BPC materials of construction

Critical performance attributes

Abstract

Single-use technology (SUT) products are widely accepted for the manufacturing of vaccines and biologics. The increase in adoption of single-use Thermo Scientific™ BioProcess Containers (BPCs) is a result of their demonstrated performance, and their cost- and time-saving benefits. The quality and purity of the plastics used to produce Thermo Scientific™ Single-Use Bioreactor (S.U.B.), Single-Use Mixer (S.U.M.), and Single-Use Fermentor (S.U.F.) BPCs and storage bags are crucial to the purity and efficacy of final biopharmaceutical drug products. Several characteristics are important to the performance of BPCs, including biological compatibility, physical and mechanical properties, and extractables and leachables (E&L). This paper details some of the important characteristics of the plastic films used to manufacture BPCs, with a discussion on E&L and Thermo Fisher Scientific's strategy for characterizing the extractables profiles of our BPC films.

Introduction

SUT products used in biologics and vaccine manufacturing provide many advantages, including fewer cleaning and sterilization processes, reduced cross-contamination risks, and shorter setup times. The advantages offered by SUT products are accompanied by regulatory requirements that focus on the suitability of SUT containers or systems. These requirements focus on the interactions between the containers and the liquids held in them. Since biologics producers use SUT products in upstream (mixing, cell culture, fermentation, and harvest/collection) and downstream (purification, bulk drug storage and transport, and fill-finish) operations, the need to fully characterize BPC products has never been greater.

The end users of SUT systems rely on their suppliers' choice of materials of construction, which, for the purposes of this discussion, are limited to the plastic films used in the production of BPCs for S.U.M.s, S.U.B.s, and S.U.F.s. The choice of film, and the processes and environments in which the film is converted into BPCs, shape the mechanical, physical, chemical, and biological characteristics of the film. For these reasons, the end user's choice of film is arguably the most important decision to be made when choosing SUT products and suppliers.

The SUT value proposition

Biologics and vaccine industries recognize the general benefits of SUT, which include speed to market, faster processes, lower capital expense, lower operating costs, reduced energy consumption, and the opportunity for power generation from the SUT product waste stream.

Government regulators also recognize the general benefits of SUT, including reduced requirements for validated cleaning protocols, lower risk of cross-contamination, and the reduced investment requirements compared to reusable/stainless steel equipment.

Higher demands on BPC films

Because of their value to end users, SUT products are used in most bioprocessing operations. This range of applications places unique demands on the BPCs, which in turn creates a need for a broad operational range. Some of the more demanding unit operations include:

- Cell proliferation
- Bulk drug storage, freezing, and shipping
- Long-term storage of process liquids or bulk drug substances
- Shipping of large volumes of process liquids or bulk drug substances, precursors, or intermediates

To properly perform all of these unit operations, the BPC film must be of extremely high purity, with well-characterized chemical compatibility and extractables profiles. The BPC film must also protect the liquid contents from pH shifts and solute concentration changes by limiting oxygen, carbon dioxide, and water vapor exchange. Finally, the BPC film must be simultaneously flexible yet durable enough to resist punctures, stress cracks, and other damage, while remaining easy to use for a full range of workflow operations. This can include storage between site transportation at frozen, refrigerated, or ambient temperatures. Low-temperature storage and transport of bulk drug substances is a challenging application for SUT products. At typical low temperatures (–20 to –80°C, or lower for certain applications), the film's mechanical properties are more important than its gas transmission properties, and perhaps even more critical than its E&L profile. Due to the unique requirements of bioprocessing operations, no single polymer has all of the required attributes. Thus, BPCs are composed of multiple layers, with each layer requiring unique resins with specific attributes. BPC manufacturers must select the appropriate resins for their multilayer designs, while ensuring that resin suppliers use appropriate manufacturing conditions with consistent quality, as well as the purity levels required for biologics or vaccine production operations. The ever-increasing regulatory scrutiny faced by vaccine and biologics producers reinforces the importance of choosing the right BPC film.

