INTERVIEW

Exploring the potential of insect cell lines for efficient AAV production

Yusuke Tomioka, Senior Engineer Upstream Biomanufacturing, Manufacturing Sciences and Technology, MilliporeSigma, Japan, speaks to **Tsuyoshi Teshima,** Director and General Manager, and **Mai Sasaki,** Researcher, Research & Development Department, Gene Therapy Research Institution Co., Ltd.

TSUYOSHI TESHIMA is the Director and General Manager of R&D at Gene Therapy Research Institution Co., Ltd. (GTRI), and is in charge of manufacturing process development, non-clinical studies, and regulatory affairs of AAV vectors. After earning a Master's degree in pharmacy from Tokyo University of Science, he worked at Japan Tobacco, Banyu Pharmaceutical, UMN Pharma, and IHI in R&D of small molecule drugs, vaccine marketing, clinical development, business development, and corporate planning. Before joining GTRI, he was active as the president of UNIGEN, a Biopharmaceutical CDMO.

MAI SASAKI is a researcher in the Research and Development Department at Gene Therapy Research Institution Co., Ltd. (GTRI), and is in charge of the development of AAV vectors using suspended insect cells. After earning a master's degree in Life Sciences from Hokkaido University, she worked at WDB Eureka Co., Ltd. and engaged in basic research on middle molecule drug discovery.

Cell & Gene Therapy Insights 2023; 9(1), 209–217
DOI: 10.18609/cgti.2023.032

Q Considering the risk of contamination of processes, such as by adventitious agents, what safety measures does your company take regarding adeno associated virus (AAV) production? How important is the quality and safety of raw materials, including cells, to your company's pharmaceutical manufacturing?

TT & MS: Unless it's impossible, we try to incorporate fully closed systems. In addition, adopting single-use materials such as bags, assemblies, sensors, etc., reduces the risk of process contamination. The quality and safety of raw materials are extremely important because they are linked to the quality and safety of the product itself.

It is essential to be able to obtain the information for providing sufficient explanations when applying to health authorities such as Japan's Pharmaceuticals and Medical Devices Agency (PMDA) and US Food and Drug Administration. Regarding requests to raw material suppliers, it would be helpful if they could respond flexibly concerning information provision that is difficult to disclose, such as by registering it in the master file (MF).

In addition, of course, consistency and stability of raw materials are also important for stable in-house manufacturing.

Q Have you encountered any regulatory concerns that have arisen from cell lines for AAV production?

TT & MS: There is concern about rhabdovirus when using Sf9 cells. Since this may be the first time Sf-RVN® will be used for commercial production, we are currently receiving questions from PMDA about the method for generating Sf-RVN® cells and the test method for the absence of rhabdovirus.

We are in the stage of incorporating information on rhabdovirus test methods, results, and cell line history conducted by Glycobac, for the consultation document with PMDA. There have been problems with animal cell lines that use serum for culture due to suspected contamination by serum-derived viruses. Chemically defined cell culture media supplied by MilliporeSigma can definitely help solve these points.

Q What are the main reasons for your company to select insect cells for AAV production?

"The high cost of plasmids and transfection reagents as a percentage of the total cost is a major challenge for production using HEK cells, but insect cells can greatly reduce this cost, which is an advantage."

TT & MS: The high cost of plasmids and transfection reagents as a percentage of the total cost is a major challenge for production using HEK cells, but insect cells can greatly reduce this cost, which is an advantage.

It has also been confirmed that insect cells produce higher AAV yields and full capsid ratio for many pipelines. We believe that AAV production using insect cells is advantageous from an economic perspective.

Q Why did your company select Sf-RVN®? Is safety against contamination one of the reasons for choosing Sf-RVN®?

TT & MS: It was possible to allay in advance any concerns about rhabdovirus, which requires a clearance test, and it was selected because it is expected to greatly shorten the development time.

Currently, cells with associated bioethical concerns are still widely used in the drug development field. On the other hand, Sf-RVN® is derived from insects, so we believe that such concerns will be unlikely in the future.

Q What are the main advantages of having a good manufacturing practice (GMP) cell bank and rhabdovirus-negative cell lines that have been tested for adventitious agents?

TT & MS: If GMP cell banks and adventitious agent tests have been completed, it is possible to reduce characterization tests when master cell banks and working cell banks are created in-house, and even considering the cost of annual licenses, we believe that it is worth it for the advantage of making the development period shorter.

We have multiple pipelines, but many of them target rare diseases for which there are currently no fundamental treatments, so we need to develop products quickly and deliver them to patients. So the shortening of the development time is very important.

Q Why did your company select EX-CELL® CD Insect Cell Medium?

TT & MS: The medium shows good performance in both low and high cell density conditions, and compared to other media on the market, the yields and ratio of full capsid of produced AAV in various serotypes are equal to or better than those when using the Sf9 platform. Therefore, we selected this platform based on a comprehensive judgment.

Q Have you compared the actual culture performance of both Sf9 and Sf-RVN® as applied to the Sf-RVN® platform?

TT & MS: We have made the comparisons.

