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Single-Use Connections Enable Advancements in Aseptic Processing

Improving Flexibility and Saving Money for Biopharmaceutical Manufacturers

by John Boehm

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Today's market demand for new drugs — combined with the difficult economic environment — is challenging bioprocessors to review their manufacturing systems and seek ways to make them more flexible, reliable, and cost effective. Increasingly, biomanufacturers are turning to single-use aseptic processing systems to meet or beat aggressive product-introduction timeframes while controlling costs.

Innovative new single-use technologies continue to be introduced, giving pharmaceutical companies greater flexibility for replacing traditional stainless tubing, equipment, and even entire process suites with plastics-based solutions. The benefits of converting to presterilized, single-use systems have been documented in many articles and case studies, but many of those benefits would be lost if manufacturers could not safely and securely connect systems and components to create a complete aseptic process.

Connecting devices and methods may appear to be a small part of these overall systems. But connection and disconnection of tubing for process fluid transfer is a critical aspect of single-use bioprocessing. Because a connector can be the deciding factor in keeping a process truly aseptic, manufacturers need to carefully consider all available options.

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Sterile Connection Options

Biomanufacturers can create an aseptic connection within or between their unit operations in many different ways. The right choice depends highly on the needs and preferences of a given facility.

Quick-disconnect couplings or fittings (sanitary and Luer-lock designs) are commonly used with a range of tubing types and sizes. These connectors provide fast, easy, and secure connections when used in conjunction with laminar-flow hoods. Tube welders, for example, provide an alternative for creating aseptic connections with thermoplastic elastomeric tubing of half-inch diameters and smaller. Such systems use heated, replaceable blades to maintain sterility while welding two separate tubes together.

Single-use steam-in-place (SIP) connectors are increasing in popularity. They create sterile connections between a range of single-use systems and stainless steel processing equipment. These solutions require a steam supply at the point of connection.

A number of available single-use sterile connectors allow a sterile tubing-to-tubing connection without needing a laminar-flow hood or tube welder. These components enable sterile fluid transfer between separate single-use systems even in gray space. For disconnecting single-use tubing, aseptic disconnect couplings with shut-off valves do not require a controlled environment to maintain fluid sterility during or after their disconnection.

Determining the best solution for a given application depends on a number of factors such as the fluid being processed, tubing selected, flow requirements, and space availability (for a laminar-flow hood, SIP system, or tube welding equipment). Additional factors to consider include material compatibility, product availability, supplier technical support, and validation — whether the connection method is already validated for use within a particular facility.

Application Examples

Fermentation Seed Trains with Stainless Reactors: Modern bioprocess facilities often feature

Figure 1: Fermentation seed train with stainless bioreactors

Figure 2: Fermentation seed train with single-use bioreactors

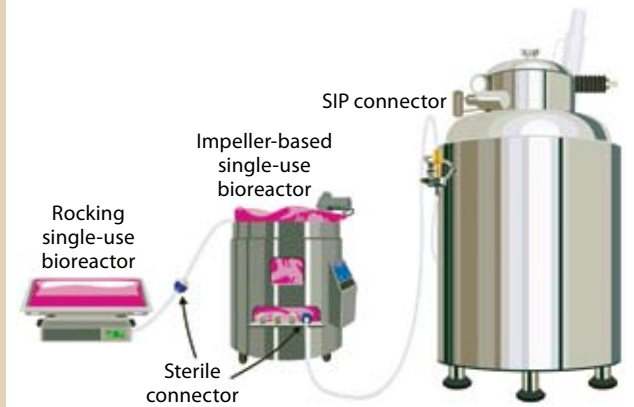
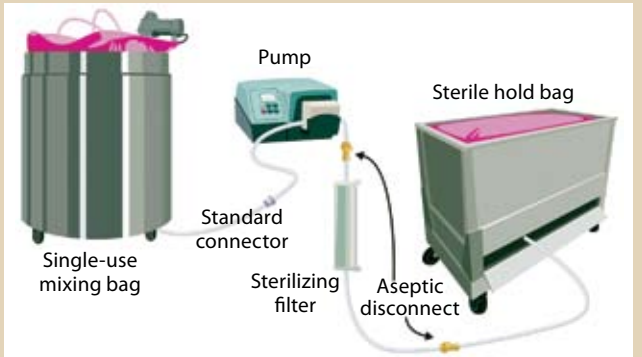


Figure 3: Buffer/media preparation



production bioreactors with 5,000-L, 10,000-L, and even 25,000-L capacities. Scaling up inoculum from a few million cells in several milliliters of culture to such large production volumes is a challenge that requires aseptic transfer at each point along the seed train.

