THE ROLE OF SINGLE-USE POLYMERIC SOLUTIONS IN ENABLING CELL AND GENE THERAPY PRODUCTION

BPSA
Bio-Process Systems Alliance
Advancing Single-Use Worldwide

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Part 1: Introduction

Intent and Scope

The Bio-Process Systems Alliance (BPSA) was formed in 2005 as an industry-led international industry association dedicated to encouraging and accelerating the adoption of single-use manufacturing technologies used in the production of biopharmaceuticals and vaccines. Corporate members include plastic equipment suppliers, service providers and users in the biopharmaceutical industry who share this mission. A key focus of BPSA's core activities is to educate its members and others through sharing of information and development of best practice guides that help suppliers, users and regulators to safeguard the quality of drugs produced with SUT.

This paper is designed to provide guidance on the manufacturing of cell and gene therapy (CGT), regulations and best practices regarding implementation of single-use components, and is largely based on experience gathered from the use of these products in the blood processing and biologics manufacturing spaces. Differences between those areas and cell therapies are highlighted throughout this paper.

Background

Successful commercialization of cell therapies relies on the development of a scalable manufacturing process that can produce products of appropriate quality on a routine basis in a cost-effective manner. While the challenges associated with establishing these robust manufacturing processes can be therapy-specific, there are a few common themes that arise from the fact that the product being manufactured is of significantly higher complexity than standard biologics, such as monoclonal antibodies or recombinant proteins.

Many cell therapies have shown significant promise in curing or alleviating a variety of diseases in clinical studies. Momentum in the field is growing as demonstrated by an investment of over $50B, $12B since the beginning of 2016 (Alliance for Regenerative Medicine [ARM]) and FDA approval of two therapies in 2017 (Kymriah and Yescarta). The pipeline is also very strong with a further 93 therapies currently in late stage clinical trials (ARM data).

Cell therapy is defined as the administration of cells to a patient to treat disease. Cell therapies can use either a patient's own cells as starting material (autologous cell therapy) or a donor cell that is expanded and used to treat several patients (allogeneic cell therapy). In many cases, cell therapies can involve genetically modifying cells. For example, a genetic modification is made to a patient's cells ex vivo and then administered back to the patient. One example of these types of therapies is chimeric antigen receptor T cell (CAR-T) cell therapies. These therapies have been successfully applied to treat a variety of cancers and work by modifying a patient's T cells ex vivo, so that they attack and kill cancer cells after administration back into the patient.

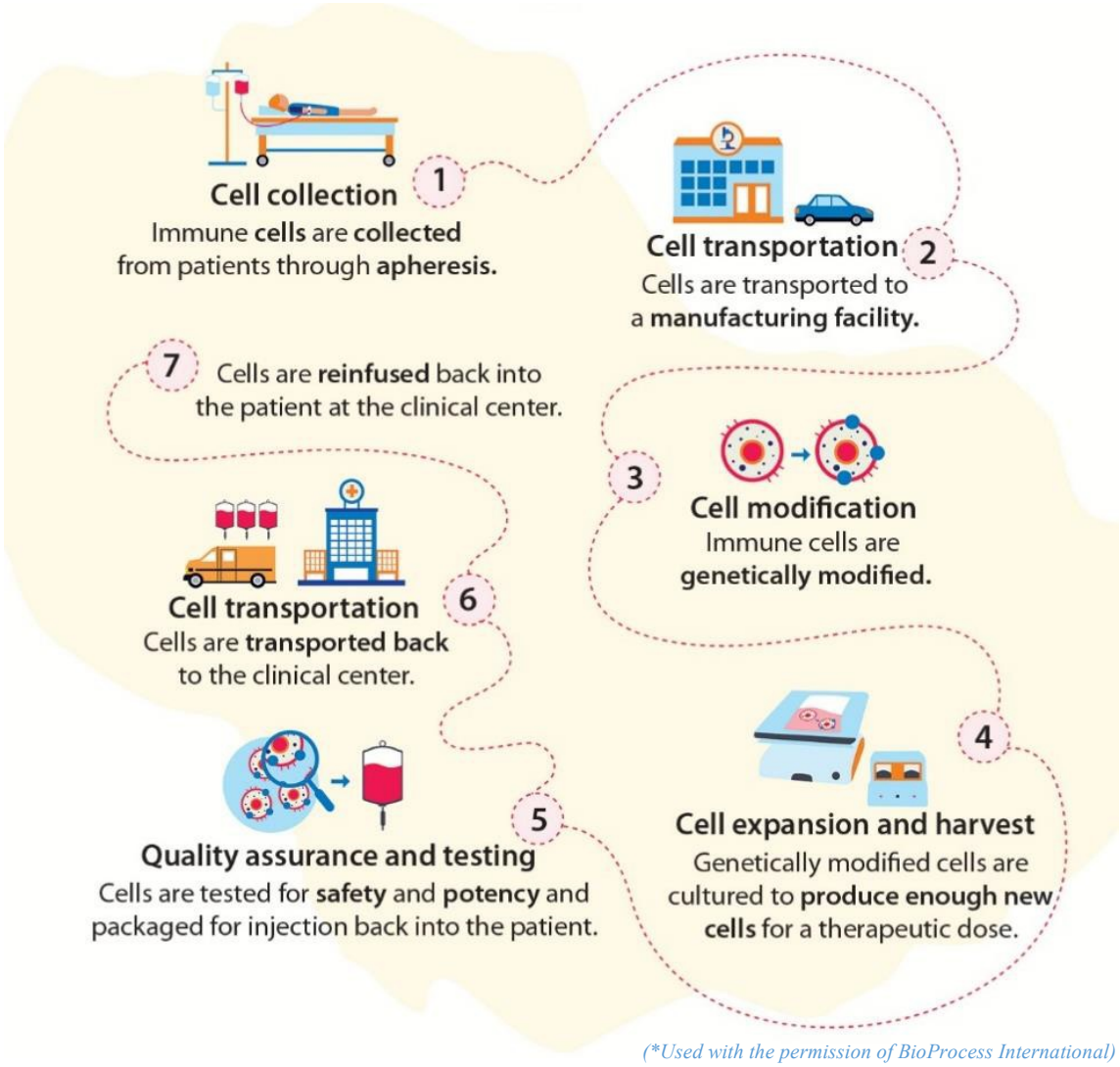
SUT, also known as disposable technologies, have gained extensive use and acceptance in the manufacture of monoclonal antibodies and recombinant proteins because they allow for greater flexibility, speed, and safety in the development of these therapeutics. Due to their widespread use in this area, a significant amount of effort has gone into development of a variety of standards involving their implementation and use in manufacturing.

