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Single-Use Technology Today – A Cornucopia of Applications

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Abstract: Whether for classic biologics or advanced therapy medicinal products (ATMPs), laboratory applications, small-scale or large-scale productions, single-use technology (SUT), and its associated consumables are used everywhere. The advantages of deploying SUTs are well known. Wherever a single-use solution is available, it is tested and ultimately used. Based on milestones in the development of single-use systems and single-use platform technologies, this article provides an up-to-date overview of products available on the market and their manufacturers/suppliers. It also discusses process examples with SUTs, design options and configurations of single-use facilities and Switzerland's pioneering role in the development and implementation of SUTs. In addition, the authors show that SUT has already been established beyond the biopharmaceutical sector.

Keywords: Biotherapeutics · Process intensification and continuous manufacturing · Single-use facilities · Single-use systems and process platforms

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1. Introduction

SUT comprises systems and equipment whose cell-, process media- and product-contacting parts are made of plastics such as polyethylene, polypropylene, polycarbonate, *etc.* and are generally ready for immediate use.[1] After being used once, single-use systems, which may have either a rigid or flexible design, are disposed of, which is why they are often referred to as disposables or single-use disposables. Single-use systems are usually purchased pre-assembled and sterilized (typically using the gamma radiation emitted by Cobalt-60).[2–4] However, due to increasing shortages in the supply of Cobalt-60 and the finite number of existing irradiation facilities, the market has begun to suffer from gamma irradiation capacity bottlenecks.[5] Therefore, more and more manufacturers are also considering X-ray irradiation for the sterilization of their single-use systems, especially now that more data has become available demonstrating the equivalence between the two irradiation methods.[6–10]

The beginnings of SUT dates back to the early 1950s. An important milestone in this context is the development of polyethylene blood bags by the Americans William P. Murphy and Carl W. Walter.[11] This was followed by small-volume cultivation systems (Petri dishes, multiwell plates, flasks) in the 1960s, hollow fibre bioreactors, Cell Factories and the first filters and filter capsules in the 1970s and two-dimensional (2D) storage bags and larger filter capsules in the 1980s and early 1990s.[12,13] At the end of the 1990s, three-dimensional (3D) storage bags became available and the first scalable single-use bioreactor, the Wave Bioreactor,[14] was launched. Incidentally, this wave-mixed bioreactor type was designed and built in Tagelswangen, Switzerland. The success of the Wave bioreactor promoted the development of numerous other single-use bioreactors and additional single-use equipment solutions in the 2000s. Among them are mixers, peripheral elements (PE) such as connectors and disconnectors, pumps, tangential flow filtration (TFF) and chromatography systems, centrifuges, membrane adsorbers, and many others. Today, users can select from a diverse range of single-use platform technologies and equipment for various unit operations offered by multiple vendors. Single-use platform technologies, which represent well-defined sequences of processes or process steps, exist for upstream processing (USP), downstream processing (DSP) and formulation and filling (F&F). Table 1 gives an overview of the product portfolios of selected manufacturers/suppliers of SUT, which are currently used for the development and manufacturing of biologics. More recently, US and European single-use vendors have been joined by Chinese companies such as LePure Biotech, Cobetter, Jet Biofil, LAB1ST, and Wuxi NEST Biotechnology, which have also started offering single-use equipment as part of their product portfolio.

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Table 1. Product portfolio from selected manufacturers/suppliers of SUT. No claim to the completeness of the table is made. The manufacturers/suppliers are listed in alphabetical order.

Manufacturers/suppliers	Single-use equipment for unit operations and platform technologies for			
	USP [1]	DSP [2]	F&F [3]	PE [4]
ABEC https://www.abec.com	x	x		
AdvantaPure https://advantapure.com				x
BioPharma Dynamics https://www.biopharmadynamics.co.uk				x
Cellexus https://cellexus.com	x			
Cell Culture Company https://hollow-fiber.com	x			
Celltainer https://celltainer.com	x			
CerCell https://cercell.com	x			
Cobetter https://cobetter.com	x	x	x	x
Corning https://www.corning.com	x			x
Cytiva https://www.cytivalifesciences.com	x	x	x	x
Distek https://www.distekinc.com	x			
Eppendorf https://www.eppendorf.com	x			x
Getinge https://www.getinge.com	x		x	
GMPTEC https://gmptec.de			x	x
G&G Technologies https://ggtechnologies.com	x	x		
Jet Biofil https://www.jetbiofil.com	x			
Kühner https://kuhner.com	x			
LAB1ST https://www.lab1st.com	x			
LePure Biotech https://www.lepurebiotech.com	x	x	x	x
Meissner https://www.meissner.com			x	x
Merck https://www.merckmillipore.com	x	x	x	x
PBS Biotech https://www.pbsbiotech.com	x			
Parker https://www.parker.com			x	x
Repligen https://www.repligen.com	x	x	x	x
SAINT-GOBAIN https://www.biopharm.saint-gobain.com				x
SaniSure https://sanisure.com	x		x	x
Sartorius https://www.sartorius.com	x	x	x	x
Single Use Support https://www.susupport.com		x	x	
Synthecon https://www.synthecon.com	x			
ThermoFisher https://www.thermofisher.com	x	x	x	x
TPP Techno Plastic Products https://www.tpp.ch	x			x
Wuxi NEST Biotechnology https://www.nest-biotech.com	x			x
3M https://engage.3m.com/purification		x		x

