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Optimised concentration and purification of retroviruses using membrane chromatography

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ABSTRACT

The ability of an anion exchange membrane to purify a γ-retrovirus was assessed and optimised with respect to different loading and wash buffers. Recoveries of infectious virus greater than 50% were consistently obtained, while specific titre was increased up to one thousand fold when compared to the material loaded. Specific proteins removed and retained by this optimised process were identified by mass spectrometry. It was possible to successfully bind and elute the equivalent of 1.27×10^8 Ifu/ml of ion exchange membrane. This could then be highly concentrated, with infectious virus concentrated to a maximum of 420-fold compared to the load.

1. Introduction:

Retroviral gene therapy vectors are a promising class of gene delivery vectors and have been used in recent successful and ongoing clinical trials. They are advantageous in their ability to stably integrate genetic information into a target cell, carry a relatively large genetic payload and have low immunogenicity [1]. Globally, as of June 2013 there are 69 phase III clinical trials involving gene therapy treatments of all types and 266 trials at all stages involving retroviral vectors. These trials have a broad range of target diseases, from hereditary conditions such as x-linked severe combined immunodeficiency (X-SCID) to cancer [2]. Retroviral vectors used in gene therapy must be of high purity, high concentration and free of replication competent virus. Current methods of production are generally limited in scalability; thus, there exists an urgent need for the development of a production and purification process that can generate batches of vector with high yield and of sufficient quality for clinical use [3]. Concentration of a retroviral vector during downstream processing allows a reduction in the burden on processes downstream [4] and improvement in transduction efficiency [5].

Macroporous chromatography adsorbents such as monoliths, membranes and microcapillary films have demonstrated their ability to be used in virus purification [6–9]. More specifically, macroporous ion exchange membranes have demonstrated high dynamic capacity for viruses and other large biomolecules such as plasmid DNA [8,10]. This large dynamic capacity is attributed to their large pores, which allow high rates of mass transfer of large biomolecules to binding sites throughout the chromatographic media relatively independent of residence time [11]. Ion exchange membranes have high dynamic capacity for lentiviral vectors and an ability to substantially concentrate them. Both the Mustang® Q and LentiSELECT anion exchange membranes have enabled successful concentration and purification of lentiviral vectors [8,9,12,13]. While no data on concentration factors achieved were reported by Kutner et al. [8], it is estimated that using a Mustang Q membrane with a volume of 0.18 ml they were able to concentrate a lentiviral vector an estimated 140-fold. This is significantly higher than the concentration factors achieved with any retrovirus by traditional chromatography, with a maximum concentration of between 1.5 and 5-fold being achieved by Rodrigues et al. [14] using a packed bed column while other membrane chromatography devices only achieved a maximum of 11-fold concentration [9], see Table 1.

Mustang Q membrane is a polyethersulfone (PES)-based membrane with a 0.8 micron nominal pore size and a surface coating of an irreversibly cross-linked polymer containing pendant Q groups [15].

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Table 1
shows selected methods of virus purification and concentration and their ability to concentrate retroviruses.

Device	Virus	Concentration factor	Reference
Streptavidin Magnespheres®	Lentivirus	2500	[18]
Mustang® 0.18 ml	Lentivirus	~140 Estimate	[8]
Fractogel® DEAE	γ-Retrovirus	1.25–5	[14]
Ultrafiltration 100 kDa	γ-Retrovirus	160	[19]
LentiSELECT 500	γ-Retrovirus	11	[9]

These data indicate the utility of membrane chromatography for lentiviral vector purification. However, there is limited information available on the purification of γ-retroviral vectors, a frequently used retroviral gene therapy vector [2,9]. With concentration of viral gene therapy vectors being so important in their dosing and efficacy choosing a purification strategy that provides both a high concentration and satisfactory purification is vital. This paper examines the utility of the Mustang Q membrane for purification and concentration of retroviral vectors, with an emphasis on a γ-retroviral vector based on a murine leukaemia virus (MLV).

2. Materials and methods

2.1. Chemicals

The following were purchased from Sigma-Aldrich (Poole, UK): Sodium Hydroxide, Hydrochloric Acid, Sodium Chloride, Ammonium Acetate, Ethanol, Bovine RNase, Trypan Blue, Bovine Serum Albumin, Dithiothreitol (DTT), Tris–HCl, methanol, glycine, Tween-20 and acetone. All chemicals used were Molecular Biology grade.