Currently, the use of SUT in cell therapy is standard practice owing in part to the fact that the field is industrializing. Single-use is perfect to facilitate this industrialization by leveraging its existing bioprocessing capabilities of closed systems and

eliminating cross-contamination. This is particularly advantageous for autologous therapies, because SUT eliminates the risk of cross-contamination between patient samples.

With more cell therapies approaching commercialization, it is important that therapy developers have a clear understanding of both the opportunities as well as the challenges associated with developing an entirely single-use manufacturing process. This paper uses the CAR-T manufacturing workflow (**Figure 1**) as an example, but the contents can be generalized to all cell and gene therapies.

Figure 1: CAR-T Manufacturing Workflow*



*(*Used with the permission of BioProcess International)*

Definitions of terms are provided at the end of this document to assist in the ongoing dialog among end users, suppliers, and regulators.

Part 2: Manufacturing Process Summary

CGT products demand additional requirements on manufacturing equipment. As CGT products are biological in nature and noting that (1) cell-based therapies cannot be terminally sterilized by filtration, and (2) cells that have direct contact with the manufacturing vessels are the final form of drug products, the general requirements on common manufacturing equipment systems become even more stringent. As discussed in this paper, requirements on sterility, biocompatibility, extractables/leachables and particulates must be carefully met to ensure final CGT products' quality, safety and effectiveness.

As CGT products are often produced in small batches, or in the case of autologous CGT products where one patient's own cells are processed in a single batch for patient's own use, SUT has become a common manufacturing platform. They often use plastic or other materials that are economically suitable and easy for operation. With such materials used in production, not only is sterility critical, but stability, physical integrity and strength of SUT are also vital to ensure CGT products' safety and effectiveness. With autologous CGT products, defects in SUT leading to manufacturing failure will put patients at significant risk, even leading to death.

Another unique aspect of CGT production, particularly for autologous CGT, is to ensure traceability of drug products throughout the supply chain and prevent any potential mix-ups. Chain of custody must be implemented from the start of cell sample collection to finish when CGT products are administered into designated patients. SUT by nature suits this purpose nicely when mechanisms are in place throughout the supply chain to ensure chain of custody.

Aseptic Considerations

Losing valuable patient samples due to contamination caused by faulty aseptic procedures must be avoided at all costs. Understanding what to look for in your single-use systems is vital to ensuring your patient gets the treatment in a safe and unadulterated manner. In this section, we will explore how to choose the right solution and what you should be looking for from your suppliers to ensure success.

Consideration of Suitability and Adoption of Bioprocess Equipment for CGT Manufacturing

Strong clinical responses have laid the groundwork for propelling cell- and gene-based therapies towards commercialization. In 2017, the industry saw two major announcements by the FDA for unanimous approval of CAR-T therapies for Novartis and Gilead. Many of these types of therapies are being fast-tracked through clinical trials under orphan status to help advance the evaluation and development of drugs. Because of these accelerated timelines, unlike the traditional timelines of monoclonal antibodies, the industry has leveraged equipment and single-use products traditionally designed and reserved for bioprocessing. CGT manufacturers have found that these products are not ideally suited for the required purpose.

There are many unique differences between traditional bioprocess manufacturing and CGT manufacturing. Before we press on, it is critical to understand these differences to ensure the development of industry standards for single-use systems specifically designed for CGT requirements. Bioprocess manufacturing consists of stable cell lines, like Chinese hamster ovary cells (CHO) that have been stably transfected with a gene of interest that allows for monoclonal or biologics production. Processes like these have been established for several decades that (1) utilize well-established upstream workflows, and (2) where downstream processes remove impurities from the final product that may have been introduced. Critical quality attributes of the final product are also well-known, and impact of specific materials that encounter the product are well-observed and documented. Unlike monoclonal antibody or biologics production where the cell is not the final product, cell therapies are the final product, which adds complexity. Upstream and downstream processes do not follow a standardized template and, furthermore, any material that is introduced to the process is likely to affect the patient, even at residual levels. Another large hurdle for CGT is that critical quality attributes are still being explored.

Although the bioprocessing industry has laid much of the groundwork for addressing the challenges and necessary changes to SUT standards, it is vital that CGT not only build on them, but further develop them. Bioprocess and CGT are distinct, and thus their end-user requirements are not identical. Some of the most common requirements for bioprocess and CGT are listed in **Figure 2**.

Figure 2: Common Requirements for Bioprocess and CGT

Activity	Implication	Bioprocess	Cell and Gene Therapy
Sterile Welding	Particulate generation	Frequently used. Particulates can be removed through multiple downstream processing steps.	Particulate generation presents a major safety issue to CGT as there are not specific downstream steps to remove particulates.
Holdup Volume	Product loss	Commonplace in bioprocessing, but loss has minimal impact considering high concentration and volume of product.	By utilizing unfit SUT for CGT manufacturing, holdup volume can greatly impact product yield.
SUT Sterility	Contamination	Common in upstream processing material and final formulation; minimal SUT sterile products found for downstream processing because of aseptic final filtration.	Leveraging SUT from bioprocess is high risk for CGT because there is no final filtration step for the product.
Materials of Construction (SUT) – Polymers and Membranes	Extractables, Leachables Biocompatibility Particulates	Many bioprocesses leverage chemically defined materials for expansion, wash and formulation. Testing for compatibility is more well-established.	CGT process employs complex media that can cause many issues to membranes like fouling, which results in product loss and protein binding, which can result in stripping of critical raw materials to support cell growth. Extractables and leachables as well as particulates are a main concern for polymers.

Note: Product defined by monoclonal or CGT material

Research vs. Clinical Processes with SUT

Apart from comparing the differences between traditional bioprocess and CGT processes, there are many important considerations amongst CGT research and commercial participants. Many in research and development are not well-versed in the nuances and requirements of SUT systems for CGT, as their focus is primarily on driving products to clinic, not necessarily improving the processes. There is no doubt that R&D groups, along with academic medical centers (AMCs), are critical for CGT. Partnerships between researchers and large pharma (e.g., U. Penn with Novartis and City of Hope and Gilead) have helped propel the industry forward. That said, all SUT systems may not be exactly suited to CGT manufacturing. It is recommended that, because of the nature of this industry where AMCs drive clinical pipelines, open communication between groups will help drive understanding and the need for new standards.

