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Automated harvesting and 2-step purification of unclarified mammalian cell-culture broths containing antibodies

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ABSTRACT

Therapeutic monoclonal antibodies represent one of the fastest growing segments in the pharmaceutical market. The growth of the segment has necessitated development of new efficient and cost saving platforms for the preparation and analysis of early candidates for faster and better antibody selection and characterization. We report on a new integrated platform for automated harvesting of whole unclarified cell-culture broths, followed by in-line tandem affinity-capture, pH neutralization and size-exclusion chromatography of recombinant antibodies expressed transiently in mammalian human embryonic kidney 293T-cells at the 1-L scale. The system consists of two bench-top chromatography instruments connected to a central unit with eight disposable filtration devices used for loading and filtering the cell cultures. The staggered parallel multi-step configuration of the system allows unattended processing of eight samples in less than 24 h. The system was validated with a random panel of 45 whole-cell culture broths containing recombinant antibodies in the early profiling phase. The results showed that the overall performances of the preparative automated system were higher compared to the conventional downstream process including manual harvesting and purification. The mean recovery of purified material from the culture-broth was 66.7%, representing a 20% increase compared to that of the manual process. Moreover, the automated process reduced by 3-fold the amount of residual aggregates in the purified antibody fractions, indicating that the automated system allows the cost-efficient and timely preparation of antibodies in the 20–200 mg range, and covers the requirements for early *in vitro* and *in vivo* profiling and formulation of these drug candidates.

1. Introduction

Therapeutic monoclonal antibodies (mAbs) and antibody-related molecules such as immunoglobulin Fc-fusion proteins represent a fast growing class of therapeutics, with nearly 50 products approved or pending registration in the US and the EU [1]. The development of these new biologics requires effective discovery platforms including high throughput antibody production and characterization. Powerful display methods such as phage display, used in combination with large combinatorial antibody libraries allow the rapid generation of a large panel of molecules [2]. In this early selection phase, when dealing with hundreds to thousands of candidates, only a few μg up to a couple of mg of antibodies prepared on robotic platforms are required to carry out the necessary biological and biophysical analysis [3]. Major developments in high throughput (HT) technology for producing a large panel of recombinant proteins have been driven initially by structural genomics and proteomics initiatives [4–6]. Back to back fully automated robotic installations for mammalian expression and purification of thousands of recombinant secreted proteins at the sub-mg scale have been reported [7]. In parallel, important progress has been made in the development of novel micro-bioreactors for HT small-scale parallel expression to support process development [8], and has been validated with small-scale disposable reactors up to culture volumes of 250 mL [9]. The downstream processing has relied mainly on modified liquid-handling systems able to carry out affinity-capture purification of recombinant antibodies or tagged-proteins [10–12]. Later on during the generation of therapeutic antibodies, the selection focuses on the 20–50 remaining candidates having shown the required biological and developability properties. Their further profiling, including biophysical screens and formulation studies, yet also confirming their

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biological efficacy in rodents or other animals, will require larger amounts, usually in the 50–200 mg range. Whereas many of the analytical platforms used for profiling these candidates have been optimized towards automation and higher throughput [13], the upstream and downstream preparative steps to produce sufficient amounts of materials rely largely on low throughput manual or semi-automated processes only. Upstream, a cost-effective and fast way to produce these quantities is to express them as recombinant antibodies transiently in mammalian cells such as human embryonic kidney (HEK)-293 cells. This is done usually in 1-L roller-bottles or in 10–100 L Wave bioreactors for gram amounts [14]. Downstream, the purification is done mainly by affinity capture (AC) chromatography such as protein-A, followed by one or several polishing steps. To reduce bottlenecks in purification, several systems based on modified bench-top chromatography instruments have increased the throughput by integrating automated multiple chromatographic steps, using intermediate pool collection between each chromatography step [15–18]. Recent improvements have been achieved by using a direct flow-through system with on-line neutralization [19] or by combining up to four purification columns [20]. Another way to increase throughput, yet keeping the advantages offered by benchtop chromatography instruments such as UV monitoring, is to include a dedicated auto-sampler for unattended tandem chromatography on the ÄKTA™ platform. The system is optimally designed to accommodate clarified sample-volumes up to 50 mL [21]. In this report, we have dealt with our downstream bottlenecks often encountered in preparing enough material for extended profiling of therapeutic antibody candidates, by building a bench-scale platform, which integrates the harvesting step and the purification in the automated process. Up to eight 1-L unclarified HEK293 cell culture-broths (CB) containing recombinant antibodies are clarified in-line by disposable filters, before being purified by tandem purification, avoiding time-consuming manual harvesting steps. The whole process runs in 19 h including the complete cleaning in place (CIP) and column re-equilibration to avoid sample cross-contamination, allowing the purification of 40 cell-cultures per week with a minimum of attendance. The automated system results in an approximatively 4-fold increase in throughput compared to the conventional downstream process relying on manual steps, yet provides material of comparable quality. The system includes additional features such as air bubbles and constant pressure monitoring, in-line pH neutralization after the capture step and efficient aggregate removal by adding an in-line filtration step before the size-exclusion chromatography (SEC).