There were no significant differences in doubling time and peak cell density. The cell diameters were larger with Sf-RVN®, and we observed a bit of a difference between Sf-RVN® and Sf9 in the cell expansion and polyhedron formation following baculovirus infection.

Although it depends on the gene of interest and serotype, we believe that it is necessary to evaluate the stability of baculovirus while confirming the differences from Sf9.

Q Are you satisfied with using the Sf-RVN® platform for AAV manufacturing?

TT & MS: We are satisfied with the concept, but there are some points for operation that should be evaluated. Currently, Sf-RVN® is only one rhabdovirus-negative insect strain that is commercially available, but it would be a 'nice to have' if the cell line had variations such as different clones and different origins, which would expand the scope of drug development. In particular, we would like to be able to select the best cell lines with high productivity for each AAV serotype and each baculovirus.

Q How do you feel about the regulatory support provided by MilliporeSigma?

TT & MS: Our company has not yet reached the point where products using Sf-RVN® have been administered to humans, but we plan to submit a clinical trial application this year in 2023, and the first administration is scheduled for 2024.

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Preparations for regulatory matters are still to come, but we strongly expect MilliporeSigma to provide sufficient support.

Q What was your impression from your evaluation of the Mobius® Bioreactor? From the perspective of commercial manufacture, what aspects of the system do you see as an advantage?

TT & MS: We are very happy that the system is very easy to operate and intuitively understandable. Since the HMI for parameters setting and operation are easy to understand, we felt that making a technical transfer to another facility would be easy, if we contracted with a contract development and manufacturing organizations (CDMO) company.

We plan to submit a clinical trial application this year in 2023 and the first administration of a product made with Sf-RVN® cells is scheduled for 2024.

We are confident in the support that MilliporeSigma will provide for regulatory matters based on the documentation and discussions with their team so far.

We expect that the introduction of new in-line monitoring technologies such as Raman spectroscopy will enable non-invasive and robust process monitoring at low cost.

Batch-to-batch differences are likely to occur because cells are living organisms, but it is thought that by having a lot of data at the time of production culture, it would be possible to quickly identify the cause of batch differences and the cause when something happens, and then to make acceptance/rejection decisions at an early stage during manufacturing.

Q Have you faced any challenges in scaling up your AAV production upstream process? What were some of the most important critical quality attributes (CQAs) in your experience and how did you manage them?

TT & MS: Scale-up studies are currently being conducted with a 3 L bioreactor and a 50 L bioreactor. Ideally, each cultivation should be able to be cultured multiple times in a smaller-scale evaluation with three or more different vessel sizes. Regarding critical quality attributes (CQAs), in the upstream process we focus on the yields and full capsid rates.

Cell conditions and the above CQAs can differ significantly between culturing in an Erlenmeyer flask and culturing in a reactor, so we would like to use a reactor as much as possible in the early process development. However, there are many challenges as it is difficult to arrange the required number of control devices due to lab space and costs, and it is difficult to prepare a huge volume of high-cost raw materials such as the baculovirus.

The available minimum reactor size for a scale-up study is larger than our expectation, and the significant expenditures of time, effort, and money per study are bottlenecks in

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the early-stage evaluation. Regarding small-scale studies, we feel that the currently available reactors suffer from limitations when applied to AAV manufacturing process development.

Q What were the results in terms of scalability when using the Sf-RVN® culture in the Mobius® bioreactor? And what are your impressions of the scalability and safety support for AAV production from MilliporeSigma? How does MilliporeSigma's total solution and technical/regulatory support contribute to the speeding up and robustness of AAV production using the Sf-RVN® platform?

TT & MS: We have observed that the scalability of both Sf-RVN® cell culture and AAV production in the 50 L Mobius® bioreactor has been fully verified. Since MilliporeSigma could bring the actual system to our company for a demonstration, and we could visit MilliporeSigma's M Lab™ and see the actual system, it is easy to imagine the installation in a manufacturing room and have an idea how it could work, and we are very grateful for this.

We greatly appreciate the company's deep knowledge and experience concerning scalability and the technical support offered during webinars and demonstrations.

Regarding regulatory support, which will come into play down the road, we look forward to MilliporeSigma's support when the time comes.

Q From the actual commercial manufacturing perspective, what do you think requires further study in terms of quality control of drug substances?

TT & MS: In both the main culture process for AAV production and the process for producing baculovirus for infection, we believe that one of the causes of lot-to-lot variability is the method for cell lines control strategy during culturing.

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Currently, we are only able to confirm the conditions before and after culturing, but we would like to be able to measure and manage cell conditions, metabolic by-products, titers, etc., in real-time.

In the manufacturing of baculovirus for infection, the titer increases until a certain period of time but then decreases rapidly after a certain period of time, so we intend to create a process in which it is possible to carry out the harvest at the time when the titer is highest. We currently do not have a sufficient observation of the process parameters that affect this instability yet, but we expect to be able to do so as monitoring technology improves. We believe that even when looking at the industry as a whole, the understanding of critical process parameters (CPPs) in AAV manufacturing is insufficient, and we would like to consider this challenge using the Sf-RVN® platform in the future.