Applying SUT to CGT

Together, the CGT industry is responsible for understanding and implementing SUT standards and ensuring correct actions are taken to ultimately provide the safest products to patients. Although vendors and developers have distinct responsibilities, it is quite important that we utilize the knowledge and experience of all operating in this field. Furthermore, it is even more pressing that, as the field begins to develop SUT products specific for CGT applications, we ensure the education of all operators working with these consumables. It is vital to understand how specific processing steps that use SUT can impact the final product. For example, testing materials with your cells/process is critical to ensure that the SUT selected does not have a negative impact on the process, cell growth, yield, cell efficacy, cell proliferation etc. Not all common bioprocess materials are suitable for use in cell therapy applications.

Equipment Requirements

SUT has been typically manufactured using flexible film-based materials. The expansion of SUT into CGT applications has led to the introduction of rigid-walled containers and enclosures. However, the role of both types of containers are effectively the same: to protect the integrity of the process and the safety of the operators. Flexible-walled containers, more commonly referred to as single-use bags, can be manufactured from a range of different polymers, the more common ones being:

1. Ethylene-vinyl acetate (EVA)
2. A range of different polyethylene formats, including but not limited to those below. Depending on the manufacturer, these differ in the types of polyethylene in contact with the product, the number of layers of materials used, and different types of outer layers used to enhance physical strength and improve puncture resistance:

   a. LDPE = low density PE
   b. LLDPE = linear low density PE
   c. ULDPE = ultralow density PE
3. Gamma irradiation stable fluoropolymers
4. Polyvinylidene fluoride, also called polyvinylidene difluoride, as the product contact layer in a multi-layer laminate film, commonly referred to as PVDF.

For rigid-walled containers, products are already on the market that are manufactured from materials including polycarbonates and polysulfone. For all SUT, regardless of material, supplier or composition, there are critical performance attributes that contribute to the process. However, there is currently no unified standardized process for the testing that is performed to determine suitability of a product against each parameter.

Most manufacturers are testing critical attributes of an SUT using a standard process. However, for many critical attributes being tested, there are multiple valid test methods. The BPSA Single-Use Manufacturing Component Quality Test Matrices list many of these, including American Society for Testing and Materials (ASTM), International Standards Organization (ISO), etc. that can be used to provide data. The SUT manufacturers can select the test method that they want, usually based on familiarity with a testing body, previous experience of that specific process, or even selecting the test method that shows their product in the best light. This makes it very difficult, if not impossible, for an end-user to effectively compare performance data from two or more suppliers when the test data presented are performed using different test methods, and therefore have different levels of impact on the final product.

Figure 3 lists some of the different tests and associated test methods that can be selected by a manufacturer for testing the identified critical attributes. This is not designed to be an exhaustive list, but is more to guide the reader to understand the testing that may be performed and the different testing bodies involved.

Figure 3: Tests and Test Methods for Critical Attributes

Critical Attribute	Objective of the Critical Attribute Test	Test Methods
Tensile strength	Measures the ability of the film to withstand load before suffering a critical failure.	ASTM D882 ASTM 638
Elongation	Demonstrates the amount that the film can be stretched before suffering critical failure. It is usually measured in two directions.	ASTM D882 ASTM 638
Tear strength	Measures the ability of the film to withstand sideways tearing forces before critical failure.	ASTM D1004 ASTM D1922 ISO 6383/1
Oxygen transmission	Measures the rate of oxygen transmission across the film under controlled conditions of temperature, relative humidity and differential pressure.	ASTM D3985 ASTM F1927 ASTM 1434
CO2 transmission	Measures the rate of carbon dioxide transmission across the film under controlled conditions of temperature, relative humidity and differential pressure.	MOCON ASTM F2476 ASTM 1434
Seal strength	Measures the amount of force required to make the welds peel apart on a single-use bag.	ASTM D1786 ASTM D882
Moisture vapor transmission	Measures the rate of water vapor transmission across the film under controlled conditions of temperature, relative humidity and differential pressure.	ASTM F1249 ASTM E96
Gamma stability	Measures the ability of the film to withstand gamma irradiation, typically up to a maximum exposure of 50kGy (Kilo-Grays). Film is subjected to some or all of the test methods detailed above, both before and after exposure to gamma irradiation. Bioburden on the film is measured to appropriate standards.	Actual exposure is determined using dosimeters in the chamber. Bioburden is tested against ISO 11737-1.
Temperature stability	Measures the maximum and minimum temperature that the film can be subject to over a predetermined time without suffering a reduction in performance. Film is subjected to some or all of the test methods detailed above, both before and after exposure to extremes of temperature.	Stability is measured against NIST-calibrated thermometers or probes.

Manufacturers may also undertake and report data on tests such as determination of the secant or Young's modulus (ASTMD822), density (ASTM 792), haze and clarity (ASTM D1003), glass transition temperature (ASTM E1640), puncture strength (FTMS 101B), dart drop test (ASTM D1709), etc.

Other compliance requirements for SUT include certification that the materials of construction of a device are free from the causative agents of BSE and TSE.

In CGT applications where the patient's cells are both the starting raw material and the end product, it is not possible to utilize a filtration step to remove extraneous particles from the finished product. It is therefore critical to assess the manufacturer's claims and testing processes to ensure that both visible and sub-visible particles are either not present or are not generated during the process, as through spallation during any pumping step using flexible tubing. As mentioned earlier, once a CGT process is exposed to particles, they cannot be removed easily, and the risk associated with infusion into the patient must be assessed.

Material manufactured using injection molding or extrusion techniques can have slip agents or extrusion agents added to the process, or to the raw material composition. These agents aid in the processing efficiency of the operation but can extract into fluids that are in contact with the surface post- processing. It is important that the manufacturer identifies and tests for these agents as part of the determination of extractables in their validation documentation.