Major polymers for single-use films

SUT BPC films are most often single-web, multilayer films. In some instances those single-web, multilayer films are also paired with a monolayer film and marketed as dual-web multilayer films. Regardless, all of these multilayer films are produced using lamination or coextrusion processes. The different film layers contribute specific attributes to the overall structure and performance of the BPC film, including:

- A suitable fluid contact surface for the variety of liquids used in bioprocess operations
- A gas and water vapor barrier to maintain pH and solute concentration
- An outer layer to protect the bioprocess liquid from the mechanical and physical stresses that typically occur in bioprocess operations

Polyvinyl chloride (PVC) film was one of the original film structures used in bioprocessing operations. PVC films can be quite brittle, and require process additives to be made suitable for bioprocessing operations. These process additives (also referred to as plasticizers) are known public health threats; and although PVC film–based bags continue to be used in a variety of health-related applications, PVC films are not commonly used in bioproduction operations.

Ethylene vinyl acetate (EVA) film was developed as an alternative to PVC. EVA films have favorable mechanical and physical properties, and continue to be used in a range of bioproduction operations. EVA film–based BPCs are more commonly two-panel, pillow-style 2D BPCs, available in small-volume sizes (up to 50 L). EVA film–based BPCs include the following features:

- An EVA fluid-contact layer
- An ethylene vinyl alcohol (EVOH) gas/water-vapor barrier layer
- A nylon, polyamide, or other engineered resin–based film outer layer, to provide durability and rigidity

These BPCs are ideal for low-temperature storage and shipment applications. They are also an economical option for certain small-volume storage and transfer applications.

Polyethylene (PE) films is a preferred fluid-contact layer because it is inherently cleaner, with lower E&L levels than EVA. PE is also inert to a wider range of chemicals. The most common variants of polyethylene that have been used for the production of single-use BPCs include low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), and ultralow-density polyethylene (ULDPE). PE resins are sometimes blended with cyclic olefin copolymers (COC) to produce COC/PE fluid contact layers. These COC/PE blends build upon the superior properties of PE fluid-contact surfaces, providing added strength and enhancing chemical resistance. PE-based BPC films also feature an EVOH gas/water-vapor layer, and an outer layer composed of nylon, polyolefin, polyester, or polyamide to impart durability and rigidity into the film structure.

Film production processes

Manufacturers of BPCs must consider the types and thicknesses of the film layers to use in multilayer films, in addition to whether to coextrude the resins as one continuous sheet of film (single-web), or to extrude the individual resins as separate layers, and then laminate them to produce the final film sheet (web). Coextruded resins can be converted to film using either a blown film or cast film process.

Coextrusion involves the use of multiple extruders to melt different types of resins and deliver them as viscous materials to a single-extrusion die, where they are joined together with a multilayer structure. The thickness of each film layer is determined by the size and operation speed of each extruder.

Film lamination encompasses a range of methods used to permanently join two or more prefabricated sheets of film. Lamination can use the adhesive properties of one or both of the film sheets to be joined. If neither film sheet possesses adhesive properties, a separate adhesive is used to join the sheets of film.

Blown films are created by feeding the extruded materials into a circular die (Figure 1). The melted plastic forms a continuous tube that is drawn from the die and simultaneously inflated with air (or nitrogen) pressure, and usually pulled upward with rollers (bubble-like expansion), often to a height of two or three stories. This is followed by a cooling step, which leads to a rapid solidification of the plastic. The expanded tube is then collapsed between rollers and wound onto a reel.

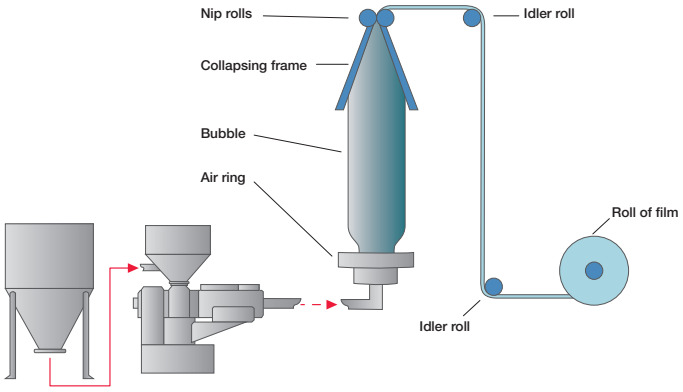

Figure 1. Process flow for blown film line.

Cast films are produced by passing the coextruded molten resins through a flat die that represents the film's final flat shape. The generated molten film sheet passes through a water-cooled chill roller to rapidly cool the film. The edges of the film are then trimmed, and the film is wound into rolls. Figure 2 illustrates the process flow for typical cast film lines.

