Influenza has been a major infectious disease that affects public health worldwide. Three pandemics of influenza occurred in the 20th century, causing tremendous loss in human lives and the economy worldwide. The first pandemic of the 21st century occurred in 2009–2010. Immunization has been the most effective and inexpensive measure in controlling the spread and seriousness of the disease.

Influenza vaccines are produced from the specific influenza viruses and the viruses have been prepared in embryonated chicken eggs for over 70 years. This egg-based production process has inherited many drawbacks. For example, the eggs may be susceptible to the virus, or the virus may not be able to grow efficiently in the eggs. The eggs should come from healthy hens and should be ordered 6 months in advance, dead and defective eggs should be detected and discarded during the production process, and the used eggs should be treated and disposed at high cost. The hemagglutinin of the viruses grown in eggs may exhibit antigenic alterations and limit vaccine effectiveness1,2. Virus harvests collected from eggs are always more or less contaminated with microorganisms that may affect the subsequent purification process and the quality of the vaccine products. And, the residual egg components in the vaccine products may cause allergic reactions in some sensitive people. Nonetheless, large quantities of influenza vaccines can not be timely produced by egg-based method to meet urgent needs, as witnessed during the 2009 H1N1 pandemic. This problem has led to the incentive actions taken by several authorities to promote development of new production methods, including cell culture.

In addition to overcoming the drawbacks associated with egg culture as cited above, production of influenza vaccine in cell culture may also offer many distinct advantages over egg culture. For example, it allows rapid initiation and scale-up of production in the event of urgent needs. It provides well-controlled, contamination-free cell cultures for use by the virus to grow, virus preparation of high purity for easier subsequent purification process, and high degree of product batch-to-batch consistency. Therefore, quality of the vaccine products will be better and more consistent from batch to batch. In fact, the vaccine products will contain much less endotoxin, a fever-causing substance derived from certain contaminated bacteria.

Cell cultures have been used in the production of polio and hepatitis A vaccines,
Figure 1. A complete set of BelloCell-500 system consisting of a BelloStage, 4 pieces of BelloCell on the stage and a control panel. The bellows and the medium are pushed up and down to affect aeration and mixing.

Figure 2. A schematic drawing of the Tide Bioreactor system. Single-use bags can be used for Vessels A and B.
among others. Active development of cell-based influenza vaccine production processes began in early 2000’s. Many cell lines, including Madin-Darby canine kidney (MDCK), PER.C6®, and Vero cells, have been used. Vaccines produced in cell MDCK and Vero with serum-free media have been shown to be safe and efficacious 3,4, and two cell-based products were approved in Europe in early 2000’s 5,6.

Currently, a variety of cell culture systems are used in vaccine production, including T-flasks, cell cubes, cell factories, hollow fibers, roller bottles, stir-tank bioreactors and shaking bags. All these systems have some drawbacks themselves. For example, roller bottles and cell factories are capacity- and space-limited, labor intensive, and prone to microbial contamination. Stir-tank bioreactors used in large-scale production require expensive and space-taking pipings for utility supply and clean-in-place (CIP) and sterilization-in-place (SIP) systems. In addition, the intensive agitation required to enhance mass transfer in stir tanks causes damage to the cells being cultured, leading to release of cellular material and impairment of the purity of the virus harvests.

Shaking bag system such as the WAVE system is an improved one that can have a culture capacity up to 500 liters. It is a single-use disposable system, in which a new, pre-sterilized bag is used each time, and does not need either the CIP or SIP system. However, like the stir-tank systems, the system is operated under extensive, foam-generating shaking and requires addition of a toxic antifoam agent for proper aeration.

Recently, a completely new bioreactor system called Tide Bioreactor system for anchor-dependent cells was introduced by Cesco Bioengineering Co., Taiwan. This system applies a special agitation mechanism that maximizes mixing and aeration without causing damages to the cells being cultured. In other words, culture medium is moved in and out of the culture vessel just like the tide moving up and down the beach, allowing the wet cells to intermittently and directly be exposed to the air. The degree of aeration and mixing is automatically controlled by mode of medium flow.