A full description of the impact and importance of the determination of extractables and leachables from SUT has been detailed in previous BPSA publications (www.bpsalliance.org) and is also the subject of a separate BPSA paper directly related to the CGT market and applications. It is recommended to perform a risk assessment based on the intended use of the component with respect to the chemical nature of process fluid, process conditions and opportunity for clearance of any potential extractables from the drug substance. Extractables characterization is recommended for the high-risk SUT components. It is worth taking time to consider that the above requirements apply not only to the product contact surface in a multi-layer film, but to all layers of construction. It has been shown that over time, materials from the outer layers in a film can leach through the product-contact layer of the film into the product.

ISO11137/11135 advises on sterility sampling plan methodology of SUT according to lot size. ISO is typically implemented for medical devices.

Sterilization Methods of Choice for SUT

Typically, SUT utilized for cell therapy manufacturing is constructed of either a laminated, flexible polymer film or a rigid plastic. Choice of sterilization method depends on the component materials and also the physical design of the product. Gamma irradiation is often the method of choice when available. Typically, these are qualified through the well-defined methods in sterility validation compliant to ANSI/AAMI/ISO 11137 VDmax25 (Sterilization of Health Care Products - Radiation-Substantiation of a Selected Sterilization Dose-Method VDmax25 kGy as a Sterilization Dose-Method VDmax). This is the use of the radioisotope cobalt 60, which offers high penetration allowing sealed single-use products to be sterilized with ease. This method kills bacteria by breaking the covalent bonds in their DNA. Typical exposure doses of between 25 and 40 kGy are used. As part of the in-process validation of each gamma irradiation cycle, the gamma dosage is measured at multiple locations within the irradiation chamber during the exposure to ensure that the minimum gamma dose required has been achieved in all locations. Depending on the single-use system manufacturer, gamma-sterilized SUT is tested post sterilization, using a combination of real-time and accelerated-aging test methods. This provides the end-user with a validated shelf life, below which the end-user can be assured that the product will remain sterile. The provision to this statement is that the packaging of the SUT must remain intact and not be compromised. The validated sterile shelf life of a specific SUT can vary widely depending on the materials of construction, complexity of the assembly, etc., but a shelf life in the range of 12 to 36 months is typical.

Certain materials are not gamma irradiation compatible and/or are made of a material that casts a "shadow" and prevents full exposure to irradiation. Also, materials in the product can be adversely affected by gamma irradiation (e.g., change in appearance, unacceptable extractable and leachable profile, or deterioration of desirable physical properties). Gamma irradiation can also become very expensive when done in small lots.

Training

Single-use products are not a panacea if used improperly. Training users in aseptic techniques, the correct unpacking,

installation, handling and disposal procedures are all vital requirements to ensure the proper handling of SUT to obtain optimal performance. Training must encompass everything from receipt, storage, opening, use in the lab and final disposal. Standard operating procedures (SOPs) that are clear and provide guidance will go a long way to ensure that successful new products hit the market.

Disposal

In the production of cell therapies using SUT, there will be significant amounts of single-use material that will require careful consideration to ensure both safe and environmentally friendly disposal. BPSA has published a "Guide to Disposal of Single-Use Bioprocess Systems." The principles and methods detailed therein apply equally to the cell therapy market.

Part 3: Regulatory Overview

Regulatory Classification

From bone marrow transplant to cord blood processing for therapeutic purposes, cell, gene-modified cell, and now gene therapy, have advanced into industrial level production and delivery. Equipment systems used for cell and gene manufacturing are critical to ensure that therapeutic products are produced and delivered to regulatory standards. Due to their original use to process bone marrow or cord blood for transplantation, historically, equipment systems have been classified as medical devices for their clinical utility. However, as cell and gene therapeutic products become regulated as biological drugs, equipment systems may no longer be regulated as medical devices, but rather as manufacturing equipment.

Unlike medical devices, when classified as manufacturing equipment, these systems are not required for pre-market clearance or approval by health authorities for commercialization. However, manufacturing equipment is still controlled through various parts of regulations (e.g., regulations 21 CFR 211 and ICH Q7A(v) in the U.S.).

These regulatory requirements are an integral part of Current Good Manufacturing Practice to ensure a drug product's quality as referenced below:

> "The minimum current good manufacturing practice for methods to be used in, and the facilities or controls to be used for, the manufacture, processing, packing, or holding of a drug to assure that such drug meets the requirements of the act as to safety, and has the identity and strength and meets the quality and purity characteristics that it purports or is represented to possess." (21CFR 210.1)

Equipment systems likewise need to perform to specifications to deliver on the critical quality attributes (CQAs) of drug products. Hence, when major manufacturing equipment is changed in an established drug manufacturing process, a predefined comparability assessment must be conducted, and new equipment must demonstrate they are able to produce comparable products. A risk-based approach to determine the impact of the change to the CQAs will also determine the actions required to support the change.

Because drug products are the entity subject for regulatory review and approval for commercialization, regulatory requirements on manufacturing equipment are enforced onto drug developers and manufacturers, i.e. on equipment users, but not on equipment suppliers. However, equipment suppliers must supply quality equipment with adequate information to enable end-users to meet regulatory requirements.

Compendial Tests

Biocompatibility

The function of the manufacturing systems is to enable production of a therapeutic agent that is safe and efficacious. Compatibility of the cells with the materials used in manufacturing and storage systems needs to be assessed to ensure the materials that constitute a manufacturing system do not negatively affect the growth or function of the cells. While currently there is no specific guidance or standard for single-use manufacturing systems used in bioprocessing or cell therapies, the industry has adopted standards for medical devices to assess the biological compatibility of plastic materials. The International Organization for Standardization (ISO) and the United States Pharmacopeia (USP) have developed similar methods to assess biocompatibility of medical devices and polymeric materials intended for making containers, parenteral preparations, implants and other systems. USP standards to evaluate biological reactivity include USP <87> Biological Reactivity Tests, *in vitro* and USP <88> Biological Reactivity Tests, *in vivo*. These tests are designed for application to plastics and other polymers in the

condition in which they are used. If the material is to be exposed to any cleansing or sterilization process prior to its end-use, then the tests are to be conducted on a sample prepared from a specimen preconditioned by the same processing.

USP <87> tests for reactivity of mammalian cell lines to elastomeric plastics and other polymeric materials with direct or indirect patient contact, or of specific extracts prepared from the materials under test. ISO 10993-5 is the corresponding test to determine cytotoxicity of materials.

