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Improving washing strategies of human mesenchymal stem cells using negative mode expanded bed chromatography

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ABSTRACT

The use of human mesenchymal stem cells (hMSC) in clinical applications has been increasing over the last decade. However, to be applied in a clinical setting hMSC need to comply with specific requirements in terms of identity, potency and purity.

This study reports the improvement of established tangential flow filtration (TFF)-based washing strategies, further increasing hMSC purity, using negative mode expanded bed adsorption (EBA) chromatography with a new multimodal prototype matrix based on core–shell bead technology.

The matrix was characterized and a stable, expanded bed could be obtained using standard equipment adapted from what is used for conventional packed bed chromatography processes. The effect of different expansion rates on cell recovery yield and protein removal capacity was assessed. The best trade-off between cell recovery (89%) and protein clearance (67%) was achieved using an intermediate expansion bed rate (1.4).

Furthermore, we also showed that EBA chromatography can be efficiently integrated on the already established process for the downstream processing (DSP) of hMSC, where it improved the washing efficiency more than 10-fold, recovering approximately 70% of cells after global processing. This strategy showed not to impact cell viability (>95%), neither hMSC's characteristics in terms of morphology, immunophenotype, proliferation, adhesion capacity and multipotent differentiation potential.

1. Introduction

Human mesenchymal stem cells (hMSC) have been emerging over the last decade as attractive candidates for cell therapy applications [1,2], mainly due to their immunomodulatory capacity and trophic characteristics, as extensively described in the literature and recently reviewed by Caplan and Sorrell [3].

However, to be applied in a clinical setting, hMSC need to comply with specific requirements in terms of quantity, identity, potency and purity [4–6]. These requirements include delivering high number of cells at low volumes (compatible with point of care delivery) [7], while maintaining cell's high viability (higher than 85%) and characteristics [4]. Given its effect on cells' characteristics, the processing time should be minimized [8] and sterility should be ensured throughout processing due to the inability of terminally sterilize cellular products. Furthermore, cells should be pure enough to be safe to administer in a clinical setting. Therefore, strategies that can successfully integrate a washing step to reduce impurity levels up to <1 ppm [6] are compulsory. Several downstream processing (DSP) technologies have been explored so far for the washing of stem cell products. In a previous work we explored the use of tangential flow filtration (TFF) to integrate concentration and washing steps in the same hollow fiber device, achieving 98% of protein removal [9], without compromising cells' characteristics. Similarly, Lonza (MD, USA) has also reported a TFF process using hollow fiber devices, where hMSC were washed efficiently while maintaining functionality [4,10]. Another study also reported the use and validation of a novel dead-end filtration to remove the DMSO from a cell suspension without fouling the filter membrane [11].

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Given the demand for highly pure biopharmaceuticals, the application of chromatography as a separation technique has been widely demonstrated. Its high selectivity and flexibility have been enabling its use for the purification of several biopharmaceuticals with different complexities [12–14]. It can be operated using radial [15] or axial flow [14], the latter being the most widely used. Furthermore, benchmark purification techniques for bioproduct's recovery and purification are currently performed using packed bed columns. Nonetheless, the use of particulate-containing feedstocks hampers the use of packed bed chromatography [16]; alternatively, expanded bed adsorption (EBA) chromatography can be used [17]. Due to its high interparticular voidage and high adsorbent surface area, amongst other advantages [16], EBA chromatography has been applied for the separation of many biopharmaceuticals, from viral vectors [18] to cells [19]. In the expanded bed, the adsorbent inside the column is fluidized from its settled state by applying an upward flow, which allows that particulate products pass through the bed without becoming entrapped [17], making it an attractive design for the DSP of hMSC.

The main goal of this study was to improve already established TFF-based washing strategies [9], further increasing hMSC's purity up to levels compatible with their use in cellular therapies, while maintaining cells' functionality. Within this context, we have assessed the applicability of using a chromatographic step that enables a single-pass operation making its integration in the already established DSP workflow for hMSC easier. We have implemented and characterized a new multimodal prototype resin based on core–shell bead technology. Furthermore, the impact of EBA chromatography on cells' recovery, viability and characteristics in terms of morphology, identity and potency was evaluated.