Essentially, the Tide Bioreactor system is composed of a culture vessel (A), a service vessel (B), and a reciprocal flow pump between the two vessels. Both vessels can be disposable. Vessel A contains both culture medium and microcarrier chips. Vessel B, which can be 10-fold or more bigger than Vessel A, contains a mixing device and serves to receive the medium from vessel A. Vessels A and B can be single-use, disposal bags. During operation, the culture medium is moved by the pump between the vessels, and mode of the movement, e.g., flow rate and holding time, is adjusted to optimize conditions for cell attachment, cell cultivation, and viral propagation. Vessel A has different capacities, and can hold culture media from as little as 500 mL (BelloCell-500, Figure 1), 2 liters (TideCell-2, Figure 2) to as much as 100 liters (TideCell-100). Based on cell mass productivity, one BelloCell is equivalent to 10 roller bottles (RB-850). Cell density in Vessel A is normally 10 times higher than those cultured by conventional method. Therefore, at the time of viral propagation, volume of the medium can be 10-fold or more than that for cell culture. Furthermore, mode of medium flow can be programmed and controlled automatically. And, the whole process can be linearly up-scaled.

Medigen Biotechnology Corporation has been evaluating the Tide Bioreactor system for its suitability for the production of influenza vaccines since early 2000. BelloCell-500 and TideCell-10 bioreactors, BioNOC-II microcarrier chips, and PlusMDCK serum-free medium were purchased from

<table>
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<th>Specification*</th>
<th>HA Content (µg/mL)</th>
<th>Total Protein/HA Ratio</th>
<th>Residual DNA (ng/mL)</th>
<th>Endotoxin (EU/mL)</th>
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*Current specification for egg-based vaccine except for residual DNA
**Assumed value

Table 1. Quality of bulks of influenza 2009 H1N1 and H5N1 vaccines produced in Tide Bioreactor system
Cesco Bioengineering. Vessel A was a modified 10-L serum bottle, and Vessel B was a single-use 50-L bag sitting on a shaker. Pre-qualified MDCK cell line and influenza seed viruses (H1N1, NIBRG-121; H5N1, NIBRG-14) were purchased from the National Health Research Institute (NHRI), Taiwan. Four batches of the H1N1 vaccine and 3 batches of the H5N1 vaccine were produced at NHRI's cGMP facility. Process parameters, including cell/virus inoculum sizes, trypsinization conditions, medium changes and virus propagation conditions, had been determined in advance. Vessel A in the Tide-10 bioreactor can hold up to 10 liters of culture medium. But, only 50% of the capacity (i.e., 275 g of BioNOC II and 5 liters of medium) for a 50-liter virus harvest had been determined in advance. Vessel A was a single-use 50-L bag sitting on a shaker. Pre-qualified MDCK cell line and EV71 vaccine using the TideCell Bioreactor system.

For the H5N1 vaccine, Vessel A was around 2 x 10^{10}/mL at the time when viral infection and propagation began. The hemagglutination (HA) titer, a measurement for virus concentration, at harvest was 1,024 as determined by the standard method. A total of 7 batches of virus harvest were purified by conventional clarification-centrifugation-diafiltration process, and detoxified with formaldehyde to make vaccine bulks. And from these, two batches (one H1N1 and one H5N1) were further processed with aluminum hydroxide gel to make vaccine products. Both products met all release criteria (Table 1), passed pre-clinical toxicity and immunogenicity tests, and were stable at 4°C for at least one year. A Phase I clinical study for the H5N1 vaccine product, Lot AT-301, will begin in the third quarter of 2012. Table 1 also indicates that the control vaccine, which was produced by using Roller Bottles, contained much more residual cellular proteins.

In conclusion, we have been able to satisfactorily produce pandemic H1N1 and H5N1 vaccines for clinical study by using the Tide Bioreactor system. This system has all the benefits associated with other single-use bioreactors. In addition, the Tide Bioreactor system offers the best mass transfer method without causing damages to the cell being cultured.

The study was conducted by team members including Queena Lin, Rayd Ho, Jianming Chen, Allen Wang and Tingwan Lin.

References


About the Author

Wenlii Lin has accumulated his hand-on experience in vaccine basic research, product and process development, cGMP production and management during the past 30 years. His career in the field began with the discovery of two compounds from the culture fluids of Streptomyces spp that inhibited the enzymatic reaction of the influenza A neuraminidase and the infection of the viruses in mice while at The University of Tokyo Graduate School. Since then, he has worked on a variety of vaccines at several institutions. Being a Visiting Associate at the Center for Biologics Evaluation and Review (CBER, then BOB), FDA, he studied the biosynthesis of the Escherichia coli capsular polysaccharide antigen. He was a Group Leader – Product and Process Improvement at Lederle Laboratories, VP-Manufacturing at North American Vaccine Corp., CEO/President at Adimmune Biotech Corp. and CEO at Medigen Biotechnology Corp. Dr. Lin’s current focus is on the development and commercialization of pandemic influenza vaccines and EV71 vaccine using the TideCell Bioreactor system.