USP <88> is designed to determine the biological response of animals to elastomerics, plastics and other polymeric material with direct or indirect patient contact, or by the injection of specific extracts prepared from the material under test. Three tests are described. The Systemic Injection Test and the Intracutaneous Test are used for elastomeric materials, especially to elastomeric closures for which the appropriate Biological Reactivity Tests, In Vitro (USP <87>) have indicated significant biological reactivity. These two tests are used for plastics and other polymers, in addition to a third, the Implantation Test, to test the suitability of these materials. A plastic class designation (Class I-VI) can be determined based on the response of animals in the prescribed tests with Class VI indicating the material is not reactive in any of the tests.

ISO 10993 series describes the tests included in USP <88> in three separate standards. ISO 10993-6 Tests for Local Effects After Implantation, ISO 10993-10 Tests for Irritation and Skin Sensitization, and ISO 10993-11 Tests for Systemic Toxicity. ISO 10993-12 describes sample preparation and reference materials. The ISO, USP or alternative qualified methods may be used to assess biocompatibility of manufacturing systems.

Although there appears to be a trend in the industry to perform only USP <88> as it is perceived to be a more extensive testing, it may be advantageous to perform USP <87> when the SUT is used for cell therapies, as the cells are the product. It may also be beneficial for cell therapy developers to perform testing on the cell type used in their product.

According to USP <1031> *Biocompatibility Materials in Drug Containers, Medical Devices and Implants*, a comprehensive biocompatibility guidance document, extracted polymers do not alter stability of product or exhibit toxicity.

Particulates

As mentioned, particulates continue to represent a distinct challenge to the field of cell and gene therapies. To date, limited to no guidance for particulate control specific to CGTs is available. Due to the emerging status of CGT, guidance documents have yet to be established. This has led the CGT industry to borrow and work with what exists for adjacent markets. Currently, the most commonly observed guidance documents followed by CGT sponsors and suppliers include USP <1> *Injections*, USP <788> *Particulate Matter in Injections* and USP <790> *Visible Particulates in Injections* in the U.S., along with the relevant EP and JP requirements for Europe and Japan, respectively.

USP <788> has been official for several years. It defines two methods for counting sub-visible particles and sets limits for containers based on size. For visible particulates, USP <1> and USP <790> offer some guidance to the industry. USP <1> sets the requirement that every final container is inspected for particulates to the extent possible, and any having the presence of observable particulate matter are rejected. USP <790> further establishes reference inspection conditions and provides quantitative limits based on acceptance sampling to meet the expectation wherein every lot is to be essentially free from visible particulates. These guidance chapters are mainly based on the premise or assumption that the final products are clear and transparent. Obvious challenges are then presented with CGT products, as most will be opaque since they contain cells as a significant part of the final product. Further complicating the matter, a final clearance or filtration step commonly incorporated in bioprocessing and pharma industries is not possible for CGT products, as the filters intended to capture the particulates would also capture the cells. If a foreign particulate is observed in the CGT product, this can have catastrophic implications for the patient. Discarding the lot, which for autologous products often equals a single product, is not desirable but may be unavoidable depending on the nature and potential impact of the foreign particulate on the patient. Therefore, while we continue to extrapolate information from other industries and reference their respective guidance documents for control of particulate matter, development of guidance documents specific to CGT will be required.

It is critical to the success of CGT products in development that they be both safe and efficacious when used to treat a specific indication in relevant patient populations. Particulate risks related to a product's final formulation are important for many different reasons. The most obvious risk is the potential for adverse events due to particulates that occur after a product

is administered to a patient. The second risk is the impact of particulates on the product quality itself, both during production and in a product's final formulation. Particulates discovered in final products also increase a product's risk of being recalled, leading to potential clinical trial delays or failure to maintain commercial inventory.

The most commonly associated risks of particulates to the intended patient center on the threat of an immediate blood vessel occlusion, in addition to avoiding possible immunological response to any foreign contaminants. Despite the different sources and composition of particulates, there are several common types of pathogenic mechanisms for potential harm to patients. These mechanisms include: inflammation due to infections caused by viable organisms; inflammatory responses caused directly or through associate leachates that trigger direct tissue injury, normal and abnormal immune responses to cellular debris; and tissue damage from thromboembolism. With CGT products, the risk to the actual product is also critical. The impact of inert particulates on cells and cell cultures will vary greatly depending on the properties of the particulate and the properties of the cell lines. Cellular adhesion can be impacted by exposure to particulates, depending on whether the particulate is taken up by the cell and the basic topography of the particulate. Particulates can also be detrimental to the viability and functionality of the cell culture. Leachables and extractables from these types of particulates can alter the pH of the environment or produce compounds that are toxic to the cells—both immediately and over time—which could directly impact product stability.

Material Tests

USP <661> Plastic Packaging Systems and Their Materials of Construction refers to a set of analytical standards defined by the U.S. Pharmacopeia (USP) to help ensure the safety of a variety of health-related products composed of and/or packaged in plastic containers. These products include pharmaceuticals, biologics, dietary supplements and devices. The polymers outlined in the USP <661> subchapters include high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polyethylene terephthalate (PET), polyethylene terephthalate G (PETG), and plasticized polyvinyl chloride (PVC). In order for plastic packaging to be approved for use with an FDA-approved therapeutic product, data must show that the material/packaging conforms to USP <661> standards and performance criteria.

USP <665> Polymeric Components and Systems Used in the Manufacturing of Drug Products is a new general chapter that addresses the qualification of polymeric components used in the manufacture of both pharmaceutical and biopharmaceutical APIs and drug products. USP<665> has been proposed and published for public comment, but as of the publication date of this white paper, has not been adopted and is currently under review with the USP.

Storage of Single-Use, Bag-Based Systems

The bags should be transported and stored in their original carton packaging (protected from light) in a protected warehouse and used before their expiration date. Recommended storage conditions are at ambient temperature (between +5°C and 40°C) and a relative humidity of less than 85%.

Although single-use bags are tested at the manufacturer's facility, improper handling can cause issues. When dealing with highly valuable patient samples, it may be worth testing at the point of use. This is typically done to detect leaks using sterile air.

Single-Use Technology Sterilization Validation and Standards

Cell therapy manufacturing shares much in common with general pharma manufacturing requirements, and that knowledge base does a good job of setting the standards for how you should be testing your single-use products. The sterility standards that apply for SUT in cell therapy manufacturing (**Figure 4**) are the same as those that apply for other classes of biopharmaceutical manufacturing.