[1]Single-use equipment for storage, transport, liquid handling, mixing, filtration, medium preparation, inoculum production, fermentation, biomass retention (perfusion devices), cell harvest; [2]Single-use equipment for storage, transport, mixing, filtration, buffer preparation, pH-shift, clarification, concentration, capturing and polishing, buffer exchange, freeze and thawing; [3]Single-use equipment for storage, filtration, mixing, transport, freeze and thawing, filling; [4]Single-use flexible tubing, transfer systems, manifolds, assemblies, flow paths, tank liners, bags, connectors, closures, valves.

2. Advantages, Challenges and Main Areas of Application of SUT

When single-use systems are correctly selected and used, their advantages dominate in comparison with their reusable glass or steel counterparts. One of the most common arguments favouring their implementation is the high level of biosafety and process safety resulting from their sterile delivery and qualification by the manufacturer.[15] Cross-contamination can be practically ruled out. In addition, single-use systems ensure product integrity and compliance with regulatory standards. Other key advantages of single-use systems are their flexibility (fast installation, minimized changeover time for multi-product manufacturing), cost savings due to reduced cleaning effort and maintenance and capital cost savings (investment into equipment and infrastructure) of up to 50%. Typically, single-use systems require a smaller footprint due to the reduction or elimination of systems for Sterilization in Place (SIP), Cleaning in Place (CIP) and Water for Injection. Also, single-use systems can be equipped with conventional and single-use sensor technology, allowing them to compete with reusable equipment in terms of instrumentation level. More remarkably, life cycle assessment studies have shown that single-use systems can be greener than their reusable counterparts if used correctly.[16] Due to the generation of plastic waste through the recurring replacement of disposable consumables, manufacturers of single-use systems have also started looking for solutions to mechanically recycle spent plastics[17–19] that would otherwise end up in landfills and incinerators. Other challenges for SUTs include: (1) the need to demonstrate the safety of extractables and leachables from plastics in contact with liquids, (2) the more difficult automation (manual nature of SUT), (3) the lack of standardization (lack of interoperability) and, finally, (4) the resulting strong dependence on suppliers.[20]

Locking in their customers is an integral part of the business model of suppliers of SUTs. Once an end-user decides on a solution, multiple incompatibilities or 'blocks' make a subsequent change in vendor difficult. This lock-in effect is partly achieved by creating more value in comparison to competitors, partly by blocks developed and owned by suppliers and, to some extent, by the rules put in place by the regulatory authorities,[21] Notable blocks used by suppliers include unique connector solutions, bag holders, mixers, and single-use bioreactors which only accept bags with supplier-specific dimensions, alongside the defined placement of ports and the use of distinctive connection designs for the drive unit and other peripheral elements. More specifically, in strategic negotiations, suppliers often adopt a razor-razor blade business model,[22] which lowers an end-user's cost of capital at the expense of increased operational costs. Additionally, the substantial effort required to re-qualify equipment to meet regulatory requirements further raises the barrier to change.

Obviously, end-users perceive any obstruction to move to another supplier as manipulative. They prefer and rely on multiple supply options to ensure manufacturability at all times, avoiding dependence on costly long-term storage of consumables, which can severely strain a company's cash flow. Single source dependency also significantly lowers the bargaining-power of end-users. This became particularly evident during the COVID-19 pandemic, where end-users frequently faced significant price increases for single-use consumables resulting from the global supply bottleneck. Following years of mergers and acquisitions, a handful of large suppliers, including Cytiva, Merck, Sartorius, and Thermo Fisher, now dominate the SUT market (Table 1). However, these giants are expected to face disruption in the coming years from larger Chinese competitors such as LePure Biotech and Cobetter, who aim to penetrate the Western market and offer a comprehensive single-use portfolio. The end-user perspective and a

strategic approach SUT users may pursue is discussed in detail by Schmidhalter *et al.*[23]

In contrast, small suppliers typically focus on producing single-use systems of lower complexity, such as lab-scale polymer-based single-use systems for the production of personalized products, or in assembling standard and customized flow sets using commercially available components. These flow set manufacturers claim to be faster, more cost-effective, and to offer greater technical flexibility, *i.e.* regarding material and component selection. Indeed, some of these smaller companies played a crucial role in sustaining biologics manufacturability during the COVID-19 pandemic. Since their business employs a me-too strategy, there is little innovation evident in their products or production operations. Manual assembly of single-use systems is still very much the status quo. Also, when collaborating with small suppliers, it is prudent to verify their capability to provide all information on the components built into their assemblies, as required by the regulatory authorities.

