Multiserotype Glycoconjugate Vaccines

Investigation into Membrane Fouling Behavior on Sterility Filters

Josh Abbott with Zhuoshi Du

Despite biopharmaceutical-industry excitement surrounding messenger RNA and other emerging vaccine modalities, glycoconjugate vaccines represent some of the most successful prophylactics developed to date. Available products include vaccines against *Haemophilus influenzae* type B, *Neisseria meningitidis*, some *Streptococcus pneumoniae* strains, and other key pathogens (**1**). Such vaccines comprise immunogenic carrier proteins linked chemically to specific polysaccharides, which can be isolated from a target pathogen or (increasingly) expressed recombinantly in microbial hosts.

As Kay, Cuccui, and Wren note in a 2019 review, glycoconjugate vaccines raise several advantages for efficacy and stability (**1**). By presenting multiple targets for immune-system detection, they tend to generate long-lived immune memory in recipients. They also can be administered to broad swathes of the population, including infants and the elderly. Although the complexity of chemical coupling historically has made glycoconjugates difficult to produce, researchers increasingly are leveraging bacterial technologies to couple glycans and carriers, helping to decrease costs (**1**).

Considerable research continues into developing glycoconjugate vaccines against new targets and novel methods for improving glycan expression. However, drug companies still need greater understanding of typical steps in glycoconjugate-manufacturing processes, including sterile filtration. In November 2023, I spoke with Zhuoshi Du, who works in the department of chemical engineering at Pennsylvania State University (Penn State). Du was the lead author on articles that explored scale-up issues and sterile filtration of glycoconjugate vaccines and was part of a team that presented a poster about sterile filtration of at the September 2023 BioProcess International Conference and Exhibition in Boston, MA (**2, 3**). Du explained, "During bioprocessing, harmful bacteria can grow. Those need to be removed using 0.22-µm or 0.2-µm sterile filters." That process is critical for "maintaining sterility in final drug substances and drug products before finished vaccines are administered to patients." As glycoconjugate vaccines grow in complexity — consider that the Pfizer vaccine product bears glycans from four *Neisseria meningitidis* strains — sterile-filtration processes are becoming more difficult, increasing the importance of their characterization (**4**). We spoke about challenges for sterile filtration of such products and about what his team uncovered while studying the Pfizer vaccine product.

Our Conversation

Tell me about the study you performed. We studied fouling behaviors of the sterile filtration process for glycoconjugate vaccine drug substances and drug product at the bench scale. We also conducted a pilot-scale study to develop a strategy for a commercialized glycoconjugate sterile filtration process. Sterile filtration processes can be very challenging, especially when working with relatively large particles and vaccines. We wanted to understand the fouling behaviors/mechanisms deeply to provide solutions from both an academic perspective and an industry perspective.

How do problems with membrane fouling affect vaccine capacity and manufacturing costs? Membrane fouling can lead to either transmembrane pressure increases at constant flux operation or a flux decline at constant pressure operation, leading to low filter capacity. Operators need to replace clogged membranes because such clogs make it difficult for filling vials and might create difficulties when ensuring the sterility of final

drug substances or drug products. But changing filters is costly and increases processing time, particularly when it is done at an industrial scale. Significant membrane fouling during a sterilization process increases manufacturing costs and can decrease production yield because a clogged membrane can retain the drug substance/product. The glycoconjugate vaccine that we worked with did not lose significant yield during our experiments, but at a larger scale, fouling ultimately can increase costs and decrease yield.

How are the serotypes that you used produced, and why do they need sterile filtration? The glycoconjugate vaccine is produced by conjugating an immunogenic carrier protein to a specific polysaccharide. During bioprocessing, any bacteria that enter the process need to be removed by using 0.22-µm or 0.20-µm sterile filters. It is important for vaccine developers to maintain sterility in their final drug product before the finished vaccines are administered to patients.

What distinguishes each of the serotypes used in your study? We used four different glycoconjugate serotypes to construct the drug product. Each glycoconjugate generates an immune response to a different serogroup of bacteria that causes meningococcal disease.