Figure 4: Biocompatibility and Sterility Standards for SUT in Cell Therapy Manufacturing

Standard	Pass Criteria
Cytotoxicity	USP <87> Certificate
TSE/BSE free	Use of raw materials which have no animal origin
Endotoxin	USP <85> less than 0.25 EU/ml by LAL
Sterility	USP <71> no detectable microbial growth

At mentioned above, cytotoxicity is always a concern, and ensuring that the problem is not the disposable containers in contact with your cells is paramount. USP <87> provides guidance to minimize that risk.

Bovine spongiform encephalopathy (BSE) is a form of transmissible spongiform encephalopathy (TSE/BSE). This is a prion disease that affects the brain and nervous system. In order to reduce the chance of contamination, it is best to try and minimize or not use any raw materials that are animal-derived. This can be done in three ways: (1) through use of products that are not of animal origin, e.g. using vegetable-derived stearates rather than bovine (tallow)-derived; (2) through certification that the animal-derived raw materials are from a source that is known to be BSE/TSE-free; or (3) if animal-derived raw materials or components must be used, they should meet or exceed the requirements specified in the Note for Guidance on minimizing the risk of transmitting animas spongiform encephalopathy agents-EMA/410/01 Rev. 3.

Endotoxin can cause fevers and other symptoms in humans who are exposed. It is caused by the Gram-negative bacteria walls that contain lipopolysaccharides (LPS). Typical testing for this is done with a limulus amoebocyte lysate (LAL) test, with USP requiring pooled testing of the production lot.

The USP <71> Sterility Testing describes the sterility testing protocol. Depending on the size, shape and application of the product, the sterility test may be performed by the membrane filtration method, the direct transfer method or the product flush method. Many of the SUT are closed chambers whose only ingress/egress is small-bore tubing. Therefore, product flush followed by membrane filtration and incubation in SCDM/FTM are commonly used, which differs from the application of USP <71> for the therapeutic product or for a medical device and is not relevant to the current lot sizes produced for most cell therapy manufacturing (<1000 units/lot).

USP <1211> Sterilization and Sterility Assurance supports USP <71> and describes the sterility testing environment with which CGT manufacturers should comply in validating sterility of their product.

SUT Qualification and Accompanying Documentation for Equipment

Any equipment system must be labeled appropriately for its intended use. With heightened requirements on traceability and prevention of mix-ups for CGT products, labeling and special coding on SUT can enable chain of custody and traceability. An integrated traceability approach such as this can also apply to ancillary materials and other manufacturing components in order to enable identification of defects and causes of failures, as well as security management throughout supply chain.

Equipment systems are supplied with accompanying documentation, such as Instructions for Use, Declaration of Conformity, etc. Suppliers also provide testing data such as extractable/leachable data, data on particulate, and oftentimes performance data to support Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ). When there are proprietary data associated with equipment systems including SUT, suppliers can prepare a Drug Master File (DMF) for end-users to cross reference in their regulatory applications. As a best practice, a regulatory support file containing all the essential information should be provided to users to facilitate regulatory inspection. Such information on file will also help users compile equipment information in their regulatory applications.

Part 4: Best Practices for Supplier Selection, Qualification and Validation to Ensure Supply Chain Security

SUT is widely used across the biopharmaceutical development and commercial production spectrum, and their adoption has forced significant changes in the way that end- users think about supplier selection, qualification, security of supply and validation. The supply chain for an SUT can range from very simple to very complex, and this presents end-users of SUT with challenges not inherent in other manufacturing processes.

Combine what can be a complex supply chain with increased regulatory scrutiny on supply chain security and risk mitigation strategies in the manufacture of a therapy, and for companies new to the use of SUT for commercial manufacturing, the number of steps and processes involved may seem overwhelming. However, there are several key points to consider, which can make the entire process more straightforward and less daunting.

Selecting the right supplier may seem to be a very easy process. “Company A has what we need, we’ve used them before and it works, so we’ll just buy it from them.” While a good strategy for procurement of materials for use in small quantities, in a non-critical process or in an unregulated

environment such as a research or developmental laboratory, this strategy cannot be applied to sourcing of critical products used in the commercial production of a lifesaving therapy.

Once a process is defined and a supplier has been selected, they must be validated and continually requalified. That qualification and validation process involves a detailed evaluation of many of the same attributes, procedures and capabilities that form the basis for selection of a supplier initially. However, it is important to understand that specification, qualification and validation is a continual process, and that regular supplier meetings and audits are designed to maintain proper quality. BPSA's Single-Use User Requirements Toolkit Pack, Quality Agreement Template for Single-Use Products, and Information for An Industry Proposal For Change Notification Practices For Single-Use Biomanufacturing Systems provide guidance to ensure this.

Qualification and validation include validating manufacturing quality across all processes the supplier has in place: the quality program, product certification, returns process, paper and site audits, risk mitigation strategy, their supply chain security program, manufacturing controls, raw materials sourcing strategy, and corrective action process. ASTM E3051 – 16, Standard Guide for Specification, Design, Verification, and Application of Single-Use Systems in Pharmaceutical and Biopharmaceutical Manufacturing, is a reference for a structured approach to this. Ask these questions: From a risk management standpoint, do they dual source? Do they make or outsource? Do they have an active continuous improvement process and a new product development program that can support the integrator's program?

Evaluation and Selection

One framework for supplier evaluation that allows each potential supplier to be evaluated equally and a sourcing decision made based on documented objective evidence, is called the 10 Cs of supplier evaluation and selection. The term 10 Cs refers to specific attributes or capabilities that any supplier or any product in any industry should be able to demonstrate to a potential customer that they can meet BOTH their immediate and future requirements. Initially developed by DPSS Consultants from the UK and first published as the 5 Cs, it has been expanded and is now widely used across multiple industries. If a potential supplier fails to meet a customer's current and future requirements on more than 30% of these critical attributes, serious consideration should be given to whether that supplier is the right partner for a long-term, sustainable relationship.

10 Cs

The 10 Cs are shown in **Figure 5**. One key discussion point in establishing an open and direct relationship with any supplier is to be totally upfront about the expectations that your company has of its suppliers and how critical they are to the success of both businesses. Clear expectations allow both the supplier and the user to benchmark performance, both above and below expectations.