Nevertheless, SUT has become mainstream in recent years, particularly in the USP of biopharmaceuticals (Fig. 1). This encompasses the manufacturing of high-value products in low-to-medium volumes (*e.g.* monoclonal antibodies {mAbs}, cell and gene therapeutics), the safe and rapid production of modern vaccines (*e.g.* virus-like particle vaccines, mRNA and DNA vaccines), and the production of toxic products (*e.g.* antibody-drug conjugates {ADCs}).[13,24–28] For such processes, mammalian cells (Chinese hamster ovary {CHO} cells, Madin-Darby canine kidney {MDCK} cells, human embryogenic kidney {HEK} cells, stem cells), insect cells from butterflies, as well as microorganisms (bacteria, yeasts) are used as production organisms. However, single-use systems are also suitable for the production of plant cell culture-based products (recombinant therapeutic proteins, cell culture extracts for the cosmetics industry) and the production of inoculum intended for the manufacturing of *in vitro* meat (novel food based on cell culture).[29,30] Common single-use systems currently applied towards this purpose include empty bags, connectors, clamps, tubing, buffer containers, filter cartridges, bioreactors, mixers, filled media bags, depth filters, waste containers, sampling systems, membrane adsorbers, chromatography systems, tangential flow filtration devices and perfusion devices.[15]

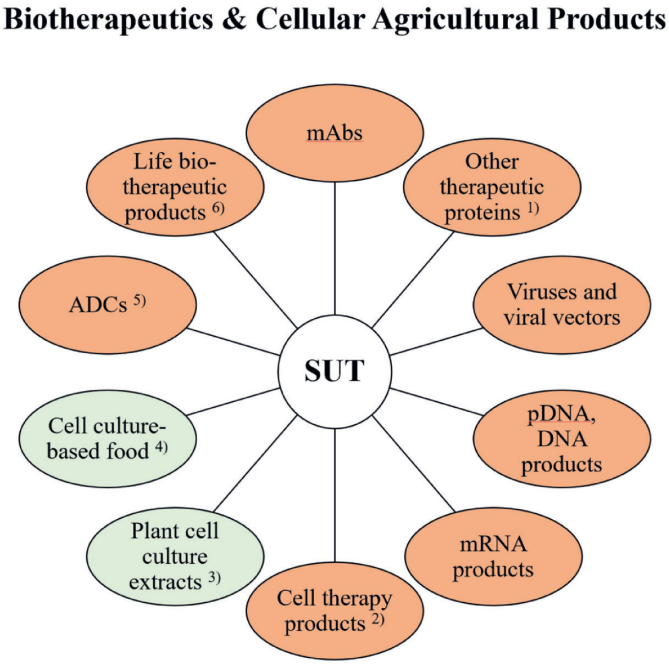

Fig. 1. Main product modalities and applications of SUT. [1)]glycosylated, aglycosylated proteins, therapeutic enzymes, recombinant vaccines; [2)]allogeneic and autologous cell therapies; [3)]*e.g.* for cosmetic applications, [4)]cultivation of meat cells; [5)]handling of toxic compounds; [6)]microbiome products.

3. Selected Applications of SUT

3.1 *Mammalian Cell Culture-based Therapeutic Protein Production: mAbs as a Case Study*

The mammalian cell culture-derived products sector dominates the SUT market in terms of both utilization and global revenue. One is almost tempted to say that innovation in the SUT sector has primarily been driven by the market success of mammalian cell culture-derived products, which today, to the authors' understanding, contributes to over 70% of global biopharmaceuticals revenue, with 430 Bio. US $ reported for the year 2023 alone.[31] This may be accredited to the complexity of recombinant protein production, its high number of production steps (over 20 key unit operations), the diversity of the unit operations, and the duration of a single batch, with the latter aspect significantly increasing the risk of batch failure due to microbial contamination. Without intensified seed preparation methodology and depending on the scale of the production bioreactor, it takes about 45 to 50 days to produce recombinant protein at the 2,000 L scale.[31–33] Key unit operations in mammalian cell culture-based USP exemplarily comprises: Out-of-freeze → Pre-cultures at working volumes (*wv*) of 50 mL to approximately 3 L over 4 stages → Cell expansion in a wave-mixed bioreactor up to *wv* of 30 L → Seed production in a single-use stirred bioreactor at 200 to 300 L scale → Product expression in the production bioreactor → Separation of biomass. Media and feed preparation followed by aseptic fill into storage bags for all stages is also part of the USP. DSP typically starts with virus inactivation, followed by protein purification steps → Chromatography 1 → TFF 1 → Chromatography 2 → TFF 2 → Chromatography 3 → Virus reduction filtration → TFF 3 → Formulation and, finally, in line microfiltration as part of aseptic fill into bulk drug substance (BDS) containers (closure systems). Also, part of DSP is the preparation of chromatography and diafiltration buffers, as well as buffers for various purposes, and their aseptic filling into storage bags.

As Fig. 2 shows and Jossen *et al.* explain in detail in their book chapter on CHO cell-based mAb production with single-use systems,[13] SUTs are available for all steps of mAb production up to 2,000 L *wv*. Complete single-use upstream lines that deliver mAb titers exceeding 3 g L^{-1} in fed-batch mode (bolus feeding) are already a reality,[34] with the increased use of intensified and continuous mAb processes[35] leading to *wv* of 2,000 L often being sufficient to address product demand.

