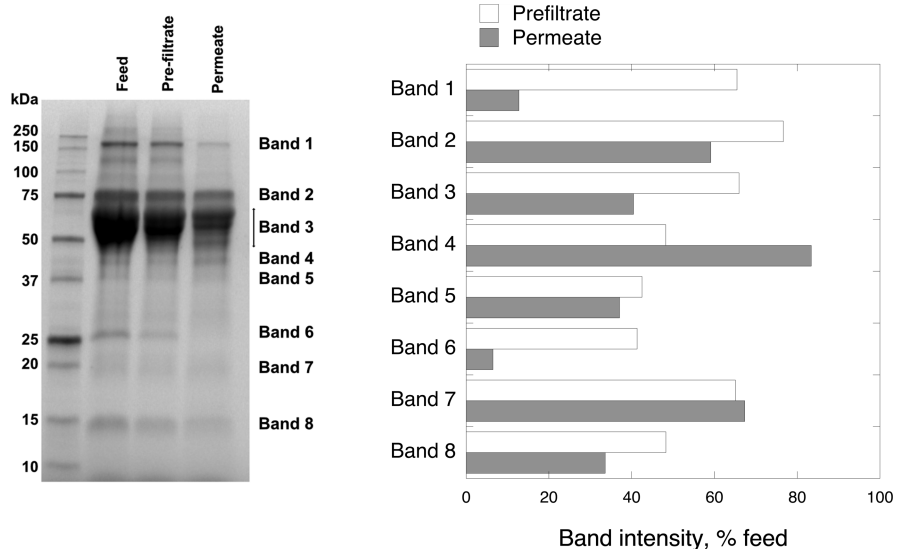

Figure 3. SDS-PAGE analysis of the feed solution and the samples after prefiltration with 11-μm thick and filtration with 33-μm thick filter papers.

prefilter paper. Unlike the DLS data, after filtration with 33-μm thick filter paper, peak distribution, and intensities were closely similar to the distribution in prefiltrate sample. This may support the speculation of lipid vesicles, observed by DLS in permeate sample.

Figure 3 shows additional proteomic analysis of the FBS sample using the SDS-PAGE technique. Results of protein analysis by SDS-PAGE confirm the complex composition of the sample and substantial changes occurring during subsequent 11-μm thick paper prefiltration and 33-μm thick paper filtration. After prefiltration, all bands decreased as compared to feed sample. Further, some of the bands were significantly decreased after 33-μm thick filtration, e.g. Band 1 and 6, while others remained essentially unchanged, e.g. Band 5 and 7. Surprisingly, Band 4 appeared to increase in intensity following the 33-μm thick filtration. It should be noted that Band 4 represents a relatively minor fraction as its intensity is substantially lower than that of other important fractions, e.g. band 3. Given the overall low intensity of the band and the semiquantitative nature of SDS-PAGE method, the significance of the observed percentage intensity increase of Band 4 between prefiltrate and permeate is inconclusive.

In order to analyze the protein composition of the samples, LC-MS/MS analysis was performed. The most abundant proteins in all samples were represented by the transport proteins (e.g., albumins, fetuins, macroglobulins, transferrins, etc.), as shown in Table 2. Distribution of the top-scored proteins was essentially similar for all samples. For full list of detected proteins, see the Supporting Information.

Cell culture media should provide sufficient conditions for cell culture performance. Alterations, in the levels of proteins and other components may lead to changes in cell culture viability and/or performance. To address the issue, the confluency, morphology, viability, and autophagy were evaluated in CHO and HEK-293 cells. CHO and HEK-293 cells are among the most common mammalian cell lines for manufacturing recombinant proteins as they are relatively easy to grow, providing stable transfection and high expression levels.[28] Figure 4 shows the CHO and HEK-293 cell cultures, cultivated for 48 h in the media, containing 5% of pristine, prefiltered and filtered FBS. In all observed groups, the cell morphology was homogeneous and the confluency rates were similar. Figure 5 shows the results of viability test for CHO and HEK-293 cells cultured in media with prefiltered and filtered

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Table 2. LC-MS/MS Analysis of 5% FBS Feed Solution, Prefiltrate, and Permeate Samples[a]

