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Affinity chromatography for vaccines manufacturing: Finally ready for prime time?

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ABSTRACT

Affinity chromatography is among the most powerful separation techniques, achieving the finest separation with high yields even in the most challenging feed streams. Incorporating affinity chromatography in vaccine purification has long been attempted by researchers to improve unit yield and purity with the secondary goal of reducing the number of downstream process operations. Despite the success in laboratory-scale proof of concept, implementation of this technique in pilot or cGMP manufacturing has rarely been realised due to technical and economic challenges in design and manufacturing of ideal ligands as well as availability of high-productivity chromatography media. This paper reviews evolving technologies in engineered ligands and chromatography media that are encouraging companies to revisit the possible use of affinity chromatography in larger scale vaccine purification. It is postulated that commercial-scale implementation of high throughput single-use affinity chromatography can significantly simplify process architecture, improve productivity and flexibility, and reduce cost of goods.

1. Rapid, global and affordable access to vaccines

Vaccination is estimated to prevent nearly 6 million deaths annually and brings other societal benefits that include reducing healthcare costs, extending life expectancy, increasing travel safety and mobility, protecting against bioterrorism, and promoting economic growth [1,2]. Despite the tremendous benefits of vaccination, there is great disparity in vaccine availability throughout the world. While vaccines have helped reduce deaths due to communicable diseases in developed countries to less than 10%, approximately 50% of deaths in developing countries are still caused by infectious diseases that are often preventable through vaccination [3]. Vaccine production is characterized by complex, diverse and often aseptic bioprocesses, and exceptional safety demands as healthy individuals are treated, leading to high investment costs in R&D and production facilities which creates barriers in affordable and sustainable global-scale supply of vaccines [4–6]. Beyond the cost concern, recent infectious disease outbreaks have raised questions about rapid and scalable manufacture of vaccines. The technologies used in vaccine manufacturing directly impact the timeline of process development, product quality, ease of process scale-up, and cost of goods (COGs). Current traditional downstream process (DSP) operations in vaccine production are time-consuming and complex with low-productivity and less-than-optimal process capability, often representing the majority of manufacturing cost [7–9]. Substantially simplified and potentially platformable vaccine purification schemes can therefore significantly shorten process development time and reduce COGs, enabling sustainable business models with affordable global prices.

2. Principle and benefits of affinity chromatography

A wide range of expression systems has been employed in vaccine manufacturing owing to the products' vast diversity and complexity. For those expressed in more complex systems (ex. single- or multi-cellular eukaryotes, transgenic organisms), traditional purification schemes typically involve multiple steps including cell disruption, precipitation (ammonium sulfate, calcium chloride, polyethylene glycol, etc.), clarification (filtration, centrifugation, depth filtration, tangential flow filtration), gradient ultracentrifugation (ex. sucrose, cesium chloride, etc.), size exclusion chromatography, and other chromatographic techniques such as ion

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exchange and hydrophobic interaction [10]. Implementation of some of these units (ex. ultracentrifugation) at manufacturing scale is challenging. Complicated purification trains are often costly with low productivities because of long processing times and low yields and constrain traditional facilities due to limited flexibility. Moreover, the diversity of antigen types (polysaccharides, virus-like particles [VLPs], live attenuated viruses, recombinant antigens etc.) demands an array of different purification techniques; the lack of process standardization requires repeated time- and resource-consuming process development activities for almost every vaccine product. New processing paradigms are therefore required to streamline, optimize, and potentially standardize the downstream operations, and be able to adjust to fluctuations in product demand or multi product situations.