2. Materials and methods

2.1. Automated instrumentation

Automated harvesting, purification and CIP were performed on two bench-top chromatography instruments ÄKTA™ pure 25 M, equipped with two system pumps capable of a flow rate of 25 mL/min and with both the U9-M triple variable wavelength and the U9-L fixed wavelength UV monitors (GE Healthcare Life Sciences, Uppsala, Sweden). Each instrument held the AC and SEC columns for the two-step purification, and performed identical operations. The two instruments were connected to a central unit containing eight disposable filtration devices, the two sample pumps S9 and sample inlet valves. The whole system was controlled by the UNICORN™ software version 6.4 (GE Healthcare Life Sciences). The instruments operated at room temperature (RT), only the two fraction collectors F9-C were kept at 7 °C in a custom-made three-door cooling cabinet (Koch-Kaelte AG, Appenzell, Switzerland).

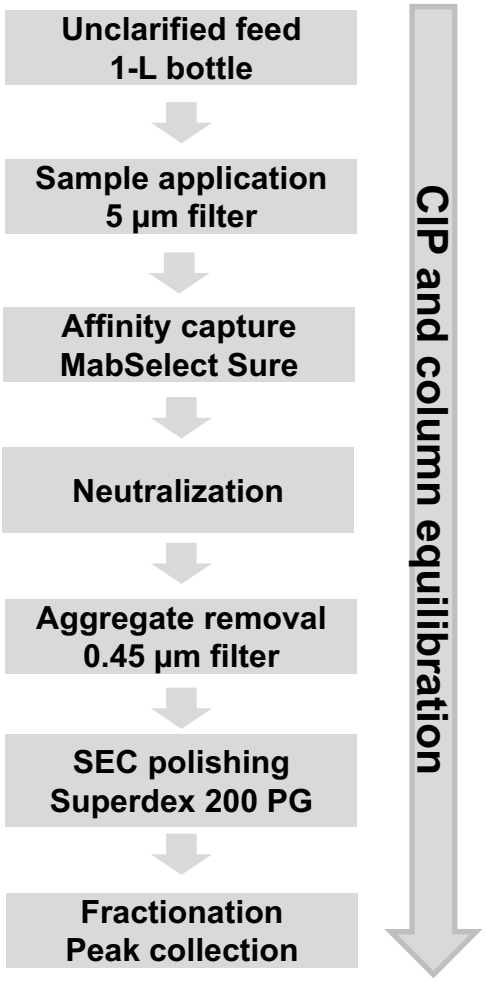

Fig. 1. Overview of the main steps of the automated process.

2.2. Automated unclarified sample application, tandem purification and cleaning-in-place – a process overview

A brief overview of the automated process is shown in Fig. 1. Operations were phased and staggered to process eight 1-L unclarified CB in 19 h. Up to four bottles were connected to the sample-inlet valve of the corresponding chromatography instrument. Two samples were loaded in parallel at a flow rate of 6 mL/min through individual single-use filtration devices ULTA™ Disc GF 47 mm, 5.0 µm pore size (GE Healthcare Life Sciences), to remove cells and cell debris before being purified in-line using tandem chromatography. MAbs were captured by protein-A affinity chromatography on two interconnected 5-mL HiTrap™ MabSelect™ SuRe™ columns equilibrated with Dulbecco phosphate buffered saline pH 7.3 (dPBS). This affinity medium was chosen for its high mAb binding and specificity properties and its alkali tolerance for efficient CIP. We used two interconnected 5-mL columns to ensure enough binding capacity. After loading, the HiTrap columns were washed with 15 column volumes (CV) of dPBS followed by a one-step elution at 5 mL/min with 50 mM Na-OAc buffer (Merck, Darmstadt, Germany). When the $A_{280\,nm}$ exceeded a threshold value of 400 mAU, the in-line pH neutralization of the eluted protein fraction was triggered by applying a gradient of 320 mM Tris base (Sigma–Aldrich, Steinheim, Germany). In parallel, the $A_{600\,nm}$ was monitored for indication of light scattering due to the presence of aggregates. The samples were then passed through a filter Filtopur S, 0.45 µm at 5 mL/min (Sarstedt, Numbrecht, Germany) for potential aggregate removal and were