Feed				
accession number	protein	gene	score	family domain
A0A4W2DDL5	uncharacterized protein	ALB	1334.55	albumin
A0A4W2GW83	uncharacterized protein	ALB	1194.80	albumin
P12763	alpha-2-HS-glycoprotein	AHSG	633.83	
A0A4W2I777	uncharacterized protein	AHSG	514.50	alpha-2-HS-glycoprotein
G3X6N3	serotransferrin	TF	458.39	
A0A4W2DZ09	uncharacterized protein	N/A	443.98	serotransferrin
Q7SIH1	alpha-2-macroglobulin	A2M	133.80	
A0A4W2EWF2	GC vitamin D binding protein	GC	124.25	
A0A4W2H192	GC vitamin D binding protein	GC	117.77	
A0A3Q1MIW0	alpha-fetoprotein	AFP	115.16	
Q2KJF1	alpha-1B-glycoprotein	A1BG	85.85	
A0A4W2D351	uncharacterized protein	FETUB	73.34	fetuin-B-type
A0A4W2DRJ5	GLOBIN domain-containing protein	LOC113883298	42.65	
P02081	hemoglobin fetal subunit beta	N/A	31.38	
A0A4W2GKV5	GLOBIN domain-containing protein	LOC113905582	27.60	
A0A140T843	beta-2-glycoprotein 1	APOH	27.19	
P00978	protein AMBP	AMBP	26.88	
A0A4W2D1I6	plasminogen	PLG	25.90	
P34955	alpha-1-antiproteinase	SERPINA1	25.82	
F1MNV5	kininogen-1	KNG1	20.66	
Prefiltrate				
accession number	protein	gene	score	family domain
A0A4W2DDL5	uncharacterized protein	ALB	1834.02	albumin
A0A4W2GW83	uncharacterized protein	ALB	1667.82	albumin
P12763	alpha-2-HS-glycoprotein	AHSG	711.21	
A0A4W2I777	uncharacterized protein	AHSG	559.35	alpha-2-HS-glycoprotein
G3X6N3	serotransferrin	TF	531.88	
A0A4W2DZ09	uncharacterized protein	N/A	511.80	serotransferrin
A0A3Q1MIW0	alpha-fetoprotein	AFP	198.99	
A0A4W2GW20	uncharacterized protein	AFP	185.57	alpha-fetoprotein
A0A4W2EWF2	GC vitamin D binding protein	GC	170.31	
A0A4W2H192	GC vitamin D binding protein	GC	158.83	
A0A4W2E185	uncharacterized protein	A2M	150.97	alpha-2-macroglobulin
Q7SIH1	alpha-2-macroglobulin	A2M	149.03	
Q2KJF1	alpha-1B-glycoprotein	A1BG	90.66	
A0A4W2D351	uncharacterized protein	FETUB	83.90	fetuin-B-type
A0A4W2DRJ5	GLOBIN domain-containing protein	LOC113883298	42.71	
P34955	alpha-1-antiproteinase	SERPINA1	37.59	
P02081	hemoglobin fetal subunit beta	N/A	33.49	
A0A4W2CXJ4	uncharacterized protein	ITIH2	30.24	inter-alpha-trypsin inhibitor heavy chain 2
A0A140T843	beta-2-glycoprotein 1	APOH	28.47	
A0A4W2D1I6	plasminogen	PLG	27.84	
P00978	protein AMBP	AMBP	27.59	
A0A4W2GKV5	GLOBIN domain-containing protein	LOC113905582	27.02	
A0A452DI25	hemopexin	HPX	25.32	
F1MNV5	kininogen-1	KNG1	24.39	
A0A4W2HBJ7	uncharacterized protein	ITIH4	23.22	inter-alpha-trypsin inhibitor heavy chain 4
F1MMD7	inter-alpha-trypsin inhibitor heavy chain H4		23.22	
A0A3Q1MA31	inter-alpha-trypsin inhibitor heavy chain H4		23.22	
P01044	kininogen-1	KNG1	22.85	
A0A4W2EPV7	anaphylatoxin-like domain-containing protein	C3	21.85	
Permeate				
accession number	protein	gene	score	family domain
A0A4W2DDL5	uncharacterized protein	ALB	1589.35	albumin
A0A4W2GW83	uncharacterized protein	ALB	1428.35	albumin
P12763	alpha-2-HS-glycoprotein	AHSG	707.58	
A0A4W2I777	uncharacterized protein	AHSG	561.66	alpha-2-HS-glycoprotein
A0A4W2DZ09	uncharacterized protein	N/A	456.20	serotransferrin
A0A4W2GWE4	uncharacterized protein	N/A	427.15	transferrin-like

