Developing Protein-Based Vaccines for SARS-CoV-2

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In 2018, SK bioscience spun off from SK chemicals, thus establishing a fully integrated vaccine company based in Seongnam, South Korea. The company signed a contract manufacturing organization (CMO) agreement with AstraZeneca in 2020 to manufacture and distribute its COVID-19 vaccine in South Korea. SK also codeveloped the SKYCovione SARS-CoV-2 vaccine with funding from the Bill and Melinda Gates Foundation and the Coalition for Epidemic Preparedness Innovations (CEPI). The 2022 SKYCovione vaccine was the first COVID-19 vaccine developed in South Korea. In addition, SK bioscience has expanded its global partnerships to develop vaccines against a number of other diseases. The company portfolio includes a 21-valent pneumococcal conjugate vaccine, a second-generation Zaire Ebola virus vaccine, and a second-generation typhoid conjugate vaccine (**1**).

We corresponded with Yong Wook Park, senior vice president and head of biological research and development (R&D) at SK bioscience, to discuss the work his company is doing to bring vaccines to people. Park has 21 years of experience in vaccines. He began his career as a researcher in 2002, focusing on bacterial-based recombinant-protein vaccines. He joined SK bioscience in 2008 as a senior researcher for viral-vaccine development. As a lead scientist, he successfully coordinated development of South Korea's first cell-culture–based influenza vaccine, helping to obtain approval and commercialization.

According to Park, SK bioscience has developed a diverse array of vaccines, including products based on recombinant proteins, inactivated viruses, live–attenuated viruses, and protein conjugates. The company has a number of protein-expression platform technologies spanning from bacteria to yeast and animal cells. Among SK's currently approved products are cell-culture–based influenza vaccines, varicella zoster vaccines, and typhoid conjugate vaccines. During the COVID-19 pandemic, SK bioscience also played a pivotal role in developing a COVID-19 vaccine based on recombinant-protein–bearing nanoparticles.

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Weighing mRNA Vaccines Against Protein-Subunit Vaccines for COVID-19

Park said that the high efficacy, rapid production, and fast distribution of mRNA vaccines enabled them to play an important role in preventing serious infection during the COVID-19 pandemic. He said that mRNA vaccines will continue to play a significant role in global healthcare and will be essential in preparing for future infectious-disease outbreaks. However, he cautioned that although mRNA vaccines have been in development for many years, no approved products existed before the emergence of COVID-19. Urgency during the pandemic led to swift emergency-use authorizations from regulatory agencies and raised concerns about mRNA safety.

Billions of people around the world have received mRNA vaccines during the COVID-19 pandemic, largely validating their safety. Nevertheless, Park pointed out that reports of side effects persist, and a 2023 study published in *njp Vaccines* found that "mRNA vaccines exhibit more adverse reactions than traditional vaccines" while also acknowledging the value of mRNA vaccines against emerging pandemics (**2**). But protein-based vaccines, manufactured using

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established platforms with proven regulatory track records, present viable alternatives for people concerned about the safety of mRNA vaccines.

Protein-based vaccines that are produced through well-established processes can help to meet diverse global demands. Park highlighted that they can be stored in refrigerators and therefore can be distributed more cost-effectively than mRNA vaccines, which require expensive freezers capable of ultracold storage. That factor makes protein-based vaccines a favorable option, especially in low-income countries.

Protein-Subunit Vaccine Production

Park explained that the manufacturing process for recombinant-protein vaccines generally uses standard bioprocess technologies. Depending on antigen characteristics, it requires planning unit operations accordingly and optimizing process parameters. The process for making recombinant-protein antigens, as with production of monoclonal antibodies (mAbs), is divided broadly into upstream and downstream processes. It involves constructing expression cell lines capable of incorporating a recombinant gene and producing a target protein. Cells are cultured in bioreactors that range in volume from thousands to tens of thousands of liters. Cultured cells then express and secrete target proteins, which are highly purified using chromatography and/or ultrafiltration.

However, the biomanufacturing process for vaccine antigens exhibits significant variability, especially when compared with the relatively standardized cultivation and purification processes for mAbs. Proteins involved in infectious diseases have diverse antigenic characteristics, unlike antibodies, which are more uniform in their features.

Park emphasized that when developing protein vaccines, it is crucial to have an interdisciplinary approach that uses expertise in virology, immunology, structural biology, computer science, and process engineering. That is especially true during the early stages of antigen discovery and design. Researchers need to understand the human infection pathways of a pathogen as well as the key proteins that cause infection and replication. Antigens are selected after scientists identify specific regions that can induce a robust immune response. Additionally, potential candidates are selected by modifying or manipulating the nucleotide sequence of the chosen protein to enhance immune responses. An optimal antigen candidate is then selected through initial screening that includes preclinical studies.