Intensified and continuous USP is based on perfusion approaches (N-1 perfusion, high-seeded fed batch, concentrated fed batch and continuous standard perfusion), which are implemented with single-use wave-mixed and stirred bioreactors.[36] In this context, continuous operation is regarded as the highest level of process intensification. The Repligen Alternating Tangential Flow (ATF®) systems, which are also available in a single-use version, are recommended for operations over 30 days. To guarantee ultra-high cell densities (greater than 100 Mio. cells mL^{-1}) in intensified mAb processes without mass transfer limitations or cell damage, users can further rely on specially designed, scalable single-use bioreactors such as the Hyperforma DynaDrive™ series (ThermoFisher Scientific), the Xcellerex™ Xbioreactor series (Cytiva) or the Mobius® iFlex bioreactor series (Merck Millipore). Merck Millipore recently launched their Mobius® Breez microreactor platform for the development of perfusion-based processes. This single-use miniature bioreactor system has four hydraulically driven microfluidic cultivation cassettes with a *wv* of 2 mL. These cassettes each consist of a three-part growth chamber containing a pH, DO, and biomass sensor, alongside 1.2 µm

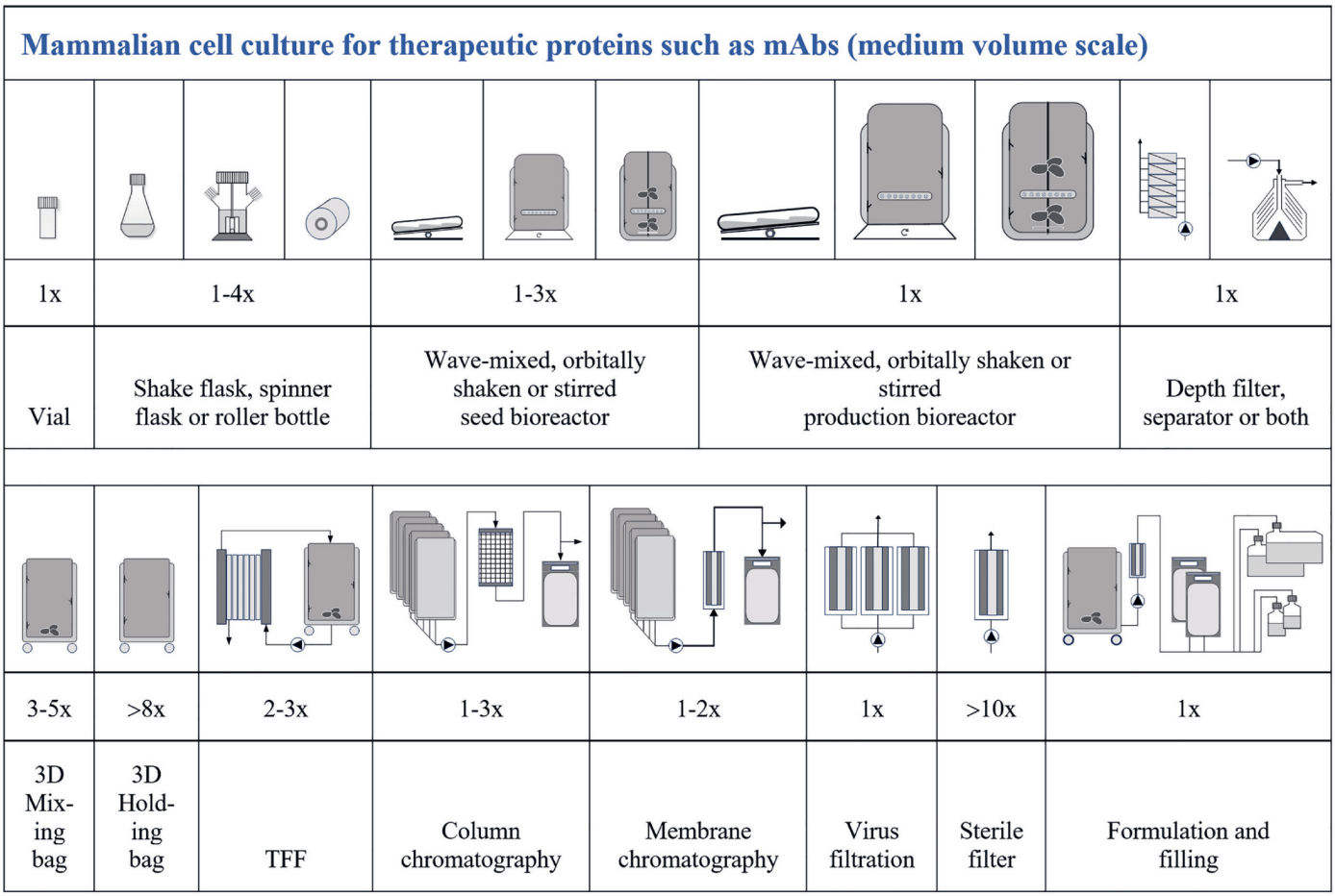

Fig. 2. Typical single-use equipment used in mammalian cell culture-based mAb production at medium volume scale (2,000 L wv).