2.2. Tissue culture reagents

Penicillin streptomycin solution, DMEM (Dulbecco's Modified Eagles Medium), Phosphate Buffered Saline (PBS), polybrene (hexadimethrine bromide), L-glutamine and Trypsin were purchased from Sigma-Aldrich (Poole, UK). RPMI (Rothwell Park Memorial Institute) and foetal calf serum (FCS) were purchased from Invitrogen (Paisley, UK). T75 and T175 tissue culture flasks and 96 well culture plates were obtained from Fisher Scientific (Loughborough, UK) and Star Labs (Milton Keynes, UK). FACS tubes were obtained from Bio-Rad (Hertfordshire, UK).

2.3. Cell lines

All cell lines were kindly supplied by Dr. David Darling of Kings College London. These include the EcoPack2 cell line for GFP carrying MLV retroviral vector production (Clontech, Saint-Germain-en-Laye, France) and the murine cell line 32Dp210 used for retrovirus titration.

2.4. Cell culture

All cell culture was performed at 37 °C with a 5% (v/v) CO_2 enriched atmosphere, passaged at 80% confluency and counted using a haemocytometer. All culture media contained 10% (v/v) FCS and 100 U/ml penicillin and 100 mg/ml streptomycin.

2.5. Production of ecotrophic MLV retroviral vector

The EcoPack2 cell line (Clonetech, France) was cultured in T175 tissue culture flasks seeded at 1×10^5 cell/ml until 80% confluence was achieved. At this point the supernatant was harvested and either used immediately or aliquoted and frozen at −80 °C.

2.6. Purification using the Mustang Q membrane

The Mustang Q membrane coin (0.35 ml membrane volume) and coin holder (Pall Europe, Portsmouth UK) were attached to a Masterflex® L/S® peristaltic pump (Cole-Palmer, London UK) equipped with a Masterflex® L/S® Easy-Load® pump head (Cole-Palmer, London, UK). MLV containing viral supernatant was produced as described above. The viral supernatant was then clarified using a 0.45 μm pore filter (Millipore, Elze, Germany) and titrated to 25 mM Tris–HCl pH 7 ± 0.1 or pH 8 ± 0.1 as required, using 1 M NaOH, with either no additional salt, 0.3 M NaCl, 0.6 M NaCl or 0.8 M NaCl, pH was determined using a sterile pH probe (Applisens ltd, NL). The Mustang Q coin assembly was sanitised at a flow rate of 3.5 ml/min using 10 ml of 1 M NaOH followed by 10 ml of 1 M NaCl and then conditioned using wash buffer containing the same NaCl concentration and at the same pH as the load or 800 mM NaCl pH 8 ± 0.1.One hundred fifty millilitres of titrated supernatant was then loaded onto the Q membrane. The Q membrane was then washed with 12 ml of wash buffer and eluted using 3.6 ml of 1.3 M NaCl, 25 mM Tris–HCl pH 8 ± 0.1. Fractions of 0.6 ml were collected and analysed for viral titre using FACS and total protein as described above. One experiment was performed using a Mustang Q XT Acrodisc®, all volumes were altered to account for the 0.85 ml Q membrane volume. All experiments were performed at room temperature, which was maintained between 18 and 21 °C.

2.7. Gradient elution experiments

150 ml of MLV containing viral supernatant was loaded onto the Mustang Q coin unit as described above. The MLV was then eluted using a step gradient from 0.9 M NaCl to 1.4 M NaCl 25 mM Tris–HCl pH 8 ± 0.1. Each step in the gradient consisted of 6 ml of elution buffer and was collected in 1 ml fractions, which were analysed for infective viral titre using FACS.

2.8. Large scale experiments using the Mustang Q coin unit

The Q membrane and viral supernatant were prepared as described above in larger volumes, up to 1000 ml. These were loaded onto the Q membrane at an initial flow rate of 10 membrane volumes a minute, during which the material that flowed through the Q membrane was collected.

2.9. Establishing infective viral titre using flow cytometry

This was performed using murine 32Dp210 cells plated in RPMI, 10% FCS, 1% P/S and polybrene at 4.4 μg/ml as in [6]. A Becton Dickinson FACScan was used to read the GFP fluorescence. The linear range of target cell GFP fluorescence to infective titre is between approximately 5 and 30%.

2.10. Total protein determination

Quantitative protein determination was carried out using a standard Bradford assay, (Sigma, Dorset UK). It was performed using 96 well plates in an EL340 plate reader from Bio Tek instruments (Bedfordshire, UK).

2.11. SDS-PAGE and silver stain analysis of protein content

All SDS-PAGE gels used were Invitrogen (Paisley, UK) 15 or 10 well 4-to-12% bis tris precast Nu-PAGE mini gels and were run according to the manufacturer's instructions. Invitrogen's Mark12™ unstained standard was used as a molecular weight marker at a 1 in 20 dilution. After running at 200 V for 35 min the gels were silver stained with the SilverSnap 2 stain kit from Pierce