GEN INTERVIEW

Joy Xiaohui Chen, PhD,

Discusses a Case Study Designed to Optimize AAV Process Development and Tool Selection

GEN: *Let's begin by finding out more about Entegris. Your company serves the microelectronics, industrial, and life sciences industries. What technologies do you bring to the life sciences table?*

Joy Xiaohui Chen, PhD, Senior Applications Engineer, Entegris

Dr. Chen: Entegris was founded in 1966 and operates out of our headquarters in Billerica, MA. We have a global footprint in North America, Europe, and Asia with 5,800 employees worldwide. We specialize in providing contamination control, critical material handling, and advanced process materials used in the semiconductor device fabrication process. Our traditional customers include semiconductor device manufacturers, OEMs that provide equipment to semiconductor device manufacturers, gas and chemical manufacturing companies, leading wafer grower companies, and manufacturers of high-precision electronics. Intel, Samsung, and TSMC are a few of our customers. As a technology-driven company, we have 2,520 active patents and an additional 1,050 pending ones.

Entegris officially branched into the life sciences industry about two years ago. We see that our core technologies can help address the unmet needs of the life sciences business. We bring 55 years of expertise in filtration and purification, fluid management, and manufacturing excellence to the life sciences table. Our single-use assemblies are used by leading COVID-19 vaccine providers due to their extremely low extractable and leachable profile, gamma compatibility, wide working temperature window, and high chemical compatibility.

Our expertise in filtration and purification to ppt (parts per trillion) levels positions us well for life sciences filtration applications. Our global manufacturing footprint, specialties in ultraclean components and materials, rapid prototyping, and customization give us unique advantages in addressing unmet life sciences industry needs.

GEN: *Entegris has been working with FUJIFILM Diosynth Biotechnologies (FDB) on a project entitled "A material characterization study for AAV process development and tool selection." Tell us a little more about the focus of the project and what brought the two companies together.*

Dr. Chen: Our customers have used Aramus fluoropolymer (FP) single-use bags and assemblies for life sciences applications such as COVID-19 vaccine manufacturing, cell and gene therapy, bulk drug substance (BDS) storage, and high-cell-density banking. With increased adoption of fluoropolymer material into life sciences applications, we want to provide our customers with more application-specific information on our products. We have data available for extractables and leachables, mechanical properties, working temperature range, permeability, chemical compatibility, gamma compatibility, etc.

However, we cannot find much information in the literature about the adsorption properties of fluoropolymers compared with different materials used in cell and gene therapy applications. With the release of the FDA's new guidance on chemistry, manufacturing, and control (CMC) for human gene therapy investigational new drug applications (INDs) in 2020, selecting optimal materials to ensure early-phase regulatory compliance has once again been brought into the spotlight.

We wanted to study the adsorptive behavior of viral vectors typically used for gene therapy using Aramus fluoropolymer materials in an effort to help biomanufacturers minimize product losses during manufacturing, simplify in-process procedures for titer determination, and reduce formulation risk with inaccurate titers. FDB is a contract and development manufacturing organization (CDMO) able to provide end-to-end services which include the development of cGMP-compliant processes for manufacturing viral vectors and the analytical capabilities needed to support batch-release testing and in-depth product characterization. The company's deep understanding of virology and its experience in viral vector manufacturing and analysis is complementary to our expertise in single-use technologies and solutions.

FDB, which has developed a triple transfection–based platform for the manufacture of AAV viral vector–based therapeutics, is always striving for the highest quality and greatest yield possible. As FDB developed this platform process, it also identified the "hold" steps in the downstream process as areas that could use better control. The collaboration between Entegris and FDB aimed to find solutions to these challenges.

GEN: *Briefly provide the key details about what was actually done during the study. What was the final result?*

Dr. Chen: To meet Entegris' project needs, the FDB team produced high-titer AAV viral vectors representative of material in the bulk drug substance stage of the manufacturing process. To visualize the product loss during storage, FDB evaluated multiple titer methods for both genome copy number and total viral particle number. Due to the variabilities in these assays, FDB chose an SE-HPLC method for particle titer determination and evaluated the particles recovered from samples taken over the time course with particle titer determination of the input material. The SE-HPLC method enables a more rapid, sensitive, and reproducible measurement of AAV viral particle titer than with prevalent qPCR methodologies.

Solutions containing accurately determined amounts of AAV particles were stored in single-use polyethylene (PE), ethylene vinyl acetate (EVA), and FP bags at room temperature. The bags were sampled at time intervals up to four days, and AAV concentrations were immediately determined by SE-HPLC and compared to the starting amount.

The results demonstrated that while the HPLC peak area related to AAV concentration in PE bags decreased by more than 15% over four days compared to that of the starting AAV concentration, the AAV concentration in FP bags showed minimal to no decrease for up to four days at room temperature, which performed similar to EVA bags for this AAV serotype.

GEN: *Why was the Aramus 2D single-use assembly able to exhibit the performance it demonstrated by the study?*

Dr. Chen: The Aramus 2D single-use assembly is made of single-resin, additive-free, and gamma-stable fluoropolymer film with low surface energy, which makes it more non-adsorptive and suitable for this application.

GEN: *What conclusions can be drawn from the Entegris-FDB study?*

Dr. Chen: ***There were four main conclusions from the study:***

1. Single-use FP bags demonstrate suitability for storage of AAV-containing solutions during the manufacturing process due to their minimal adsorption of AAV particles, in contrast to more commonly used PE bags. Single-use FP bags also demonstrated on-par adsorption performance with EVA bags along with the conical-tube control in this study. Choosing fluoropolymer material can help avoid or minimize product loss during AAV manufacturing processes and reduce the complexity of quantification and formulation risks during final fill and finish.

2. The collaboration between Entegris and FDB has highlighted the need for a more rapid, sensitive, accurate, and precise method to determine AAV concentration during manufacturing processes.

3. During this collaboration, FDB has developed a more rapid, sensitive, and precise SE-HPLC method to quantify particle titers for their viral vector platform, which performs better than the qPCR method.

4. There is a need for the development of more amenable in-process analytical methods to support the rapid development and scale-up of AAV manufacturing processes while maintaining compliance with regulatory requirements.

I also want to acknowledge Cari Sadowski, Principal Scientist, Science & Innovation Group Leader at FUJIFILM Diosynth Biotechnologies for her critical role in our joint project. ■

Cari Sadowski, Principal Scientist, FUJIFILM Diosynth Biotechnologies

View the webinar
www.entegris.com/aav