filters for cell retention, microchannels, and microvalves. A detailed explanation of the Mobius® Breez's operating principle, alongside data on CHO cell-based perfusion processes in which high and ultra-high cell densities were achieved, may be found in the publication by Schwarz *et al*.[37]

The current focus on mAb process intensification in USP has also impacted DSP, even if there has been less activity to date than in the upstream area. Here, process intensification contributes to savings on high-priced resin and buffer volumes.[38,39] Two main approaches may be identified in DSP: (1) The increase or more effective usage of chromatography resin binding capacity and (2) the continuous operation of DSP steps, with the latter covering continuous chromatography for protein A capture and polishing applications, continuous low-pH virus inactivation, continuous virus filtration, and ultra- and diafiltration steps in continuous flow mode. It is worth mentioning that fully continuous, single-use DSP process platforms such as those of Sanofi Genzyme in Framingham or Bayer in Leverkusen have already been successfully established.

While end-to-end production using SUTs is limited to 2,000 L scale facilities, SUT has also made its way into commercial production facilities operating at scales of up to 25,000 L. These facilities, henceforth called hybrid facilities (see also Section 4), make use of SUT to the fullest extent offered by the supplier-specific solutions. While the production step is conducted in a stainless-steel bioreactor, upstream operations covering all pre-culture and seed train steps up to 2,000 L scale may be single-use-based. This includes using single-use mixers and bags for media and feed preparation and storage. Some companies adopted ABEC's mid-scale single-use bioreactor technology for commercial production, pushing the limit of the nominal volume for single-use bioreactors to 6,000 L.[40] Common SUT DSP solutions implemented in large-scale manufacturing facilities include systems for buffer preparation and storage and for BDS formulation, aseptic fill, and storage.

3.2 *Virus and Viral Vector Production*

Although viruses, as well as viral vectors, may be produced with both adherently growing cells and cells growing in suspension (*e.g.* Vero, MDCK, HEK293, *Spodoptera frugiperda* 9 {*Sf*9}, *Saccharomyces cerevisiae*, *Escherichia coli* cells) up to the cubic meter scale, the subsequent discussion will focus on virus and viral vector production by means of non-anchorage dependent host cells growing in suspension, given that production technologies have generally evolved towards facilitating this approach. More information on how adherent cells may be used to produce similar products is discussed in Section 3.8. Virus and viral vector products have two main applications, *i.e.* human and veterinary vaccination and as gene therapy vectors. Besides these, viruses are also used as biopesticides and for phage-therapy purpose. The related process schemes share the characteristics that the USP consists of two separate stages which are (1) the production of host cells, also called producer cells, for the proliferation of a virus or viral vector, and (2) the production of seed virus to infect the host cells or the production of plasmids carrying the viral genes to transfect the producer cells. This commonality exists regardless of whether the product is a virus designated for vaccination (*e.g.* influenza vaccine), a viral vector for gene transfer (*i.e.* an adeno-associated virus {AAV} or lentivirus vector),[41] a bacteriophage (*e.g.* mono- and polyvalent phage) preparation to treat bacterial infections[42] or a host-cell specific virus for controlling bio-pests (baculovirus). Depending on the application, cells of human, animal, or

microbial origin serve as host cells for the proliferation of the virus or production of the virus vector. Merten *et al*. provide a detailed overview of the various systems used for this purpose and their technical requirements.[43] The transient expression of recombinant proteins by means of *Sf*9 cells in combination with the baculovirus expression vector system (BEVS) is based on a similar process principle.[44]

In general, the proliferation of eukaryotic host cells follows the same process scheme as described for mammalian cell-based protein expression in Section 3.1. A notable difference is the addition of a step where the production cell line is infected with the target virus or co-infected with several viruses once reaching a defined host cell concentration. Once assembled, the cells may release the virions directly or otherwise require lysis to do so. From an equipment standpoint, the same single-use systems as used for the mammalian recombinant protein production (Fig. 2) may be used for this purpose. This is also true for the isolation and purification of the virus or viral vector product, which typically consists of TFF, followed by two orthogonal chromatography steps.[45]

However, there are obstacles associated with the proliferation of prokaryotic host cells in single-use bioreactors for phage preparation production purposes. While anaerobic bacteria can be effectively cultivated in such systems, the cultivation of aerobic bacterial host strains still faces limitations, as discussed in detail in Section 3.3.

3.3 *Microbial Therapeutic Protein*

The production of recombinant proteins by microorganisms primarily uses the same or similar unit operations as those based on mammalian cells (Fig. 2), although additional production mode-dependent unit operations may be required, such as disruption of cells in the case of intracellular accumulation of product or protein refolding in the case of inclusion-body-based manufacture of protein.[46] On the other hand, microbial-derived products are not subject to specific viral safety requirements. Furthermore, USP using microbials requires significantly less time due to their significantly higher specific growth than mammalian cells, shifting the bottleneck from USP to DSP.