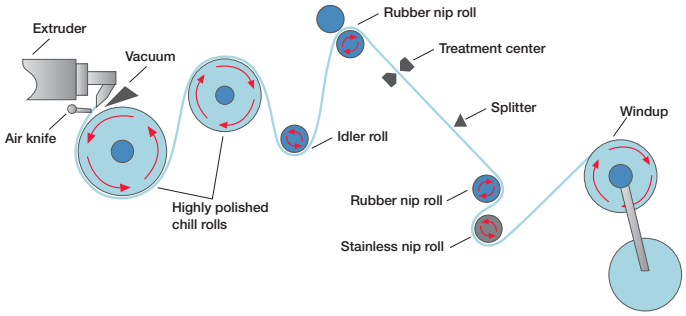

Figure 2. Process flow for cast film line.

Each of these processes has its own advantages. Unlike cast film processes, which are more easily produced in International Organization for Standardization (ISO)-classified clean areas, blown film processes are less likely to be produced in an ISO-certified clean area. To compensate for this, producers of blown films often use 0.2 micron filtered air to create the film tube, arguing that using filtered air produces a cleaner film with lower bioburden levels than cast films. This is valid in situations where cast films are not produced in an ISO-classified environment. However, in situations where film casting occurs in a clean room, bioburden levels are often significantly lower when compared to blown film. Higher bioburden levels will require higher gamma irradiation doses to attain a 10^{-6} sterility assurance level (SAL), which may generate more E&L.

The importance of E&L testing

An extractable is any compound that migrates from a resin, material, component, or system when exposed to exaggerated conditions, such as organic solvents, elevated temperatures, and extremes in pH, over time. Extractables studies characterize the SUT product's materials of construction using simulated worst-case conditions.

Responsible SUT suppliers will provide their customers with qualitative and quantitative extractables data for use in assessing the risk of using the suppliers' SUT products in their manufacturing processes. Risk assessment methods vary, but are most commonly utilized when SUT products are used in critical process operations, such as cell proliferation or process steps involving drug substances. In these instances, extractables data of the highest quality are required, emphasizing the importance of film choice.

A leachable is any substance that migrates from in-process equipment or containers into the process liquid, as a result of direct contact with a material, component, or system under actual product-use conditions. Leachables studies characterize the SUT product's materials of construction under as used conditions that are typical to bioproduction operations. Because biologics and vaccine producers often employ SUT products during critical operations such as cell proliferation and bulk drug storage/shipping, the BPC film must have a neutral (or at least well-understood) impact on any bioprocess fluids exposed to those BPC films.

The influence of resin additives on E&L profiles

Additives are commonly used in the production of the plastic resins that are used to manufacture SUT products. Slip agents, anti-blocks, antioxidants, plasticizers, antistatics, heat stabilizers, UV stabilizers, colorants, color stabilizers, fillers, and lubricants serve varying purposes but are most often used to enhance the functionality of the resin or the resin-to-component (film) conversion process. Different plastic polymer resins use different amounts and combinations of these additives.

Additives can negatively influence the E&L profile. Some plasticizers can act as immunomodulators, and a few are known public health threats. Certain lubricants can cause product aggregation, and degradants from certain antioxidants have shown cytotoxic effects *in vitro*. Several suppliers of SUT products have experienced some or all of these occurrences. Such occurrences heighten the awareness of government regulators, which impacts both suppliers and end users of SUT products.

SUT supplier responsibilities

Suppliers of SUT products use a range of strategies to characterize and monitor their products and processes. All established BPC suppliers perform extensive material analyses during the initial development of BPC films, as well as conducting routine product monitoring and process verification of BPC films. BPC suppliers pursue different strategies related to their supply chains, manufacturing capacity, and capabilities, in order to improve the quality and performance of their SUT products. BPC suppliers have varying degrees of technological expertise on films. Suppliers with multiple manufacturing sites, a best-in-class approach to component sourcing, an uncompromising quality focus, and extensive technical expertise offer several advantages to end users.

E&L testing strategies

Historically, suppliers of BPC products have employed various methods of conducting extractables testing, which were often internally developed and unique to each SUT supplier. These protocols varied by the solvent type and concentration, incubation times and temperatures, and analytical methods. This variability makes it difficult to directly compare extractables profiles for SUT materials of construction. In 2010, the Bio-Process Systems Alliance (BPSA), a trade association of SUT suppliers, developed extractables testing guidance intended to help end users directly compare the films used in SUT BPCs. Some BPC suppliers openly resisted adopting this guidance. More recently, the BioPhorum Operations Group (BPOG) developed a protocol intended to serve as the standard guidance for suppliers, although it has yet to be universally adopted.