Figure 5: The 10 Cs

Number	Attribute	Explanation
1	Capacity	Does the supplier have adequate engine room to produce your goods? Capacity includes equipment, human resources, materials and space. Can your supplier adjust their capacity in line with your requirements?
2	Cash	Does your supplier have adequate financial standing and resources? This is especially important if you expect your business to grow.
3	Clean	Does your supplier have an appropriate sustainability policy?
4	Commitment	Quality is a key requirement for any business. Does your supplier have the commitment to maintain suitable quality performance?
5	Communication	What tools will you utilize to communicate with your supplier? Another key point is who will communicate with whom? For example, consider how you will manage problem resolution and issue escalation.
6	Competency	Does your supplier have the skills to deliver the materials you require?
7	Consistency	Does your supplier guarantee and deliver a consistent product every time and are they on time with their deliveries?
8	Control	Is your supplier in control of their policies and procedures? Can they ensure that their performance can be consistent?
9	Cost	What is their cost of goods and do they have their own supply chain under cost control?
10	Culture	Does your supplier share the same cultural values as your organization? Does it make sense that your supplier shares similar values and attributes to avoid strains in future relationships?

The 11th C for Biomanufacturing

In addition to the 10 Cs above, which can be applied to any industry, supply of a product that is used to manufacture a therapeutic product or patient therapy is also subject to the 11th C: Change control.

As part of the supplier selection process, it is essential to understand both the supplier's ability to manage any changes to raw materials, manufacturing processes or product design, as well as understand the procedures they have in place to mitigate any risk associated with change to you as their customer. Questions to review with them could include:

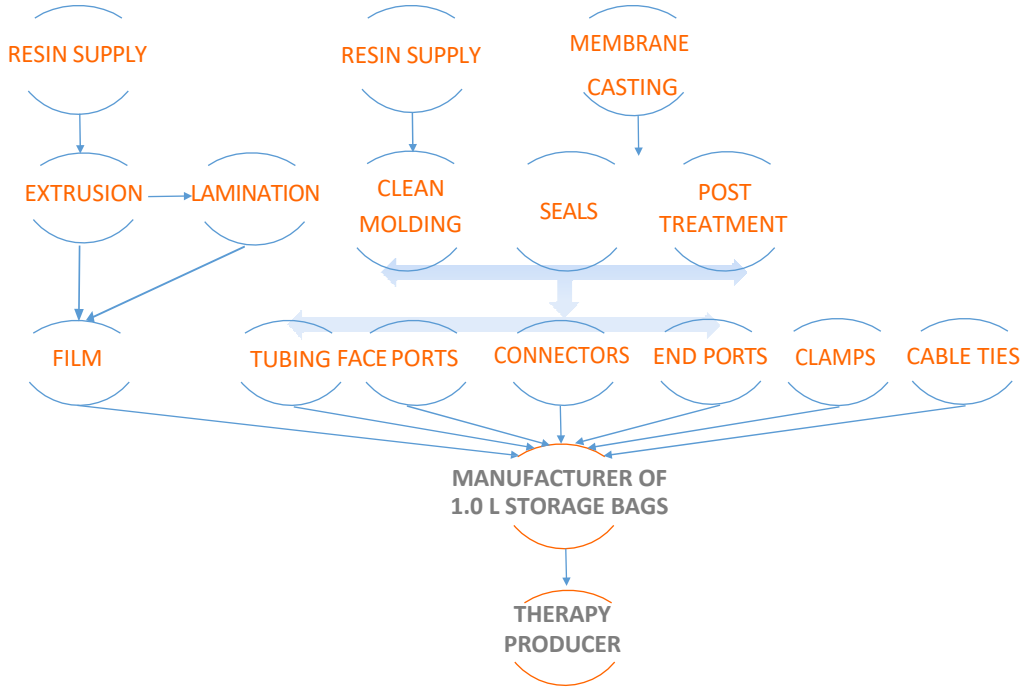
- What is your change notification policy in terms of time?
- What is your right to final buy policy?
- What is your change management process?
- What are the allowed exceptions to this process?

It is very important that the supplier is supplying SUT that meets an end-user's process requirements and the regulatory requirements *consistently*. Then, the CGT provider must assess the risks associated with trusting the supplier to do this. Determine if the supplier has appropriate risk mitigation in place relative to the gravity of the situation, as ultimately, the patient's fate could be in that vendor's hand and a mishap that goes unnoticed by the supplier could have serious consequences. Other points to be considered when selecting a supplier, and which, like the 10 Cs above, have a direct impact not only on supplier selection, but also help to provide a secure and robust supply chain of the critical products needed to commercialize a therapy process include: dual manufacturing, dual sourcing, disaster recovery plan, and their procedures for qualifying and maintaining their own supply chain security. For example:

- Does the supplier have a dual manufacturing strategy?
- If they have a single production facility, what is their disaster recovery plan in the event of a catastrophic failure?
- Do they have a dual sourcing strategy for critical raw materials?
- How does your intended supplier qualify their suppliers? Will they share that process with you?
- How frequently do they evaluate and audit their supply chain?

As an example, below in **Figure 6** are some but not all of the products and raw materials that are used to produce a product as simple as a 1.0L storage bag. However, each SUT product, supply chain step and individual process should be validated and fully traceable to the finished product.

Figure 6: Products and Raw Materials for Manufacture of 1.0L Storage Bag



Manufacturing Validation

And last but not least in the supplier evaluation process is the validation of the supplier's manufacturing processes. Manufacturing validation extends across all aspects of the production operation: from raw materials supply, incoming inspections, operator training validation and facility/utilities/equipment validation, to validation of the manufacturing process, its packaging, labelling and shipping methods. The latter three are especially critical if the product is fragile, easily broken or temperature/humidity-sensitive.

The focus on product quality continues through the validation of the manufacturing process, facility, equipment and personnel who manufacture the assemblies. This should include:

- parts and raw materials;
- primary manufacturing steps, which may include processes such as extrusion, injection molding, rotational molding, vacuum molding, casting, etc.;
- assembly, which may include cutting, heat sealing, ultrasonic welding, solvent welding as well as manual assembly of components;
- personnel;
- facility;
- equipment validation, calibration and maintenance programs (IQ, OQ and PQ);
- environment; and
- finished product, which will include, but is not limited to, final testing, quality parameters, release criteria, sampling and testing methodology, certification, returns policy, packaging testing and specifications, labelling, etc.