Q When you consider adaptation to future process improvements and potential regulatory compliance, what new process optimization technologies would you like to expect from your suppliers of cell culture media and cell lines?

TT & MS: When we think about improving the speed of development, we are wondering if a simpler and less hands-on process is required. At present, we believe that if some supplements which are added during the process, such as cholesterol, have been configured as one cell culture medium product in advance, and if satisfactory shelf life and stability are pre-evaluated, it will support faster process start-up. In addition, we believe that systems that can measure multiple parameters, such as Raman spectroscopy, are very powerful for evaluation of the impact on CQAs in process development. We feel that it is very beneficial to have a package or service that has already been demonstrated for the measurement of these CPPs.

Q What new upstream process technologies for AAV production do you expect to see when considering future process improvements and next-generation technology adaptations?

TT & MS: In our current AAV production process lines, it is necessary to proceed with multiple culture lines at the same time, such as the baculovirus production operation and the main process operation. In addition, at our company, multiple drug pipelines are manufactured at different times in the same manufacturing facility, so it takes time to fumigate the facility, clean the lines, and complete the changeover.

One of our Sf-RVN® AAV formulations involves the preparation of four types of baculoviruses, then the production of three types of AAV using those baculoviruses, and then

the three completed AAVs are mixed into one product, all of which require a total of seven manufacturing cycles.

We would welcome MilliporeSigma's proposals for novel facility designs that could reduce risk of cross-contamination and time expenditure in the future. In addition, we expect perfusion technology, continuous manufacturing, and the development of cells that stably express specific genes, etc.

Our goal is to lower the financial and time expenditures on the way to market, and to reach a future where products can be delivered to patients quickly and at low cost.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Acknowledgements: None.

Disclosure and potential conflicts of interest: The authors have no conflicts of interest to disclose.

Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

Article source: This article is based on an interview with Tsuyoshi Teshima and Mai Sasaki.

Interview submitted: Feb 28 2023; **Revised manuscript received:** Mar 7 2023; **Publication date:** Mar 20 2023

DOI: 10.18609/cgti.2023.032

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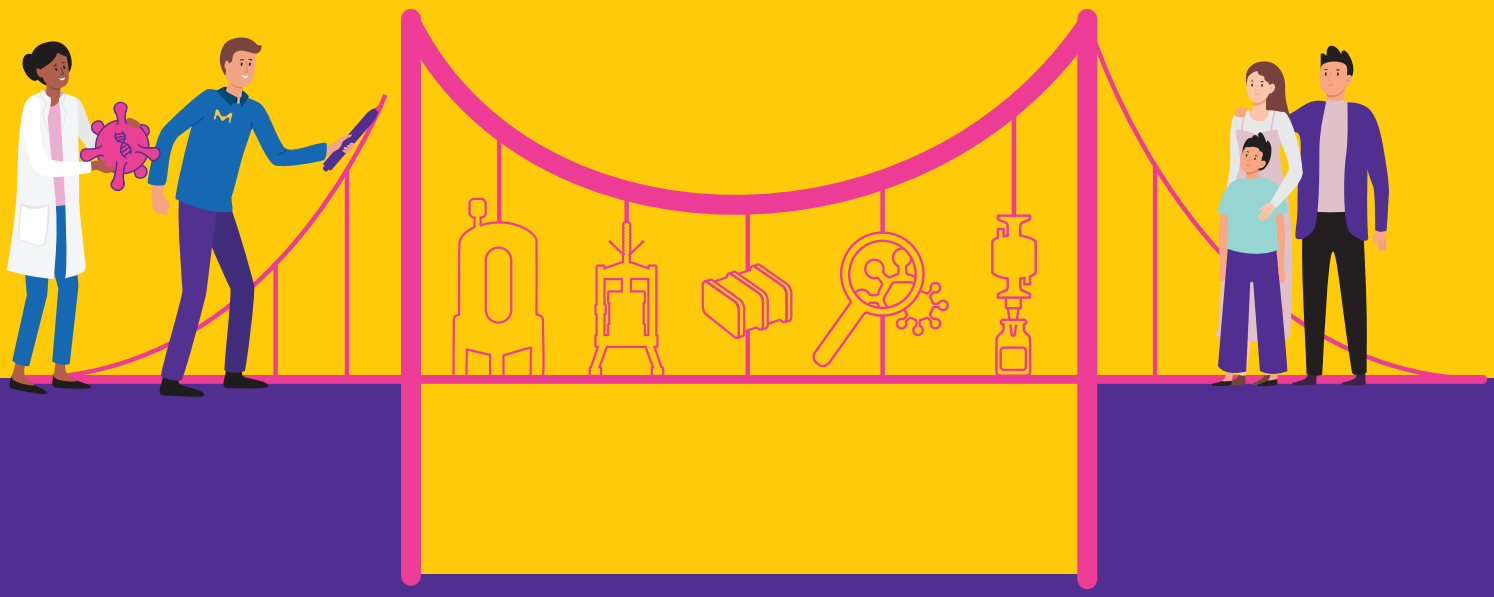

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