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Removal of xenotropic murine leukemia virus by nanocellulose based filter paper

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ABSTRACT

The removal of xenotrpic murine leukemia virus (xMuLV) by size-exclusion filter paper composed of 100% naturally derived cellulose was validated. The filter paper was produced using cellulose nanofibers derived from Cladophora sp. algae. The filter paper was characterized using atomic force microscopy, scanning electron microscopy, helium pycnometry, and model tracer (100 nm latex beads and 50 nm gold nanoparticles) retention tests. Following the filtration of xMuLV spiked solutions, LRV $\geq$5.25 $\log_{10}$ $TCID_{50}$ was observed, as limited by the virus titre in the feed solution and sensitivity of the tissue infectivity test. The results of the validation study suggest that the nanocellulose filter paper is useful for removal of endogenous rodent retroviruses and retrovirus-like particles during the production of recombinant proteins.

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1. Introduction

Therapeutic proteins (monoclonal antibodies; recombinant proteins; hormones; coagulation factors) occupy a unique niche in biotechnology, and there are hundreds of therapeutic protein products on the market today. The viral contamination of therapeutic protein production lines occurs rarely but, when it happens, the consequences are grave. The virus contamination incident at the Genzyme factory, Massachusetts, in 2009 testifies to the scale of the problem [1]. The threat of viral contamination is directly related to the safety of the final product for the end users, which can be due to both *active* and *dormant* infection, since viruses may possess both oncogenic and autoimmnune potential.

The common types of the viral contaminants, the sources of viral contamination and the various risks associated with using cell lines for production of vaccines and therapeutic proteins have been thoroughly discussed by Merten [2]. Viral contamination of production lines can indirectly jeopardize the safety of patients by creating a drug shortage, which may occur during decontamination of production facilities. Because there are no preservatives with viricidal activity to include in the final product and because the infected cell lines cannot be cured with chemicals, the possibility of virus contamination is indeed a serious issue for the biopharmaceutical industry. Even though the industry persistently strives to employ the most rigorous routines to eliminate the risks of endogenous and adventitious viral contamination, several instances of viral contamination have been recorded in the literature over the years such as Porcine Circovirus (PCV1) [3]; Cache Valley Virus (CVV) [4,5]; Reovirus (Reo3) [5,6]; Epizootic Haemorrhagic Disease Virus (EHDV) [7]; Human Adenovirus (HAdV) [8]; Mouse Minute Virus (MMV) [9–11]; and Vesivirus 2117 [1,12]. A number of other viruses have also been detected in the past in various rodent antibody production tests, such as Reovirus (Reo3), Simian Virus 5 (SV5), Sendai Virus, Pneumonia Virus of Mice (PVM), and Lymphocytic Choriomeningitis Virus (LCMV) [13].

Therapeutic proteins can be derived using various biological expression systems, such as cell lines [14], transgenic animals (milk) [15], transgenic plants (tomato, tobacco, rice, potato, turnip, canola) [16], or human blood [17]. Cell lines occupy a crucial place in the production of therapeutic proteins, and both rodent- and human-derived cells, e.g. 3T3, CHO, BHK, HeLa and HepG2, can be used for protein expression. In this respect, Chinese hamster ovary (CHO) cells are some of the most commonly used cells (70% of all recombinant protein products produced by cells) [14].

There is an innate concern related to the use of CHO and murine hybridoma cells for protein expression because rodent cell

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cultures produce endogenous retrovirus (at least four types of infectious viruses [18,19] or retrovirus-like particles) [20–22]. This is because their genomes contain multiple copies of retrovirus-like sequences [19,22–24]. Since the retrovirus genome persists within the genome of the cells, retroviruses are transmitted vertically from one cell generation to another. Indeed, non-infectious retrovirus-like particles have been observed in all CHO cells by electron microscopy, including intracytoplasmic type A particles and budding type C particles [21,22,25–28]. While type C particles produced by CHO cells are generally non-infectious [21,22,28], they nevertheless may sporadically become expressed as infectious viruses [13]. Furthermore, they silently possess an oncogenic potential stemming from their morphological and biochemical resemblance to tumorigenic retroviruses: the murine retroviruses have been shown to be oncogenic in primates [29], and recombination between retroviruses may lead to oncogenic viruses in mice [30–32]. Therefore, regulatory agencies require excessive virus removal/inactivation capacity (including non-replicating particles) during manufacturing and a demonstrated wide safety margin for all cell culture-derived biologicals for *in vivo* use [13,33,34].