Part 5: Conclusion

In order to realize the full benefits of single-use technologies, an unprecedented level of communication and information exchange between system and equipment suppliers and end-users is required. More collaboration is needed by the therapy manufacturers, integrators, component suppliers and regulators than currently exists with traditional drug manufacturing systems. This increased collaboration must work through all aspects of the design, testing, manufacture and validation of the single-use technologies and the therapies with which they are used for many years after initial approval. This creates a pathway for the industry to share information and to partner at multiple levels. ■

Part 6: Terms and Definitions

Autologous cell therapy	Cell therapies which use a patient's own cells as starting material.
Allogeneic cell therapy	Cell therapies which use a donor cell that is expanded and used to treat several patients.
Biocompatibility	The ability of a material to perform with an appropriate host response in a specific situation.
Bovine spongiform encephalopathy (BSE)	A fatal neurodegenerative disease in cattle that may be passed on to humans by consumption of infected meat.
Cells	"The fundamental unit of life. The living tissue of every organism is composed of these fundamental living units. Unicellular organisms, such as yeast or a bacterium, perform all life functions within the one cell. In a higher organism, a multicellular organism, entire populations of cells may be designated a particular task." (per ISPE)
Cell therapy	Also called cellular therapy or cryotherapy, this is a therapy in which cellular material is injected into a patient. Typically, this means intact living cells.
Chain of custody	The chronological documentation, or paper trail, that records the sequence of custody, control, transfer, analysis and disposition of a material. In autologous CGT, this usually refers to the patient's own cells.

Chimeric antigen receptor T-cell (CAR-T)	In CAR-T therapies, immune cells are removed from the patient, modified to include new proteins that allow them to recognize the target cancer, grown in cell culture systems to produce sufficient quantities of cells that can produce a therapeutic effect, then placed back into the patient.
Critical quality attribute (CQA)	These serve as the benchmarks that most quality by design implementation revolve around. They include chemical, physical, biological and microbiological characteristics, or properties that can be consistently measured and quantified.
Cytotoxicity	The quality of being toxic to cells. This can cause effects ranging from cell death to impaired viability.
Direct transfer method	This method required that the test article first pass through a size exclusion membrane capable of retaining microorganisms before the membrane is transferred to the test medium.
Endotoxin	A toxic heat stable lipopolysaccharide present in the outer membrane of Gram-negative bacteria that is released from the cell upon cell lysis.
Extractable	Chemical species that can be released from a product contact surface under controlled laboratory conditions which include extraction solvent, temperature, contact time, etc.
Gram-negative bacteria	Bacteria that do not retain the crystal violet stain used in the gram-staining method of bacterial differentiation. They are characterized by their cell envelopes, which are composed of a thin peptidoglycan cell wall sandwiched between an inner cytoplasmic cell membrane and a bacterial outer membrane.
Injection molding	A process where a material is heated to its melt point, forced under pressure into a mold, and then allowed to cool. During this process, the melted material, typically a plastic, assumes the shape and dimension of the mold into which the material is injected.
Leachable	Chemical species that migrate from a product contact surface into an associated drug product under normal conditions of use or during accelerated drug product stability studies.
Lipopolysaccharides (LPS)	Large molecules comprised of a lipid and a polysaccharide composed of O-antigen, an outer core and an inner core joined by covalent bonds. They are found in the outer membrane of Gram-negative bacteria.
Membrane filtration method	A method introduced in the late 1950s as an alternative to the Most Probable Number methods for microbiological analysis of liquid samples, specifically drinking water.
Monoclonal antibody	Antibodies made by identical immune cells that are all clones of a unique parent cell. mAb's have monovalent affinity in that they bind to the same epitope.
Orphan status	A drug or biological product used to treat a rare disease or condition, known as the orphan disease.
Particle	Loose mobile matter or embedded matter that is unintentionally present in/on the single-use component/assembly and potentially may contact or may end up in the process/product fluid.
Particulate	1. Particle (see above), or

	2. Particulate matter in injections and parenteral infusions consist of mobile un-dissolved particles other than gas bubbles unintentionally present in the solutions.
Polymeric materials	Polymers are large molecules (macromolecules) manufactured from millions of repeated linked units, each a simple molecule. Also used to refer to plastics, which are large chain polymers.
Puncture strength tests	These are used to determine the puncture or rupture characteristics of a material. This is generally a test where a material is compressed by a probe or other type of device until the material ruptures or until the stretch limit is achieved.
Single-use technology (SUT)	"Consist of fluid path components to replace reusable stainless steel components. The most typical systems are made up of bag chambers, connectors, tubing and filter capsules." (*per* BPSA).
Slip agents	A range of ingredients that helps other ingredients spread over skin and penetrate into it.
Sterility	Free from microorganisms.
Supplier	"An organization or individual, internal or external to the user, associated with the supply and/or support of products or services at any phase throughout a systems lifecycle." (*per* ISPE)
Therapeutic agent	Compounds with a beneficial and desirable effect when consumed or applied.
Thromboembolism	Obstruction of a blood vessel by a blood clot that has become dislodged from another site in the circulation.
Transmissible spongiform encephalopathies (TSE)	A group of progressive, invariably fatal, conditions that are associated with prions and affect the brain and nervous system of many animals, including humans, cattle, and sheep.
Young's modulus	Also known as the elastic modulus, a mechanical property of linear elastic solid materials. It defines the relationship between stress (force per unit area) and strain (proportional deformation) in a material.

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Disclaimer

The information in this document is intended to capture the current state of the single-use technology industry regarding CGT. This information is offered in good faith and supported by the expertise of its contributors. However, BPSA, its members, and contributors do not assume any responsibility or obligation for the reader's compliance to the content of this document. This is not a standard, but a set of recommendations. Manufacturers, suppliers and end-users should consult with their own legal and technical advisors relative to their SUT use and participation.

About BPSA

The Bio-Process Systems Alliance (BPSA) was formed in 2005 as an industry-led international industry association dedicated to encouraging and accelerating the adoption of single-use manufacturing technologies used in the production of biopharmaceuticals and vaccines.

For more information, visit www.bpsalliance.org