2. Materials and methods

2.1. Cell culture

In this work, human bone marrow-derived mesenchymal stem cells were used (hMSC; STEMCELL™ Technologies, Grenoble, France). All reagents used to perform the cell culture were purchased from Life Technologies™ (Carlsbad, USA), unless otherwise stated.

hMSC were routinely expanded using xeno-free MesenCult®-XF Medium (STEMCELL™ Technologies) supplemented with 2 mM L-glutamine and propagated in tissue culture flasks (Thermo Scientific™ Nunc™, Massachusetts, USA), previously coated with MesenCult™-SF Attachment Substrate (STEMCELL™ Technologies). Cells were expanded at 37 °C in a humidified atmosphere of 5% CO_2 in air. 50% of the culture medium was exchanged at day 5. At 70–80% cell confluency, the medium was removed and cells were washed with Dulbecco's phosphate-buffered saline (DPBS) and incubated with TrypLE™ Select (1×) dissociation reagent for 10 min at 37 °C. After detachment, cells were resuspended in MesenCult®-XF medium, and centrifuged at 300 × g for 5 min at 20 °C. The cell pellet was resuspended in MesenCult®-XF medium and transferred to new pre-coated culture flasks, at an inoculum cell concentration of 5×10^3 cell/cm^2.

For downstream processing experiments, after detachment cells were ressuspended in DMEM supplemented with 10% (v/v) Fetal Bovine Serum (FBS).

2.2. Downstream processing (DSP)

2.2.1. Chromatography

A multimodal resin prototype based on a similar concept to Capto™ Core 700 (GE Healthcare, Uppsala, Sweden), was used to perform the DSP experiments. The resin beads consist on agarose-based porous particles with a deactivated outer layer and an inner core that has been modified with octylamine ligand.

2.2.1.1. Resin prototype size distribution. Bead size was estimated using a phase contrast inverted microscope (DMI 600 B, Leica Microsystems GmbH, Wetzlar, Germany) using the software Leica Application Suite LAS AF. One perpendicular diameter of each bead was measured from 20 beads, of three different samples, from beads before and after sieving.

2.2.1.2. Batch protein adsorption characterization. To characterize the protein removal kinetics throughout time, 5 g of resin were weighed and incubated with 5 mL of DMEM (Life Technologies) supplemented with 5%, 10% or 20% (v/v) of FBS (Life Technologies). These different medium formulations were equilibrated for 60 min with the beads with a constant rotational stirring (33 rpm) in a roller mixer (SRT9, Bibby Scientific Ltd., Staffordshire, United Kingdom). Sampling was performed at 2, 5, 10, 15, 30, 45 and 60 min. These samples were centrifuged at 300 × g for 5 min at 20 °C and the supernatant was kept for further analysis.

To determine the binding capacity of the resin (amount of adsorbed protein *per* mass of resin), DMEM supplemented with 2%, 5%, 10%, 20% or 30% (v/v) of FBS was incubated in a 1:5 (w/v) ratio of beads:medium for 120 min (saturating conditions) with a constant rotational stirring (33 rpm) in a roller mixer (SRT9, Bibby Scientific Ltd.). Sampling was performed after the incubation period, where the suspension was centrifuged at 300 × g for 5 min at 20 °C and the supernatant kept for further analysis. The matrix was regenerated following the manufacturer's instructions.

The adsorption isotherm for proteins onto the adsorbent was fitted to a Langmuir isotherm Eq. (1):

$$q = \frac{q_{\max} C}{K_D + C} \quad (1)$$

where q is defined as the amount of total protein (mg) adsorbed *per* mass of resin (g), $q_{\max}$ the maximum adsorption capacity, C is the equilibrium total concentration of un-adsorbed protein in the liquid phase and K_D is the dissociation constant.

2.2.1.3. Packed bed and expanded bed chromatography. The chromatographic resin was used either in a packed bed radial column or in an expanded bed column. All chromatographic experiments were performed at 20 °C using ÄKTA explorer 10S (GE Healthcare) controlled by UNICORN™ (GE Healthcare). Special attention was given to the flowpath to avoid points of high shear; in order to do so, the flow restrictor and internal filters were removed. The cell suspension was injected via a 50 mL superloop (GE Healthcare) and monitored using a UV 280 nm detector.

To perform radial chromatography, the beads were packed into a Micro FC column (Proxcys BV, Nieuw-Amsterdam, The Netherlands), with 60 mm bed height and 5 mL of bed volume, using a 100 μm frit. According to the bed height, cells were injected inside the column at 46 cm h^{-1}, in order to ensure a comparable residence time with expanded bed chromatography.

Expanded bed chromatography was performed in a regular Tricorn 10/50 column (GE Healthcare). Initially, the system was sanitized by recirculating NaOH 1 M in 30% (v/v) in 2-propanol (VWR International, Pennsylvania, USA) for 60 min. The resin was equilibrated for at least 1 h before the experiments at the desired expansion flow rate with DPBS, until the pH was stabilized at 7 and the bed stability was attained. A control experiment was performed by incubating a similar cell suspension and resin's amount for the same period of time in batch mode under orbital shaking at 20 °C.

Experiments using different bed expansion ratios were performed using 12 g of resin. As depicted in Fig. 1, q was determined

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