What materials did you use, and how did you select them? Our most important feeding materials are the four different drug substances or individual serotypes that we used, along with the formulation buffer that we used to form the drug product from those drug substances in-house. We explored the behavior of a number of sterile filters with different chemistries and morphologies. We selected sterile filters specifically to determine the impact of membrane chemistry and pore morphology on filtration behavior. In terms of the sterile filtration experiments, we used a peristaltic pump to maintain constant flux and a pressure regulator to maintain constant pressure during our different operation modes.

Can you summarize your experimental method and explain why you proceeded that way? In terms of our sterile filtration experiments, we operated at either constant pressure or constant flux. In some of the experiments, we used a fluorescently labeled glycoconjugate vaccine, challenged the sterile filters, and then cut the fouled membrane cross-sectionally. Then we used confocal microscopy to observe fouling behaviors, which enabled us to achieve a better understanding of the underlying fouling mechanisms. The drug product has a mixture of four different glycoconjugate vaccine drug substances. By labeling each serotype with a different fluorescent label, we could determine the contributions of individual drug substances in the drug product on filtration behavior.

What factors make sterile filtration a challenging process for multivalent glycoconjugate vaccines? Because sterile filtration is a size-based separation, bacteria should be retained by the filter while the glycoconjugate vaccine passes through the membrane and is collected on the filtrate side. But from the dynamic light scattering (DLS) measurements for the glycoconjugate vaccine, we observed species that are actually larger than the nominal pore size of the sterile filter, leading to membrane fouling and low filter capacity. The multivalent glycoconjugate vaccine drug product is a mixture of four drug substances, which adds to the complexity of the fouling during the sterile filtration process.

What characteristics do you seek out in membrane filters? For instance, do you seek out particular materials of construction? What performance parameters are most important to consider? There are a number of different commercial sterile filters on the market that have different chemistries, such as those made from polyethersulfone or polyvinylidene fluoride. Some also have different pore morphologies, such as having a symmetric or asymmetric pore size distribution through the depth of the filter. It can be difficult to judge which parameters are the most important to consider because all of them can affect filtration performance — understanding the contributions from each of those factors was a key goal of our research. Our goal is to identify filters that have high volumetric capacity while also providing high yield of the glycoconjugate vaccine.

What did these experimental results reveal? We found that both the drug substances and drug product show substantial fouling, but that the fouling behavior is quite different among different serotypes. Our experiments revealed that low buffer conductivity can cause a significant increase in fouling for several individual drug substances. We successfully performed confocal microscopy to locate foulants within the membrane to verify that all four drug substances contribute to the drug product fouling and there is no evidence of any significant intermolecular interactions between the individual drug substances.

How will these results influence further work in the field, including your own moving forward? Sterile filtration is crucial to ensuring drug-product

and drug-substance sterility, no matter what kind of biotherapeutic product or modality is being used. Our sterile filtration study using one glycoconjugate vaccine provides information and background knowledge about sterile filtration that enabled us to devise industrial-scale strategies for this important process. We ask ourselves several questions based on the results of this study to inform our future work: What is the scale-up problem that can occur when shifting from a small-scale to pilot-scale operation? What kind of difficulties would we face because of the complexity of our drug product associated with interactions between the different drug substances? What are the challenges in filtering a mixture that is complex when compared with single drug substances? And what techniques should we use? This includes the benefits of using microscopic methods and particle size characterization in addition to actual sterile filtration experiments. When approaching a new project, you try to envision a theme and a baseline early in the process.

Acknowledgments

This work was accomplished at Penn State with support from Du's advisor, Dr. Andrew Zydney, and through sponsorship and collaboration with Pfizer.

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optimization should be performed to increase yield and efficiency, and the degree of optimization plays a pivotal role in determining the success of vaccine development.

Moreover, traditional vaccine-manufacturing methods, such as those used for protein vaccines, are time-consuming compared with those for mRNA vaccines. Such methods can pose difficulties in responding to infectious threats such as COVID-19 promptly. To address the problem, proactive developers should establish production processes, accumulate production experience for many different antigens, and secure platform technologies that can be applied swiftly to new antigens. Introducing the latest production technologies for faster development and manufacturing is also key.

SK bioscience has secured most of the necessary manufacturing platform technologies for vaccine production. The company also has diverse antigen production experience and the expertise to develop processes rapidly by understanding characteristics and requirements from research to production scale. SK bioscience intends to respond swiftly to potential future outbreaks of new infectious diseases and to contribute to global healthcare.

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