Traditional facilities accomplish that scale-up using a dedicated series of stainless steel bioreactors linked together with valves and rigid piping. To prevent contamination between production runs, a clean-in-place (CIP) system is designed into each bioreactor, vessel, and piping line to remove residual materials. Sterility assurance at the start of each culture is provided by an SIP system comprising steam pipes, temperature sensors, and condensate collection piping. Both CIP and SIP systems require extensive validation testing, and their valves and piping can create additional validation challenges. These systems also must be revalidated following significant maintenance or changes to the connected network of piping and valves.

Advances in single-use system technology allow bioprocess engineers to replace most storage vessels and fixed piping networks with single-use storage systems and transfer lines. Disposables eliminate the need for CIP validation of many components and reduce maintenance and capital expenses by eliminating expensive vessels, valves, and sanitary piping assemblies. Figure 1 depicts a production suite with stainless steel bioreactors, with integrated single-use technology for cell culture media storage and key transfer lines. Media storage systems arrive at the facility presterilized by gamma irradiation and may be fitted with integrated filters, sampling systems, and/or connectors. Using SIP connectors allows operators to make sterile connections between these presterilized single-use systems and stainless steel bioreactors for aseptic transfer of media.

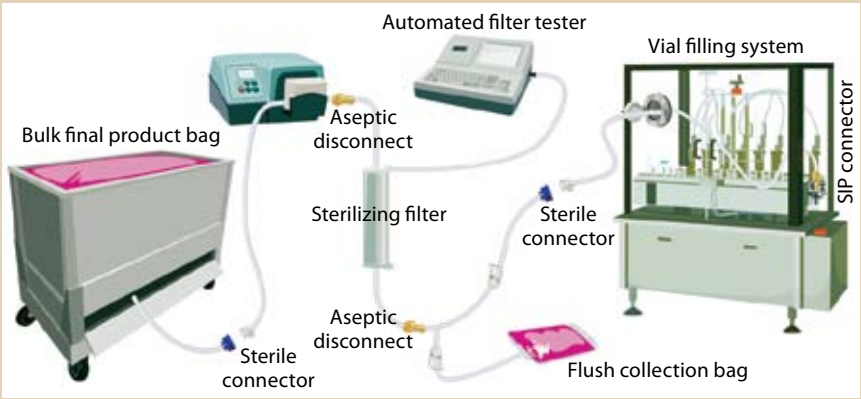
Similarly, single-use transfer lines may be used to transfer inoculum between bioreactors using either a peristaltic pump or head-space pressure. These lines can reduce the number of reusable valves required for transfer as well as eliminate problem areas for CIP/SIP validation. Terminating each presterilized transfer line with a single-use SIP connector provides sterility assurance equal to that of traditional fixed piping but at lower capital costs.

Fermentation Seed Trains with Single-Use Bioreactors: With the increasing acceptance and availability of single-use bioreactor (SUB) technology, users can create flexible seed train systems consisting entirely of different-volume SUBs or a combination of those and traditional stainless steel bioreactors for high-volume production. It is not practical to have a train of SUBs assembled, sterilized, and installed as one complete system, so single-use connections can be used to create a secure aseptic process that connects the components once they are installed within a process suite. That can be done with quick connectors, tube welders, or sterile connection devices. If smaller-volume SUBs are connected with larger-volume traditional reactors, quick-connects or sanitary fittings can be used in conjunction with laminar-flow hoods, or SIP connectors can be used to establish sterile media transfer. Figure 2 shows a model facility incorporating disposable and stainless steel bioreactors as the two largest vessels.

Buffer/Media Application: In-house media/buffer preparation requires in-line sterile filtration after mixing and before storage. Many manufacturers use filter integrity testing to ensure product purity and minimize product loss. Integrity testing verifies that filters are functioning properly and can remove minimum-sized particles from filtrate. Such tests can be conducted prefiltration, postfiltration, or both — and they identify potential breaches in sterility and determine whether rerunning a batch process may be necessary.

Bioprocess facilities historically used hard-plumbed systems with replaceable filter elements in stainless steel

Figure 4: Final fill operation



housings that required time-consuming set-up, validation, and postproduction cleaning. Introducing disposable components into these integrity test systems reduces the risk of contamination while improving process flexibility. Single-use components used in filtration and filter integrity testing include disposable capsule filters, bags, tubing, clamps, and connectors. Connectors are the key interface between components within these systems, and they also provide quick and easy integration of the subsystem into a larger production process.

Disposable components and systems can replace some or all stainless steel equipment used in culture media and buffer preparation (Figure 3). Single-use tank liners or specialty mixing bag systems substitute for fixed-in-place mixing tanks. Bag systems with integrated capsule filters replace stainless steel filter housings and sterile hold tanks.

After process fluid has been filtered into a sterile hold bag, the used filter can be quickly detached for postfiltration integrity testing using valved aseptic disconnect couplings. The resulting sterile disconnection enables technicians to confidently remove filters without risk of contaminating the contents stored in the holding bag — while also keeping the filter wetted for integrity testing.

