A HIGH-THROUGHPUT SINGLE-USE PLATFORM FOR VACCINE BIOPROCESS DEVELOPMENT

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Vaccination is the predominant tool in the prevention of infectious disease. Considering the SARS-CoV-2 (Covid-19) pandemic, the need for a development platform, capable of rapid candidate screening and/or a vaccine scaffold capable of adaptability to new disease targets, has never been more apparent. VLPs, expressed in the methylotrophic yeast *Pichia pastoris,* offer an exciting alternative to current manufacturing methods due to their potential as scaffolds for foreign antigen display. The hepatitis B core antigen (HBC) can spontaneously self-assemble, forming icosahedral particles that are inherently immunogenic. Tandem Core HBC (TC-HBC) VLPs have been genetically modified in the major insertion region (MIR) enabling display of up to two epitopes of interest when assembled. For TC-HBC VLPs to be considered a viable vaccine candidate, their bioprocessing must be optimized. Currently, there are various issues to address, including problems with formation, solubility, and immunogenicity.

The aim of this work was to develop a single-use vaccine development platform and explore the potential of HBC as a vaccine scaffold. Influenza was used as a model pathogen owing to its persistence as a public health threat. The equipment and methods developed in this work were considered to enable: (1) thorough investigation of three HBC VLP candidates to identify a universal bioprocess, irrespective of surface displayed epitopes; (2) formation of a small-scale high-throughput platform which could be implemented for rapid screening of new disease targets or to allow fine-tuning of processes for epitope-dependent optimisation.

The ambr®250 modular was used to investigate three influenza specific candidates (HBC -HA2,3M2E, -LAH3,K1 and -3M2E,K1), exploring fermentation induction strategies and to identify epitope related differences. Following this, the most readily soluble candidate (HBC-LAH3,K1) was selected for further optimisation combining ambr®250 experimentation with statistical Design of Experiments (DoE). An improved process was identified, enabling an increase in VLP titre, a 34% increase in biomass compared to the initial condition, and a 6% decrease in process time compared to methanol only induction. This improved feeding regime was applied to the production of the other two VLP constructs, resulting in higher biomass and soluble HBC yield for all.

Subsequent downstream process studies on VLP primary recovery were necessary to account for the reduced volumes associated with miniaturised fermentation studies, and to bridge the gap between upstream processing and purification. Building on previous work, a high-throughput, small-scale cell disruption method was investigated using Adaptive Focused Acoustics® (AFA). A single-use 96-well plate workflow was demonstrated, enabling suitable VLP release and recovery with a ~99.7% reduction in sample volume, in comparison to high pressure homogenisation (HPH).

Finally, chromatography screening was undertaken using PreDictor® plates to rapidly identify separation conditions for the various vaccine candidates. Studies were conducted to investigate suitable resins and binding/elution conditions and to determine the influence of the physicochemical properties of the displayed epitopes on separation performance. Multiple resins were identified as being suitable for VLP purification, and results were useful to manipulate chromatographic separation (5mL column scale) conditions to achieve improved product yield and purity profiles.

Overall, this research suggests that a high-throughput vaccine development platform can be realised through the integration of numerous small-scale single-use equipment, techniques, and methodologies. Namely, the use of the ambr®250 bioreactors, AFA® cell disruption in 96-well plates and 96-well PreDictor™ resin plates. Combined with statistical DoE, this platform can be used to rapidly optimise production and purification conditions for novel vaccine technologies, such as HBC Tandem Core VLPs.

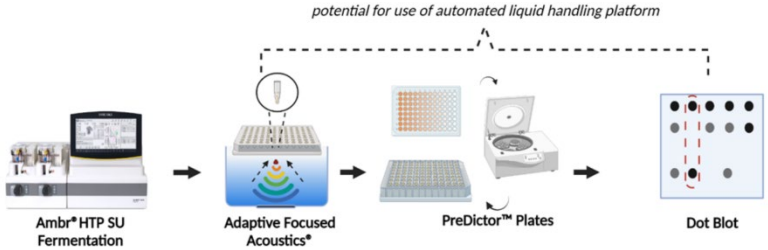

Figure 1. The small-scale, single-use, high-throughput workflow for rapid bioprocess development established and demonstrated in this work.