This protocol provides a method for directly comparing SUT materials of construction from different suppliers, and offers a new definition of simulated worst-case conditions. Tables 1 and 2 describe the BPOG extractables testing guidance, and the differences between BPOG and BPSA protocols, respectively.

Table 1. BPOG extractables testing guidance.

Product	Solvents	Surface area/ volume (SA/V)	Incubation	Time points	Assay	Reporting units	Sterilization
Mixing bags	WFI 50% EtOH 0.1 M H_3PO_4 0.5 N NaOH 1% PS-80 5 M NaCl	6:1 (cm^2/mL)	To 25°C, all else 40°C (on orbital shaker)	To, 24 hr, 21 day, 70 day	HPLC-PDA/MS, GC-FID/MS, HS-GC-FID/MS, ICP/MS conductivity, TOC, pH	µg/cm^2	Gamma irradiation at 50 ±5 kGy or Steam-sterilized at maximum time, temperature, and number of cycles
Storage bags							
Bioreactor bags							
Tubing							
Sterile filters		1:1 (cm^2 EFA/mL)		To, 24 hr, 7 day		µg/cm and µg/cm^2	
Process filters				To, 24 hr, 21 day			
Connectors		6:1 (cm^2/mL)		To, 24 hr, 7 day		µg/cm^2	
Sensors				To, 24 hr, 21 day			

WFI: water for injection, EFA: exploratory factory analysis

Table 2. BPSA/BPOG protocol comparison.

Previous studies	BPOG study
Gamma-sterilized at 25–40 kGy	Gamma-sterilized at 25–40 kGy
Static incubation SA/V = 2.2 cm^2/mL	Dynamic incubation at 80 rpm SA/V = 6 cm^2/mL
Solvents: • WFI • 2 M HCl • 3 N NaOH • 4 M NaCl • 20% EtOH in WFI	Solvents: • WFI • 0.1 M H_3PO_4 • 0.5 N NaOH • 5 M NaCl • 1% PS-80 • 50% EtOH in WFI
Analyses: • Headspace GC-MS • GC-MS • Ultra-performance liquid chromatography–photodiode array mass spectrometry (UPLC-PDA-MS) • ICP-MS • pH, conductivity, TOC	Analyses: • Headspace GC-MS • GC-MS • LC-MS (ESI +/– and APCI +/–) • ICP-MS • pH, conductivity, TOC
Time points: 1, 30, and 90 days at 60°C No isolation of individual samples	Time points: • 0 at 25°C • 0, 1, 21, and 70 days Isolation of individual samples by canisters

Our extractables testing strategy

Thermo Fisher Scientific has adopted both the BPSA and BPOG testing guidelines. The United States Pharmacopeia (USP) is also finalizing guidance for extractables testing, which we will also test upon its completion. We follow all applicable BPC testing guidelines (Table 1) as part of our corporate commitment to making the world healthier, cleaner, and safer. Our philosophy and goal as a supplier of SUT products is to be an extension of our customers' organizations, by becoming a partner in addition to their preferred supplier of SUT BPC products. We do our best to enable them to do their best in producing life-saving medicines. In the spirit of this philosophy, we intend to continue testing our films and components to the latest standards and protocols, in order to provide our customers with the most comprehensive and meaningful extractables testing data possible.

We support the efforts of BPOG and believe there is fundamental value in creating user-driven testing standards that allow for more directly comparable test methods and analysis. This work also highlights that it is essential to include experimental controls and replicates in the design of quantitative test methods. A logical next step should include point-of-use product testing that evaluates the quality and purity of the complete BPC assembly after gamma irradiation, at defined storage intervals, and with performance criteria specific to the intended use.

Manufacturers of SUT products should perform an L&E assessment for all primary resins used in the manufacture and assembly of BPCs. Third-party suppliers that provide components specifically for SUT (e.g., connectors, filters) should also generate L&E supporting data for the purpose of enabling end users to make informed risk-based decisions specifically for their application needs.

BPSA and BPOG test data

In 2013, Thermo Fisher Scientific published extractables testing data for our Thermo Scientific™ CX5-14 and Aegis™ 5-14 films. This information is available to our customers upon request. In 2015, we began testing on Thermo Scientific™ ASI 26/77 films, and in 2016 began testing on Aegis5-14 and CX5-14 films. Unknown volatile and semivolatile organic compounds will be identified via the Automated Mass Spectral Deconvolution and Identification System (AMDIS) to deconvolute the complex chromatograms and extract "clean" single-compound mass spectra.