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Table 2. continued

Permeate				
accession number	protein	gene	score	family domain
A0A3Q1MIW0	alpha-fetoprotein	AFP	145.41	
A0A4W2GW20	uncharacterized protein	AFP	142.66	alpha-fetoprotein
A0A4W2EWF2	GC vitamin D binding protein	GC	128.91	
A0A4W2E185	uncharacterized protein	A2M	124.67	alpha-2-macroglobulin
Q7SIH1	alpha-2-macroglobulin	A2M	121.91	
A0A4W2H192	GC vitamin D binding protein	GC	119.23	
Q2KJF1	alpha-1B-glycoprotein	A1BG	93.91	
A0A4W2D351	uncharacterized protein	FETUB	87.48	fetuin-B-type
P06868	plasminogen	PLG	51.99	
A0A4W2D1I6	plasminogen	PLG	51.99	
P02081	hemoglobin fetal subunit beta	N/A	38.63	
A0A4W2DRJ5	GLOBIN domain-containing protein	LOC113883298	35.33	
A0A4W2CXJ4	uncharacterized protein	ITIH2	31.61	inter-alpha-trypsin inhibitor heavy chain 2
P34955	alpha-1-antiproteinase	SERPINA1	29.86	
P00978	protein AMBP	AMBP	28.98	
A0A4W2HBJ7	uncharacterized protein	ITIH4	24.75	inter-alpha-trypsin inhibitor heavy chain 4
F1MMD7	inter-alpha-trypsin inhibitor heavy chain H4	ITIH4	24.75	
A0A4W2D408	anaphylatoxin-like domain-containing protein	C3	23.62	
A0A3Q1LXP4	beta-2-glycoprotein 1	APOH	23.19	
F1MNV5	kininogen-1	KNG1	20.93	

[a]Proteins with the highest score are listed.

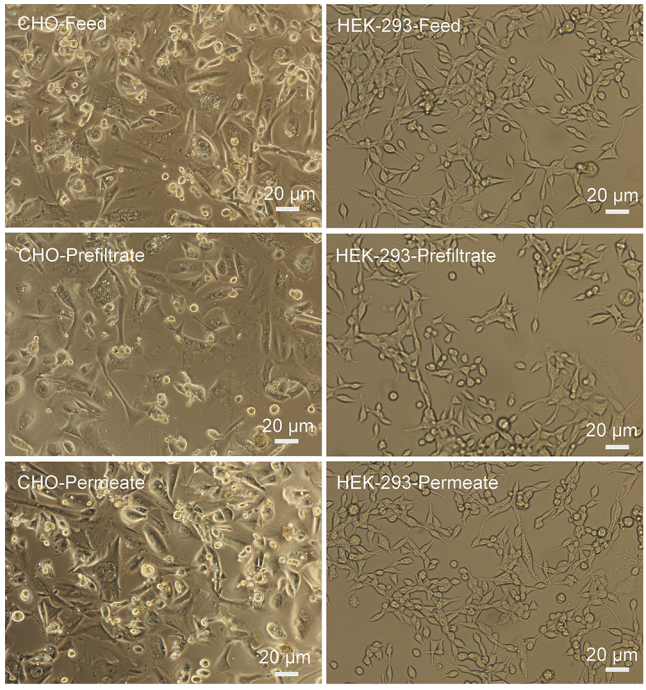

Figure 4. CHO and HEK-293 cells cultured for 48 h in PowerCHO and HyClone SFM4HEK-293 media, supplemented with 5% FBS before and after prefiltration with 11-μm thick prefilter and filtration with 33-μm thick filter papers.

FBS. No significant changes were observed in cell viability for prefiltered samples. The ISO-10993-5 *Tests for in vitro citotoxicity* defines the acceptable cell viability level at 70%.[29] Thus, it is concluded from the results of this study that the cell viability of CHO and HEK-293 cells is high and within acceptable limits of cytotoxicity, despite the overall lowered amount of proteins in the final sample. The relatively high cell viability was sustained by the presence of main protein fractions verified by LC-MS/MS analysis.

However, it should be noted that sequential filtration of FBS through 11- and 33-μm thick filter papers had a moderate, yet statistically significant ($p < 0.05$), negative effect on cell cultivation, which was manifested by slightly decreased cell viability, as seen in Figure 5. Deficiency of nutrients, resulting from reduced protein content in each step, may lead to autophagy activation, which in turn initiates recycling of damaged organelles and some nutrients to favor cell survival.[30] As it is seen from Figure 5, the differences in cell viability were not statistically significant between feed and prefiltrate pair or between prefiltrate and permeate samples. However, the cumulative effect on reduced cell viability between feed and final permeate was statistically significant.