As such, the production of recombinant proteins using microbials serves as the second-largest contributor to global biopharmaceutical sales, making it an attractive business opportunity for developers of single-use systems.[47] Despite this, SUTs do not play the same role in microbial production as in mammalian cell-based production. This is because existing single-use bioreactor platforms suffer from oxygen transfer and cooling capacity limitations, making single-use bioreactors unsuitable for classical microbial recombinant protein production,[48,49] with such processes typically being realized by cultivating *E. coli*, *S. cerevisiae*, or *Pichia pastoris* to high cell densities of 350 to 450 g L^{-1} wet weight.[50] Accordingly, only pre-culture or low-volume seed culture steps are typically conducted in USP using SUTs. A further distinction that must be considered when transitioning from stainless steel to polymer-based equipment, is that for microbial processes the medium is usually heat-sterilized, while for mammalian cell-based processes it is sterile-filtered, placing limitations on the type of equipment which can be used for this purpose.

Single-use bioreactors are suitable for microbial fermentations with low oxygen demand or for anaerobic fermentation, however. Here, oxygen demand and heat formation can be controlled by modulating the feed rate of the carbon source. Even so, to the authors' knowledge, the number of high value added products manufactured by anaerobic organisms that can afford SUTs' impact on operational costs is still very limited, with the production of collagenase with *Clostridium histolyticum* potentially being one such example. Nevertheless, the use of single-use bioreactors and single-use biomass separation technologies could provide a suitable response to regulatory challenges, such as those arising from the fermentation of spore-forming organisms, for instance, in the production of live microbiome biotherapeutics.[51] Note, more details on the manufacturing of bacteriophage preparations are given in Section 3.2.

The scenario is somewhat different when it comes to product purification. The manufacturing of microbial recombinant proteins is hardly platform-based; production protocols, particularly for DSP steps, are typically tailored to each specific product. Moreover, due to the short fermentation duration, DSP often dictates the overall batch cycle time. Leveraging technology that enables rapid product transitions and flexibility in the sequence of unit operations is, therefore, highly desirable for multiproduct facilities where product changes are frequent, even though it might not be possible to identify suitable SUTs for all conceivable microbial DSP steps at this time. As such, a partial replacement of stainless-steel equipment by SUT, *e.g*. for TFF and chromatography, might already provide a suitable economic advantage through reduced batch cycle time, lower CIP/SIP cost, and lower operational and quality control effort.

As mentioned in Section 2, the application of SUTs offers the advantage of reducing the risk of both product cross-contamination and microbial contamination, which can otherwise result in hidden operational costs. However, despite its operational and risk-reducing advantages, the use of single-use equipment in the production of microbial recombinant proteins is often limited to applications such as buffer preparation mixers, hold bags for buffer and intermediate products storage, in line filtration between production steps, and the aseptic filling of BDSs into bags or bottles. Occasionally, manufacturers of recombinant proteins using microbial expression platforms leverage the benefits of prepacked chromatography columns. As for TFF, membrane filters are typically cleaned and re-used for multiple batches of the same product, a practice rarely observed in cell culture-based manufacturing.

Finally, the microbial manufacturing of recombinant proteins at mid- and large scale rarely makes use of SUT, apart from single-use sampling devices, filters, -occasional transfer lines, and during automated aseptic filtration and filling of BDS into bags or bottles.[15] The cost of more complex single-use assemblies is simply too high for microbial products of lower value or no pharmaceutical application.

3.4 *Plasmid Production*

Plasmid DNA (pDNA) is used to treat infectious diseases, genetic disorders, and cancer, *i.e.* as a vaccine, gene therapeutic, and immunotherapeutic, respectively. It also serves as a starting material for manufacturing pharmaceuticals, such as viral vectors, *e.g*. AAV and lentiviral plasmids, cell- and gene therapeutics, and mRNA under Good Manufacturing Practice (GMP). Given its broad usage, it is unsurprising that several studies estimate the global pDNA market to have been worth 2 Bio. US $ in 2024,[52,53] growing at a relatively high compound annual growth rate of over 20%. The production of large quantities of pDNA is usually platform-based and uses *E. coli*. It is, therefore, characterized by process specifics that deviate from conventional recombinant protein production. For this reason, the manufacturing of this modality is discussed separately.

Traditional process schemes comprise the following unit operations:[54,55] Fed-batch fermentation of an *E. coli* strain with high plasmid copy number capability and containing the target plasmid of interest to high cell densities → Harvest and washing of cells by centrifugation → Alkaline cell lysis in the presence of NaOH and SDS → Neutralization and precipitation with $CaCl_2$ to remove RNA resulting in flocculation → pDNA recovery, *i.e*. the flocculated material is removed either by depth filtration, centrifugation, or an alternative proprietary procedure → Normal

flow filtration and TFF to remove protein and RNA → The removal of any remaining endotoxin, protein residues, genomic DNA, open circle pDNA and RNA in two consecutive chromatography steps, one of which is often performed with a hydrophobic interaction resin → The resulting product solution, which contains covalently closed circular pDNA (cccpDNA, also called supercoiled pDNA), is then concentrated by TFF → Formulation and aseptic filling complete the process.

