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Concentration of Soluble and Membrane Proteins by Pressurized Ultrafiltration with Vivaspin® 100

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Abstract

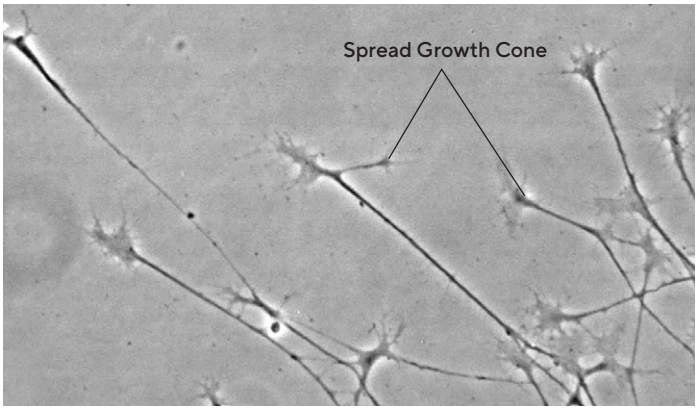
One of the most crucial steps at every stage of the protein purification protocol is concentration of the sample. This step enables the gathering of important data including specific activity, yield, recovery, and the degree of fold homogeneity. Protein samples can be concentrated using centrifugal ultrafiltration devices with membranes that have a specific molecular weight cut-off (MWCO). For small volumes of protein solutions ranging from 0.1–20 mL there are many devices available on the market, such as those from the Vivaspin® family. Similarly, it is possible to concentrate large volumes ranging from several hundred milliliters to hundreds of liters, using tangential flow devices such as Vivaflow® or Sartocon® Slice. However, if the sample to be concentrated is in the volume range of a few hundred milliliters, there is a problem since this falls between the range of the widely available low and high volume devices. In this study, we have tested the Vivaspin® 100, a centrifugal or pressure-driven ultrafiltration device, which fills this gap, allowing for the concentration of up to 100 mL initial sample volumes. We demonstrate the unique suitability of Vivaspin® 100 for concentrating intermediate volumes of both soluble and membrane proteins.

Introduction

The axons of neurons in the mammalian central nervous system (CNS), including the human brain and spinal cord, fail to regrow following injury. Recent evidence from a variety of studies points towards axon-growth inhibitory factors in the environment of damaged axons as being responsible for the lack of axonal regeneration. In our laboratory, we have purified an axonal growth inhibitory protein that induces the collapse of axonal growth cones in culture (Figure 1). We have used this protein, as an example of a membrane protein, to test the ultrafiltration performance of Vivaspin® 100 in the presence of detergent. We have also purified a diffusible protein that induces growth cone collapse of retinal ganglion cell growth cones and inhibits axonal outgrowth in collagen gel culture. This soluble protein was also used to test the performance of the Vivaspin® 100.

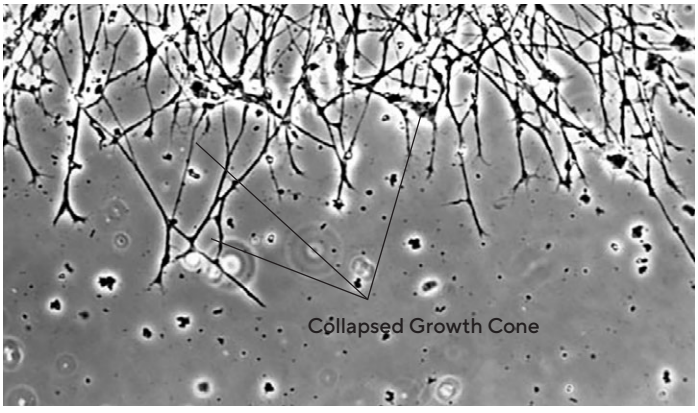
Figure 1

A. Spread Growth Cone



Note. Neuronal outgrowth from an explanted dorsal root ganglion showing axons with normal or spread growth cones.

B. Collapsed Growth Cone



Note. Neuronal outgrowth from an explanted dorsal root ganglion showing axons with collapsed growth cones following addition of 100 mL of an extract of embryonic membrane protein.