The moderately deteriorated cell performance was further manifested by increased levels of autophagy biomarkers, i.e. LC3 protein. The microtubule-associated protein 1 light chain 3 (LC3) is the only known marker for autophagic vacuoles and exists in two forms, LC3-I and LC3-II.[31] LC3-II is a proteolytic product of LC3-I and is associated with autophagosomal membranes. Detecting LC3 by immunoblotting or immunofluorescence is a reliable method for monitoring autophagy and autophagy-related processes, including autophagic cell death. LC3-II intensity was compared to the intensity of β-actin, which is one of the main cytoskeletal proteins playing a significant role in cellular death.[31] Despite the phenotypical identity of cells cultured in media with pristine, prefiltered, and filtered FBS, the last two initiated autophagy activation; see Figure 6. Autophagy was more promoted in HEK-293 cells compare to the CHO cells, cultured in similar conditions in appropriate medium. It is concluded that autophagy biomarkers were slightly elevated due to the lower level of total protein following nanofiltrations since over 45% of protein loss was recorded.

In conclusion, sequential nanofiltration using nanocellulose-based 11- and 33-μm thick filter papers showed improved flux

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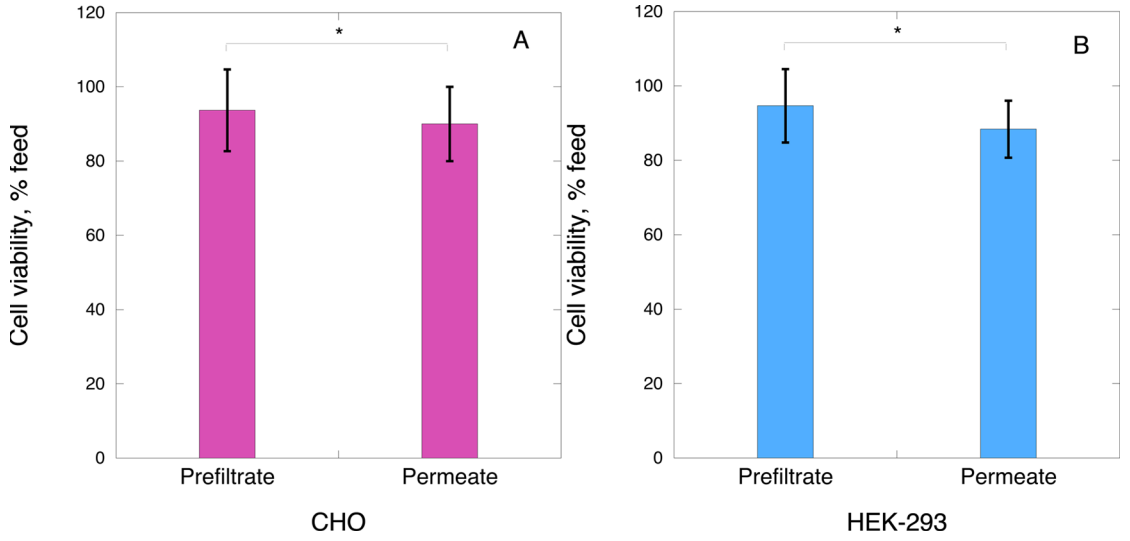

Figure 5. Viability of CHO (A) and HEK-293 (B) cells cultured for 48 h in PowerCHO and HyClone SFM4HEK-293 media, supplemented with 5% FBS before and after prefiltration with 11-μm thick prefilter and filtration with 33-μm thick filter papers. *, $P < 0.05$ ($n = 6$) compared to feed.