RNAse treatment serves as an alternative to $CaCl_2$ mediated precipitation of RNA, with a fully SUT-based laboratory scale pDNA production process described using this approach.[56] Controlled feeding of the carbon source and supplementation of the inlet air with pure oxygen allow oxygen limitation to be avoided, even at an optical density exceeding 100. Dreher *et al*. successfully cultivated *E. coli* to a high optical density of 175, measured at a wavelength of 600 nm, in a 50 L stirred single-use bioreactor.[48] The carbon source was fed at a rate that supported a growth rate of 0.1 h^{-1}. According to Aldevron's website, pDNA has also been produced at 300 L scale under nutrient-limited conditions using microbial single-use bioreactors, with 100% of the consumables being single-use-based.[57]

Obviously, downstream unit operations such as depth filtration, in line filtration, chromatography steps, TFF, mixing, and filling can be performed using SUT. However, whether this applies to pDNA recovery after cell lysis depends on the methodology used. The closed-system approach provided by SUT definitely permits faster product changeovers and has the potential to reduce the required cleanroom classification, thereby improving operational efficiency and cost-effectiveness.

3.5 *Production of ADCs*

In 2023, the global sales volume of ADCs, also known as bioconjugates, surpassed 10 Bio. US $ for the first time, representing a notable share of approximately 2.5% of the global biologics market.[58] However, manufacturing ADCs is particularly challenging due to the need to handle highly toxic compounds. This complexity extends to the cleaning of equipment, as the required detection or quantification limits for these toxic substances often falls below the capabilities of existing analytical methodologies. Consequently, the use of SUT presents an ideal solution for the production of such compounds, minimizing cross-contamination risks and simplifying cleaning validation processes.

Furthermore, the use of organic solvents to dissolve the typically lipophilic payload and facilitate the chemical coupling reaction also poses a potential challenge for ADC manufacturing. This is partly due to technical limitations, such as the potential lack of robustness in polymer-based equipment components, and partly due to regulatory concerns, as strict control over product contamination from extractables is necessary.[59] However, the industry has demonstrated that these risks can be effectively managed through the meticulous selection of product-contact materials and robust quality assurance measures.[60] Additionally, progress is being made to tailor systems specifically for ADC processes. For instance, MilliporeSigma (a division of Merck) has developed the more robust Ultimus® film and introduced mixing bags with dip pipes to enable more efficient reagent addition.[61] Fig. 3 illustrates the new Mobius® ADC reactor, a scalable single-use mixer specifically designed for ADC manufacturing. Given these innovations and risk management strategies, it is unsurprising that some ADC production facilities now operate end-to-end single-use processes on a kg scale.

Fig. 3. Mobius® ADC reactor offering scalability from 1 to 500 L (with kind permission of Merck Millipore).

A generic cysteine conjugation ADC process typically includes the following sequence of unit operations: Reduction of the antibody → TFF to remove the reducing agent → Selective reoxidation → mAb drug-linker conjugation followed by quenching excess drug-linker → Chromatography using prepacked columns to remove by-products and reaction reagents → Buffer exchange by TFF → Formulation → Final in line microfiltration before aseptic fill into BDS containers.[59] As shown in Fig. 4, SUT solutions are available to run all these unit operations.

In summary, end-to-end production of ADCs based on SUT increases operator safety, reduces the risk of microbial contamination during processing and simplifies the disposal of product- and reagent-contact surfaces and cytotoxic waste, while managing the associated regulatory and technical risks.

ADCs and mRNA

>1x	>5x	>5x	>5x	2-3x	1-3x	>5x	1-2x
Bottle	2D bag	3D Holding bag	3D Mixing bag	TFF	Column chromatography	Sterile filter	Formulation and filling

Fig. 4. Typical single-use equipment used in the clinical and commercial production of ADC and mRNA products.

3.6 *Production of mRNA Products*

Although the security of supply has long been identified as a potential business risk in every SWOT analysis regarding the transition to SUT, the COVID-19 pandemic revealed significant shortcomings in the solutions provided by manufacturers and the preventive measures implemented by end-users. During this period, ongoing and planned clinical and commercial manufacturing of non-COVID biopharmaceuticals faced prolonged bottlenecks in the supply of single-use devices. Nevertheless, SUT proved indispensable for the rapid setup and commissioning of new manufacturing facilities capable of producing large quantities of mRNA SARS-CoV-2 vaccines in record time. The production and sale of prophylactic mRNA vaccines by a small number of companies drove the global annual mRNA market revenue to an estimated 50 Bio. US $ in 2021.[62] By 2023, it had re-adjusted back to 10 Bio. US $, aligning with the revenues reported for ADCs.[63]

The manufacture of mRNA vaccines in single-use systems was not only the most optimal solution for flexibility during the pandemic but also remains the solution of choice for mRNA production in general. Microbial contamination or contamination by ubiquitous RNAse can readily degrade mRNA products. SUT provides the advantage of enabling end-to-end closed and aseptic manufacturing, thus ensuring the integrity and safety of mRNA products.