Affinity-based development and manufacturing platforms have the potential to address many of the constraints raised above. Affinity chromatography (AC) separates the molecule of interest from the crude process stream based on highly specific interactions between the target and the immobilized ligand (Fig. 1). With the right ligand, sufficient purity can be achieved in one step. This high purification efficiency allows the process architecture to be simplified to a single AC capture step accompanied only by a clarification (before) and a polishing step (after), enabling a platform approach where by coupling a different affinity ligand on the chromatography media, a new molecule can be purified with only minor or no modification to the entire downstream scheme. In principle, a well-established platform has the potential to decrease time-to-market, increase R&D productivity and control costs [11–13]. Monoclonal antibodies (although much simpler and more characterized compared to most vaccine antigens) exemplify the tremendous success of this strategy where recurrent application of different assets provides the preconditions for investing significant R&D efforts. This has contributed to fine-tuned operational performance, and substantial return is achieved from improved cost-efficiency and better product and process characterization. In the vaccine industry, however, the panoply of antigen types has held back the establishment of true manufacturing platforms. In recent years, novel antigen delivery systems such as viral vectors, RNA/DNA vaccines and lipid-based carriers have emerged for which AC can be potentially developed for each delivery system to help build manufacturing platforms. Moreover, affinity-based platforms are also suitable for purifying free and/or conjugated carrier proteins (cross-reacting material [CRM], tetanus toxoid, diphtheria toxoid, *Neisseria meningitidis* and *Haemophilus influenzae* protein D) that are recurrently used in polysaccharide (glycoconjugate) vaccines. Sophisticated affinity ligand design and chromatographic support coupling system can lead to a truly revolutionary paradigm shift in the industry as application of affinity-based purification will enable a low-footprint, intensified and generic downstream process independent of the type or specific antigen of interest. In such approach, most of the focus of downstream development would shift from resource-intensive process development to potentially externalized, fast and cost-effective ligands generation for any given antigen with high probability of success which could be plugged onto the generic DSP.

3. Laboratory-scale proof of concept

Over the past decades, various AC techniques including immunoaffinity, lectin, immobilized metal affinity chromatography (IMAC), and heparin were investigated for purification of different types of vaccines at laboratory scale. Promising purification performance along with higher yield compared to other separation methods such as ultracentrifugation demonstrated that AC is a simple and specific method for vaccine purification. Table 1 highlights examples of virus applications for the most popular types of affinity chromatography along with their advantages.

Besides the four major types of AC mentioned above, other affinity ligands have also been explored. Dye ligands have been

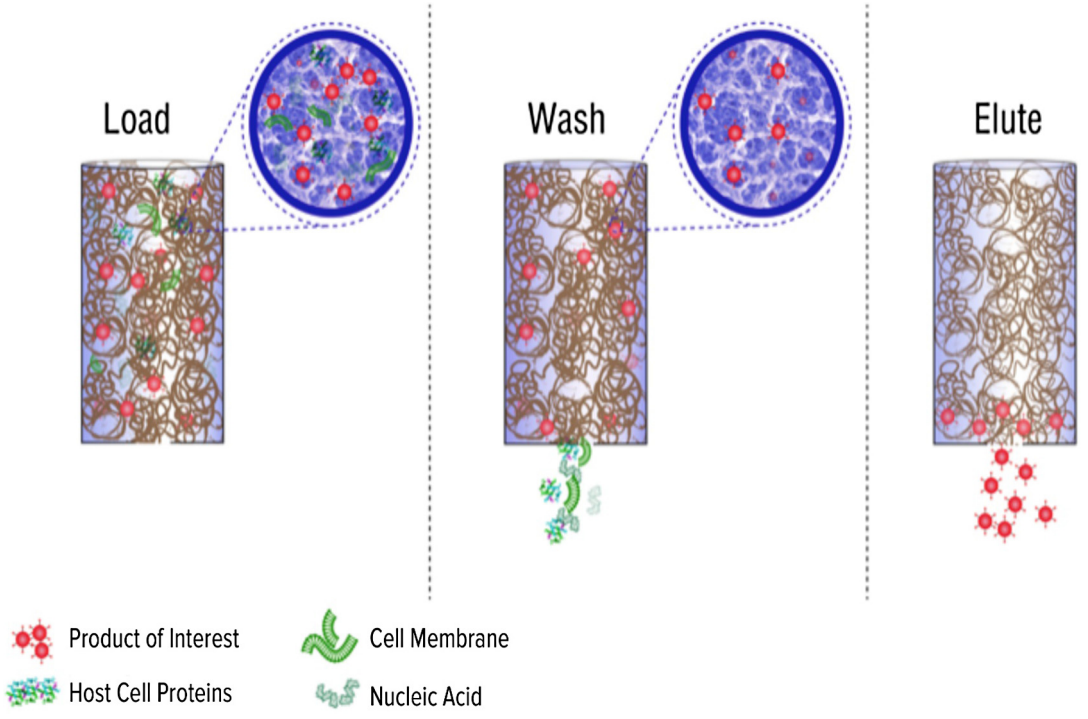

Fig. 1. Principle of affinity chromatography. Affinity chromatography separates the product of interest from a mixture based on highly specific interactions between the target and the immobilized ligand. Under binding condition, the target molecules bind to the ligands while impurities including cell membrane components, host cell proteins and nucleic acids flow through the column. Optimized washing condition removes non-specifically bound impurities. Bound target molecules are then released from the affinity media under elution condition.

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