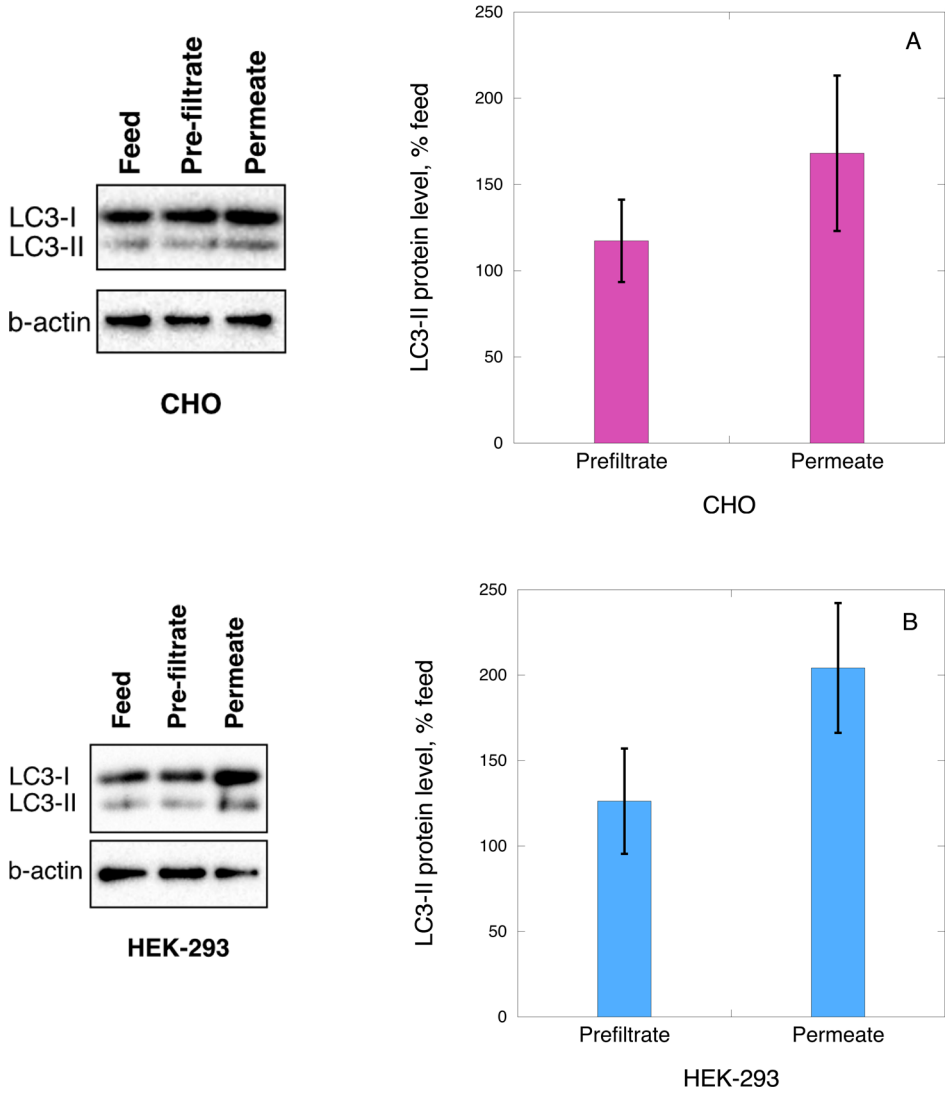

Figure 6. Autophagy marker protein levels in CHO (A) and HEK-293 (B) cells, cultured in Power and HyClone SFM4HEK-293 media, supplemented with nonfiltered, prefiltered, or filtered FBS (1:20, v/v). The results in bar plot are the average with standard deviation ($n = 3$).

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and high virus retention properties (LRV > 5) during filtration of FBS. Proteomic analysis showed that prefiltration and subsequent virus removal filtration of FBS (1:20, v/v) with 11- and 33-μm thick filter papers removed a number of large Mw proteins and protein aggregates. The cell viability of CHO and HEK cells was relatively high and confluency was adequate, albeit moderate signs of autophagy could be detected due to nutrient depletion.

EXPERIMENTAL SECTION

Materials. Bacteria E. coli (Migula) Castellani and Chalmers (15597) C-3000 strain and bacteriophage MS2 (15597-B1) were purchased from the American Type Culture Collection (ATCC). Agar (214530) was obtained from BD (Franklin Lakes, NJ). Tryptone (LP0042B) and yeast extract (LP0021B) were obtained from Thermo Fisher Scientific. NZCYM broth (N3643), maltose monohydrate (M5885), phosphate buffered saline (PBS) (P4417), total protein reagent (T1949), 2-mercaptoethanol (2-ME) (M3148), and HEK-293 cells (85120602−1VL) were purchased from SigmaAldrich. Any kD Mini-PROTEAN TGX stain-free protein gels (4568125), tris/glycine/SDS running buffer (1610732), 4× Laemmli sample buffer (1610747), and Precision Plus Protein unstained protein standards (1610363) were purchased from Bio-Rad. HyClone SFM4HEK-293 Media (10500283) was obtained from Fisher Scientific. PowerCHO2 medium (12-771Q) was purchased from BioNordika. GH-CHO (DHFR-) cells (Q420) were purchased from BioSite. L-Glutamine (25030081), fetal bovine serum (FBS) (10082147), penicillin−streptomycin (PEST) (15140122), and AlamarBlue cell viability reagent (DAL1025) were purchased from Thermo Fisher Scientific. Immunoblotting antibodies against LC3B and β-actin (13E5) were obtained from Cell Signaling (Danvers, MA, USA).