After removal, filters can be tested using automated equipment by bubble-point, gas diffusion, or pressure decay methods. Once testing confirms filter integrity, stored cell culture media may be released for continued processing. Extended use of single-use components to filter integrity testing is an additional way manufacturers can streamline a bioprocess and speed products to market.

Suite-to-Suite Transfer: When upstream production is complete, a protein-containing medium must be aseptically transferred to a different location to be purified and formulated for final filling. This suite could be in a room next door or much farther away. Traditional bioprocess facilities accomplish this transfer using stainless steel manifolds with piping or reusable hoses as transfer lines. Such equipment requires validated CIP and/or washing procedures before use and sterilization before each media transfer.

With disposable components, process engineers simplify that process by incorporating single-use transfer lines between a bioreactor and transfer vessel. Using presterilized connectors and tubing, medium can be moved from a production suite to a preparation suite without the need to sterilize stainless steel piping or equipment.

In addition to faster production, such systems provide greater flexibility in determining which process to run in each production suite. This enhanced mobility eliminates many restrictions that hard-plumbed piping can place on a manufacturing facility. The risk of cross contamination in suite-to-suite transfer can be high, especially in multiproduct facilities. Using sterile connectors to interface key processes can reduce those risks and improve the speed and safety of drug development.

Formulation and Filling: Product safety concerns and overall value are highest during final formulation and dose filling. Like other applications, traditional filling processes involve stainless steel equipment connected through reusable valves, rigid tubing, and steel piping. Once again, single-use technology can be integrated in the form of bulk storage containers, filters, tubing, connectors, and even disposable filling manifolds to minimize contamination risk and reduce operational downtime.

Figure 4 depicts a filling operation using a disposable manifold system with a sterile filter to transfer drug product between a bulk storage bag and filling equipment in an isolator. To address product quality and reduce waste, both pre- and postfiltration integrity testing may be combined during this final stage. To conduct prefiltration testing, a filter assembly is connected to the sterile hold tank using an aseptic connector. Once connected, the filter is wetted by the product formulation, then prefiltration testing can be performed.

After filter integrity is confirmed, the line to the flush bag is clamped off and the filter assembly is aseptically connected to an isolator transfer line. That line can be preinstalled on a vial filling system using an SIP connection to minimize downtime. The flow clamp is then opened for filtration to begin. Once filtration is complete, the filter can be removed from the assembly using aseptic disconnect couplings to maintain sterility until postfiltration integrity testing can be performed. By conducting both integrity tests, operators will be assured that product purity has been maintained in the final drug formulation so it is ready for release.

Best Practice Guides

With the range of solutions available, it can be difficult for manufacturers to determine the best approach for evaluating or qualifying connection

technology for their processes. The BioProcess Systems Alliance (BPSA) Technical Committee formed subcommittees to address best practices for major categories of single-use technologies including films and containers, filter capsules, tubing, and connectors and fittings. Because plastic connectors have different characteristics from traditional stainless steel valves, a team of connection experts from industry leaders developed consensus guidelines on quality test methods used to address key performance attributes.

The result of that collaborative effort is a matrix that outlines tests common to connectors and fittings, testing frequency, and a summary of available references and industry standards (**1**, **2**). Burst, integrity (leakage), bacterial challenge/soiling, biocompatibility, particulate, physicochemical, flow rate/pressure drop, and sterilization process compatibility testing are included. This matrix is an excellent reference for drug manufacturers selecting, qualifying, and validating connection solutions. Representatives from BPSA member companies also can provide valuable support and assistance to users during technical evaluation.

Future Growth

Connection and disconnection of tubing for fluid transfer are critical to aseptic processing with single-use systems or disposables in combination with traditional stainless steel equipment. Advanced connection technology allows different subsystems or processes to be combined for increased flexibility and efficiency from upstream fermentation through downstream filling. Because a connector can be the deciding factor in maintaining process sterility, users need to carefully choose their connection solutions.

Industry support from organizations like BPSA — along with continuing product development from suppliers — will further address aseptic processing needs of faster product changeover for small-batch production. Higher-throughput systems and tubing ranges will create even more efficient operations and give manufacturers the flexibility they need to speed their products to market. Advancements in connection technology are thus helping address industry demands for rapid drug development, process reliability, and improved manufacturing efficiencies.

References

1 BPSA Guidelines and Standards Committee. Bio-Process System Alliance Component Quality Test Matrices. *BioProcess Int.* 5(4–5) 2007: www.bioprocessintl.com/multimedia/archive/00077/41200790_77498a.pdf.

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John Boehm *is bioprocessing business unit manager at Colder Products Company (1001 Westgate Drive, St. Paul, MN, 55114; 1-651-645-0091; john.boehm@colder.com; www.colder.com) and vice chair of the bioprocessing systems alliance (BPSA), and he served as education chair and lead for BPSA's Connectors and Fittings Subcommittee. He has a bachelor's degree in mechanical engineering and a master's degree in business.*