SK bioscience developed its COVID-19 vaccine by leveraging computerized antigen design. Park's team collaborated with Neil King's team from the Institute for Protein Design (IPD) at the University of Washington in Seattle. King has conducted extensive research on computer-designed nanoparticle vaccines that can present multiple antigens, optimizing the size and structure of nanoparticles to induce a strong immune response. SK bioscience worked with the IPD to create a virus-like particle comprising two components that self-assemble to form a regular icosahedral nanoparticle. Subsequently, SK demonstrated the candidate's clinical efficacy and established a robust biomanufacturing platform capable of producing and supplying those vaccines at a sufficient scale.

Key Considerations for Biomanufacturing

Recombinant-protein antigens can be obtained from several types of expression hosts, including bacteria, yeast, and animal cells. Each host has advantages and disadvantages, Park explained. Bacterial hosts offer relatively simple and fast upstream processes with high yields that result in cost-effective production. However, proteins produced by microbial hosts may have different characteristics than those of wild-type viruses, potentially leading to lower immunogenicity. On the other hand, animal cells require complex and time-consuming upstream processes with higher associated costs, but they often exhibit superior immunogenicity because of posttranslational modifications (PTM) that closely resemble those of wild-type virus proteins. Yeast expression systems fall between their bacterial and animal counterparts.

Several factors need to be considered before selecting a protein expression system, including

cost-effectiveness, production speed, yield, scalability, regulatory requirements, company preferences for specific expression systems, intellectual property, and PTM requirements. Depending on the characteristics of a target protein, different factors may carry more weight in the selection process for a particular expression system.

Downstream Processing: Affinity chromatography processes with protein-A resin are used commonly during mAb purification. This is a standardized approach that applies to proteins that contain a crystallizable fragment (Fc) receptor. However, for viral proteins, the type and characteristics of antigenic proteins can differ depending on the pathogen and thus require different methods and materials.

Different chromatography resins can be used, depending on the physicochemical properties of the antigenic protein. Ion-exchange, hydrophobic-interaction, affinity, and other chromatography resins all have utility depending on the circumstance. Media selections can be tailored to create a purification process suitable for the specific characteristics of a protein of interest.

Scalability: The manufacturing process for protein-based vaccines is generally scalable to meet the required productivity level. It is similar to the scalability of mRNA vaccines and mAb production processes. However, unlike standardized processes for mRNA vaccines or antibody production, the manufacturing process for protein vaccines can be influenced by differences in production technologies. Factors such as the type of protein expression system used and the method of purification can affect scalability.

Although developers can optimize most processes for large-scale production, they must consider the demand for a product and quality characteristics when expanding a manufacturing process. For example, unlike antibodies, vaccines typically require a small amount of protein per dose, making large-scale cultivation, as is commonly needed in antibody processes, unnecessary.

The Role of Adjuvants

Protein-subunit vaccines are composed of specific antigens that induce immune responses. However, their intrinsic immunogenicity may be low compared with that of inactivated or live–attenuated vaccines. In addition to proteins acting as antigens, such vaccines may contain other proteins, polysaccharides, and nucleic acids that strongly stimulate a human immune response, thus enhancing efficacy. However, protein vaccines, which are predominantly purified to include only the actual antigen protein, can exhibit relatively low efficacy. Therefore, adjuvants often are used to enhance the immune response to protein vaccines. Adjuvants stimulate a potent and sustained immune response, helping a person's body recognize and "remember" the vaccine antigen.

Park added that the use of adjuvants can reduce the amount of antigen protein used in a vaccine, increasing productivity and inducing a strong defense. Depending on the adjuvant, vaccines can induce both humoral and cellular immune responses. Therefore, vaccine development should involve careful adjuvant selection based on antigen characteristics. The formulation of vaccines, including adjuvants, should be developed meticulously. For the successful formulation of a final product, developers must ensure compatibility and stability. They also must confirm that the physical and chemical properties of an antigen remain unchanged when mixed with adjuvants.

> Developers should establish production processes, accumulate production experience for many different antigens, and secure platform technologies that can be applied swiftly to **NEW ANTIGENS.**

Overcoming Manufacturing Challenges

Park told us that the most challenging aspects of protein-vaccine manufacturing are ensuring appropriate production quality and yield at the needed speed. For recombinant-protein vaccines, regulatory agencies impose stringent requirements and demand tight specifications, particularly in terms of purity and sterility. Achieving such specifications often leads to complex processes, resulting in increased costs and diminished productivity. Therefore, when developing manufacturing processes, it is crucial to select an appropriate expression system and choose cost-effective production technologies. Process

*Continued on **page 16***