The National Institute of Standards and Technology (NIST) library is being used for search and spectral comparison, and authentic samples will be used to confirm the unknowns. Unknown nonvolatile organic compounds will be analyzed in the fragmentation pattern using Thermo Scientific™ Mass Frontier™ software, resulting in realistic structural proposals. Authentic samples will be used to confirm the proposed structures. A formal status update on our progress to identify the unknowns will be presented in mid-2018. Testing of internally sourced components began in 2017, and will be completed in 2018. Extractables data on externally sourced components have been solicited from suppliers and manufacturers in response to BPOG member requests.

Testing findings

Thermo Fisher Scientific has found the following to be true in the testing of BPCs:

- Different testing methods will generate different results. End users must choose between testing methods when comparing different films; different testing methods cannot be considered equivalent to each other, as the results may vary greatly.
- There was a general drift toward lower pH, and higher conductivity and total organic carbon (TOC), due to migration of additives and their oxidized degradants.
- Previous studies at higher temperatures resulted in the loss of most volatile organic compounds (VOCs) observed in the BPOG study. Placement of individual samples in canisters prevented cross-contamination of samples.
- Dynamic incubation (80 rpm), higher SA/V (6:1), and the inclusion of atmospheric pressure chemical ionization (APCI) generated a rich profile of semivolatile organic compounds (SVOCs). Nonvolatile organic compounds (NVOCs) increased the number of extractables, compared to in previous studies.

Our films

Thermo Fisher offers four films that have been engineered specifically to meet the demands of the biopharmaceutical industry. The Aegis5-14 and CX5-14 films are single-web, five-layer coextruded films with a ULDPE layer, an EVOH gas/vapor barrier layer, and an outer polyester layer. ASI 26/77 film is a "bag within a bag" dual-web, multilayer coextruded film. The inner web (ASI 26 film) features a ULDPE fluid-contact web. The outer web (ASI 77 film) features an LLDPE inner layer that is coextruded with an EVOH gas/vapor barrier layer, and a nylon outer layer. The fourth film, ASI 28, is a coextruded film with an EVA contact layer, and an EVOH gas/vapor barrier layer sandwiched between two layers of LDPE. Figure 3 provides descriptions of the cross-section structures and materials of construction of the most common BPC films. Details on the various features of BPCs available based on these different resins are shown in Table 3.

Our films have passed biocompatibility testing per USP and ISO protocols, bacterial endotoxin testing (USP 85), physicochemical testing (EP 3.2.2.1 and USP 661), and particulate testing (USP 788). Our films' mechanical properties impart durability and puncture resistance, as well as flexibility. These mechanical, biological, and physical properties (Table 4) complement each other to address the unique requirements of the bioproduction workflow.

Table 3. Key features of Thermo Scientific BPCs.

	Aegis5-14 and CX5-14	ASI 26/77	ASI 28
Materials of construction	ULDPE/ EVOH/ polyester	ULDPE/LLDPE/ EVOH/nylon	EVA/LDPE/ EVOH/ LDPE
Construction type	Coextruded/ cast	Coextruded/blown	
Total thickness	14 mil	12 mil (5 mil inner layer, 7 mil outer layer)	12.5 mil
Available sizes	50 mL—3,000 L	100 mL—5,000 L	100 mL—50 L
Ports	Face and seam ports		Seam ports
Chamber type	2D and 3D		2D