Materials and Methods

Ultrafiltration

For concentration of both the adult rat inhibitory membrane and the soluble embryonic lens protein, a Vivaspin® 100, with 100 kDa MWCO PES membranes was used. In separate experiments, 100 mL of adult rat membrane protein and soluble embryonic lens protein was dispensed into the concentrator body. The device was pressurized to 4 bar with nitrogen gas and, to maintain activity and viability, was incubated on ice during concentration. The protein content of the filtrate and retentate samples was measured using the Advanced Protein Assay (Sigma).

Collagen Gel Culture

400 µL of rat tail collagen 1 (Sigma) was mixed with 40 µL of 10X Dulbecco's Minimal Essential Medium (DMEM) and 25 µL of 7.5% sodium bicarbonate and vortexed for 1 minute. A small drop of the collagen was pipetted into the well of a 4-well Nunc tissue culture plate. A small, 600 μm^2, retinal explant and one embryonic day 6 chick lens were placed together on the drop of collagen, separated by a distance of 8 mm. Another drop of collagen mixture was pipetted onto the tissues so that they were completely surrounded within collagen. The collagen was allowed to polymerize by placing the culture plate in a CO_2 incubator for 15 minutes at 37° C. The culture wells were then filled with growth medium (3% chick embryo extract in 1X DMEM).

Growth Cone Collapse Assay

Growing axons produce hand like structures at their tips, known as growth cones. The addition of inhibitory proteins to cultures of dorsal root ganglion or retinal neurons induces a change in the morphology of growth cones from a spread structure to a collapsed stump. Thus, the growth cone collapse assay can be used to detect the presence of these proteins and quantify the amount of inhibition present in a sample. Membrane proteins need to be purified in the presence of detergents in order to keep them in solution. However, detergents must subsequently be removed before their activity can be tested on growing neurons. This can be achieved either by dialysis, ultrafiltration or with the use of Vivapure® membrane adsorbers. To mimic the natural environment of membrane proteins it is useful to incorporate them into liposomes.

Liposomes were obtained by mixing the proteins with a combination of phospholipids (phosphatidylcholine and phosphatidylserine) and dialyzing them against PBS. The liposome mixture was then added to cultures of growing neurons, which were incubated for one hour and then fixed using 4% paraformaldehyde in PBS, pH 7.4. Growth cone collapse activity was measured as the percentage of the total number of axons with collapsed growth cones.

Results

Following concentration of the adult rat brain membrane and soluble embryonic lens proteins, the growth cone collapse activity remains in the Vivaspin® (Table 1). Both the adult rat brain membrane protein and the soluble embryonic lens protein were concentrated 10-fold. The protein concentrations before and after ultrafiltration are shown in Table 2.

Conclusions

The excellent performance of Vivaspin® 100 was reflected in the high recoveries of active protein. In this respect, most of the active protein content was recovered in the retentate with minimal loss. Additionally, there were no differences in the performance of Vivaspin® 100 when concentrating either soluble or membrane proteins.

Table 1: *Growth Cone Collapse Measurements*

	Negative Controls		Retentate	
Sample	**Percentage of Spread Growth Cones (%)**	**Percentage of Collapsed Growth Cones (%)**	**Percentage of Spread Growth Cones (%)**	**Percentage of Collapsed Growth Cones (%)**
Adult rat brain inhibitory membrane protein	99	1	3	97
Soluble embryonic lens protein	100	0	2	98
Phosphate buffered saline	98	2	NA	NA
Liposomes only	99	1	NA	NA

NA—Not applicable

Note. The number of spread and collapsed growth cones in neuronal cell cultures supplemented with Vivaspin® 100 retentate samples or negative controls (including Vivaspin® 100 filtrate samples) were counted to determine the levels of axon growth inhibition.

Table 2: *Concentration of Axon-Growth Inhibitory Factors with Vivaspin® 100*

Sample	**Initial Protein Concentration (mg/mL)**	**Retentate Protein Concentration (mg/mL)**	**Filtrate Protein Concentration (mg/mL)**	**Protein Recovery (%)**
Adult rat brain membrane protein	4.6	45.1	0.5	98
Soluble embryonic lens protein	3.4	33.7	0.7	99

Note. Initial, retentate and filtrate concentrations were used to determine protein recoveries.

Note

Vivaspin® 100 is part of the Vivaspin® product family. Literature published up to c.2022 may reference the use of Vivacell 100, which is a name previously used for the same centrifugal | pressure-driven ultrafilters. When these devices were renamed, there was no change made to fit, form or function, so results collected using Vivacell 100 devices remain valid also for Vivaspin® 100.

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