Clearance of retrovirus as well as other endogenous and adventitious viruses during manufacturing processes is generally achieved by two mechanisms, viz. virus inactivation and virus removal [35–37]. Virus inactivation is aimed at reducing the infectivity of viruses by chemical or physical means. Low pH treatment, harsh solvent/detergent treatment, heat, and irradiation are commonly used retroviral inactivation methods. The virus removal is intended to physically separate viral particles from the intended product and includes mainly three methods: (i) filtration (size-exclusion filtration; electrostatic interception filtration); (ii) chromatography (protein A chromatography; ion exchange chromatography); and (iii) precipitation (Cohn fractionation, ethanol precipitation). Ideally, the size exclusion virus removal filtration is the method of choice because it is not constrained by the processing parameters such as pH, ionic strength, isoelectric point of virus, or detergents [17,35–38]. In general, the benefits of using size-exclusion filtration include flexibility and ease of use; virus removal predictability; removal of viral markers, i.e. ease of validation; no use of toxic/mutagenic chemicals for inactivation; low impact on protein yield, integrity and functionality; inertness and non-interference; adaptability to large range of products; avoidance of developing chemical resistance (e.g. Reo3, SV40) [17].

We have previously described a filter paper consisting of 100% naturally derived cellulose nanofibers [39,40]. The filter paper is composed of high crystallinity cellulose of algae origin and features a surface area of larger than 100 m^2/g [41]. The cellulose substrate material was shown useful in composite electrochemically-controlled, nanofiltration-combined solute removal hemodialysis membranes [42–45]. By adapting paper making hot press technology, a filter paper was developed featuring a virus removal capacity LRV $\geq$ 6 for Swine Influenza A Virus [39]. The goal of this study is to further validate the performance of the filter paper with respect to removal of xenotropic murine leukemia virus (xMULV) particles.

2. Experimental

2.1. Materials

Crystalline nanocellulose from Cladophora algae was supplied by FMC Biopolymer (G-3095-10 batch; USA). Polystyrene latex beads (100 nm; #L9902) and gold NPs (50 nm; #753645) were used for retention tests as supplied by Sigma Aldrich.

2.2. Microorganisms

The ICH Q5A recommends murine retroviruses as specific model viruses for rodent cell substrates. Therefore, xenotropic MuLV (xMuLV) was used as supplied by Charles River biopharmaceuticals GmbH, Germany.

2.3. Filter paper preparation

The preparation of the filter was done as described before [39,40]. About 300 mg of Cladophora was dispersed in deionized water using high-shear ultra sonic treatment (750 W; 20 kHz; 13 mm probe; Vibracell, Sonics, USA) for 10 min at 71% amplitude. The dispersed sample was then drained on a nylon filter having an average pore size distribution of 100 nm (R01SP09025; 90 mm; GE Water and Process Technologies). The sample was then dried under load using a heat-press (Rheinstern, Germany) at 105 °C to produce a flat paper sheet.

2.4. Filter paper porosity

The total porosity of the membrane was calculated from the ratio between the bulk and true density as follows

$$\varepsilon_{\%} = \left(1 - \frac{\rho_{bulk}}{\rho_{true}}\right) \times 100 \quad (1)$$

where $\varepsilon_{\%}$ is the total porosity, ρ_{bulk} is the membrane bulk density calculated from membrane dimensions and $\rho_{bulk} = 1.64\ g\ cm^{-3}$ is the true density of Cladophora cellulose [41]. The thickness of the produced membrane was measured using a digital 10^{-3} mm precision caliper (Mitutoyo Absolute, Japan). The thickness of each membrane was measured in 10 different positions, and in total 16 membranes were evaluated.

2.5. Pore size distribution

The pore size distribution of the produced membrane was measured according to the Barret-Joyner-Halenda (BJH) method [46] from nitrogen gas adsorption isotherms using ASAP 2020 (Micromeritics, USA) instrument.

2.6. Scanning electron microscopy (SEM)

Following the filtration of latex beads and gold nanoparticles, the filter paper was studied with a scanning electron microscope (Leo 1550 FEG-SEM, Zeiss). The membranes were sputtered with Au/Pd prior to analysis to avoid charging of the samples.

2.7. Particle retention test

A suspension (5 μl) of uniform polystyrene latex beads (2.5% solids) and gold nanoparticles (250 μl) was diluted in 10 ml of water. The diluted dispersions were filtered through a Cladophora cellulose filter paper (26 mm in diameter) in a Büchner funnel. The suction pressure was adjusted to 10–15 kPa using a laboratory type water aspirator.

2.8. Atomic force microscopy (AFM)

The atomic force microscopy images were acquired on the paper samples in air with Dimension Icon (Bruker, Germany) instrument. The AFM probes (ScanAsyst-Air™) were purchased from Bruker, Germany. The sample was mounted on a magnetic holder using a double adhesive tape. The images were acquired in the peak-force

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