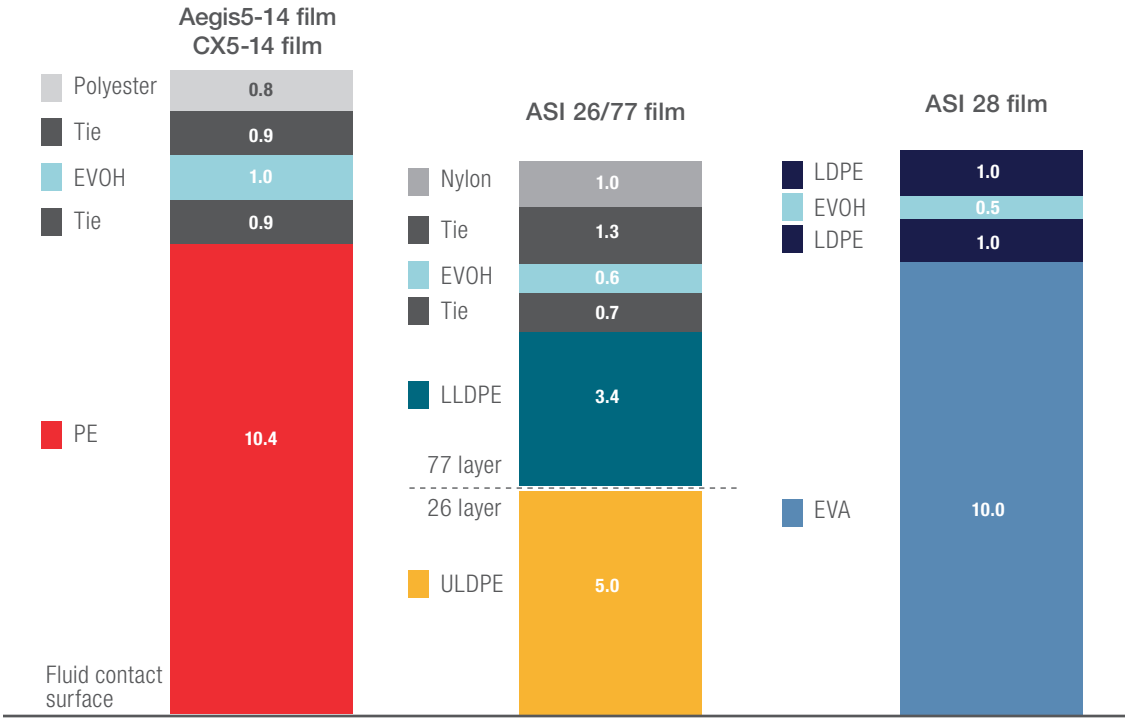

Figure 3. Common BPC cross-sectional structures and materials of construction (dimensions in mils).

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Table 4. Biological, mechanical, and physical property data for Thermo Scientific BPCs.

	Aegis5-14	CX5-14	ASI 26/77	ASI 28
Biocompatibility (USP <88>)	Pass	Pass	Pass	Pass
Biocompatibility (USP <87>)	Noncytotoxic	Noncytotoxic	Noncytotoxic	Noncytotoxic
Water vapor transmission rate in g/100 sq. in./24 hr (ASTM F1249)	0.023	0.023	0.022	0.011
Oxygen transmission rate in cc/100 sq. in./24 hr (ASTM D3985)	0.023	0.024	0.041	0.28
Carbon dioxide transmission rate in cc/100 sq. in./24 hr (MOCON method based on ASTM D3985)	0.087	0.089	0.110	0.58
Tensile strength in psi (ASTM D882)	2,392	2,316	3,015	2,118
Elongation in % (ASTM D882)	487	476	486	639
Yield strength in psi (ASTM D882)	1,362	1,238	1,973	828
Puncture resistance in lbf (ASTM F1306)	25	26	11	11
Glass transition temperature in °C (ASTM E1640)	–31	–28	–27	–28

Conclusions

SUT offers many benefits to biopharmaceutical manufacturers. As with any other container or equipment used for the production of biologics, careful consideration must be given to any factor that may affect the quality or efficacy of the final products. For single-use BPCs, this requires careful selection and evaluation of the polymer resins used to produce the BPC films. It also necessitates identification of reliable SUT product suppliers who have quality systems and procedures in place to ensure security of supply back to the resin manufacturer.

Thermo Fisher Scientific has been supplying BPCs to the biopharmaceutical industry for over 20 years. Our Aegis5-14, CX5-14, ASI 26/77, and ASI 28 film–based BPCs are used in both upstream and downstream bioprocessing operations. As a result, their performance has been repeatedly proven, and their durability, puncture resistance, and cleanliness have been well demonstrated. Our newer Aegis film offers the same performance characteristics as the CX5-14 film, but with an even cleaner extractables profile, while the ASI 28 film offers yet another cost-effective option for common biologics and vaccine workflow applications. With such a broad portfolio of high-quality films, all of which have been extensively validated and come with full documentation, we are able to deliver single-use bag solutions based on optimal films for every biopharmaceutical production application. Customers also have the option to qualify Thermo Fisher Scientific as a primary and secondary supplier with global manufacturing facilities for increased supply chain security. With more high-quality choices, we are providing our customers with greater flexibility and the potential for improved productivity.

Find out more at **thermofisher.com/bpc**

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