Filter Preparation. The nanocellulose-based filters were prepared by hot-pressing a nonwoven cellulose wet-cake mass formed by draining a nanocellulose dispersion over a supporting membrane. For brevity, the detailed procedures for filter-making and characterization can be found in previous publications from our group in open access sources.[24,25,27]

Model Virus Clearance. MS2 bacteriophage was used as a model small-size virus to quantify virus clearance capacity of the produced nanocellulose-based filter paper by well-known PFU quantification method. For brevity, the detailed procedures for virus filtration setup, virus and host bacteria propagation, and clearance quantification can be found in previous publications from our group in open access sources.[24,25,27] The prefiltration through 11-μm thick filter was conducted at 1 bar and through 33-μm thick filter at 3 bar at room temperature according to previously described procedure.[25] The integrity of the filter and filtration setup was tested by assuring that the flux is within specification range by buffer flushing prior to filtration.

Proteomic Analysis. Proteomic analysis of FBS supplemented cell media before and after filtration was conducted using total protein biuret assay, polyacrylamide gel electrophoresis (PAGE), dynamic light scattering (DLS), and size-exclusion high performance liquid chromatography (SE-HPLC), and tandem liquid chromatography−mass spectrometry. For brevity, the detailed procedures for listed proteomic characterizations can be found in previous publications from our group in open access sources.[24,25,27] Additionally, for the determination of levels of specific protein biomarkers, i.e. LC3B and β-actin, Western blotting was performed with immunoblotting antiobodies. The immuno-reactive bands were imaged with ChemiDoc XRS+ (Bio-Rad) and quantified with Image Lab software (Bio-Rad).

Cell Culture and Quantification of Cell Viability. Chinese hamster ovary (CHO) dihydrofolate reductase deficient (DHFR-) cells were cultured in PowerCHO 2 medium, supplemented with 5% FBS, L-glutamine, and penicillin−streptomycin (10 000 U/mL) in an incubator at 37 °C, 5% CO_2 in a humidified atmosphere.

HEK-293 cells were cultured in HyClone SFM4HEK-293 medium, supplemented with 5% FBS and penicillin−streptomycin (10 000 U/mL) in an incubator at 37 °C, 5% CO_2 in a humidified atmosphere.

The viability of cultured cells was determined by the AlamarBlue assay. The cells were collected in Eppendorf tubes and centrifuged at 1000 rpm for 5 min. The supernatants were removed from the tubes, and cells were resuspended in 500 μL of AlamarBlue stock solution diluted 1:10 in cell culture medium, transferred to a 24-well tissue culture plate, and incubated at 37 °C, 5% CO_2 in a humidified atmosphere for 2 h. Aliquots of 100 μL were transferred from each well to a 96-well plate, and the fluorescence intensity was measured at 560 nm excitation wavelength and 590 nm emission wavelength with a spectrofluorometer (Tecan infinite M200).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsabm.0c01372.

Full list of identified proteins by LC-MS/MS analysis (PDF)

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Author Contributions

L.M.: Investigation, formal analysis, conceptualization, writing−original draft. M.E.M.: Investigation. A.M.: Conceptualization; writing−original draft and review and editing; supervision, funding acquisition.

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Notes

The authors declare the following competing financial interest(s): A.M. is the inventor behind the IP pertaining to virus removal filter paper.

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ABBREVIATIONS

ATCC = American Type Culture Collection
BSA = bovine serum albumin
BSE = bovine spongiform encephalitis
CHO = Chinese hamster ovary
DMEM = Dulbecco's modified eagle's medium
DLS = dynamic light scattering
FBS = fetal bovine serum
GMP = good manufacturing practice
EMA = European Medicines Agency
HEK = human embryonic kidney
HPLC = high performance liquid chromatography
LC3 = light chain 3
LC-MS/MS = liquid chromatography−mass spectroscopy/mass spectroscopy
LRV = log10 removal value
PVDF = polyvinylidene difluoride
PFU = plaque forming unit
SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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