The generic procedure for the production of a prophylactic mRNA vaccine typically involves two independent process sequences: mRNA production and purification and the production of lipid-based nanoparticles (LNPs), followed by the integration/encapsulation of the mRNA. Both processes employ SUT.[64] Steps for mRNA production and purification comprise: *In vitro* transcription and 5'-capping of mRNA → TFF to remove unused small molecule reagents → Chromatography to purify the target mRNA product → TFF for buffer exchange and mRNA concentration → Aseptic filling of target mRNA into containers for intermediate storage. Steps for the production of LNPs and mRNA integration/encapsulation include: The production of LNPs → mRNA integration/encapsulation by means of microfluidic mixing → TFF to concentrate mRNA containing LNPs and to remove any remaining small molecule impurities → Final in line filtration before aseptic fill into BDS containers.

As the lipids solutions are prepared at high ethanol concentrations, lipid mixing should be performed using a mixing tank, which is not prone to the formation of leachables. Glass vessels may be used instead and disposed of after use. In conclusion, the production of mRNA and ADCs shares a common reliance on SUTs (Fig. 4).

3.7 *Autologous CAR-T Cell Production as an Example of an Autologous Cell Therapy Product*

In contrast to the production of all previously discussed product modalities, where living cells are primarily used as production systems, CAR-T cell production uniquely positions the cells themselves as the final product. The production of chimeric antigen receptor (CAR)-T cells is personalized, utilizing the patient's own cellular material. These genetically modified cells are designed to target and effectively treat cancers such as malignant lymphoma, blood cancer, and multiple myeloma. CAR-T cells bind to cancer cells *via* their specific targets and initiate an attack. As long as targets are present, the CAR-T cells continue to proliferate and actively combat the cancer.

Autologous CAR-T cell manufacturing is a highly complex process.[59,60] The use of SUT is essential to ensure patient safety and is easily implemented due to the low volume requirements for producing autologous products. Fig. 5 illustrates typical single-use equipment utilized in clinical and commercial CAR-T cell production facilities. Bag-based single-use systems, including static and wave-mixed configurations, dominate.

Before the CAR-T cell production process begins on a clinical scale in a GMP facility, such as the one for Novartis' Kymriah® in Stein, Switzerland, the patient's peripheral blood mononuclear cells (PBMCs) are typically collected through a leukapheresis procedure performed in hospital apheresis units. Following collection, the PBMCs are either cryopreserved on-site and then shipped to the production facility or delivered directly to the facility as a fresh product, depending on logistical considerations and clinical requirements.[66] In principle, CAR-T cell manufacture includes the following steps: PBMC thawing and washing → T cell enrichment (*e.g.* immunomagnetic bead separation) and activation *via* polyclonal stimulation using soluble anti-CD3 antibodies, immobilized CD3 and CD28 antibodies or paramagnetic beads coated with CD3 and CD28 antibodies → CAR-T cell expression by genetic modification with viral vectors (retroviral or lentiviral vectors) → CAR-T cell expansion → Cell harvest, concentration and washing → Medium exchange → And finally, cryoformulation of the patient-specific CAR-T cell product.[67] A CAR-T cell manufacturing process typically takes 7 to 14 days, provided the viral vector stock required for CAR-T cell expression is already available.[68] For detailed procedures for producing frozen viral vector stocks, readers are encouraged to consult the review by Levine *et al*.[69] and the book chapter by Delgado *et al*.[70] As outlined in Section 3.2, viral vector production extensively utilizes single-use equipment, including consumables for routine cell culture work, single-use bioreactors, filtration systems, separators, and chromatography systems, among others. Once the CAR-T cells are manufactured, they undergo rigorous quality control and release. The final product is then transported to the treatment site in a liquid nitrogen container to ensure stability and viability, where it is administered to the patient.

At present, the CAR-T cell manufacture is either manual (with 2D systems such as static flasks and bags) or semi-automated,[71] with a clear trend towards (a) automating individual process steps (*e.g.* by using liquid handling units or dynamic single-use bioreactors such as Wilson Wolf Manufacturing's G-Rex®, Cytiva's Xuri™ Cell Expansion System or Sartorius' Biostat® RM TX Bioreactor) and (b) automating the entire process chain (*e.g.* Milteny BioTEC's CliniMACS®, Lonza's Cocoon® Platform or Cytiva's Sefia™ Cell Therapy Manufacturing Platform).[72,73] If a single bioreactor cannot produce the doses required to treat the patient, several bioreactors are operated in parallel instead (scale-out approach).

3.8 *Allogeneic Human Mesenchymal Stem Cell-based Therapeutic Production*

Of the 30 human mesenchymal stem cell (hMSC)-based therapeutics currently on the market, the majority are produced using cells isolated from adipose tissue, bone marrow, or the umbilical cord.[24,74] Most of these therapeutics, such as Alofisel®, Cartistem®, and Prochymal®, adopt an allogeneic approach to treating a patient's (*e.g.* orthopedic, cardiological) indications. This approach produces multiple doses for several patients from a single batch, making it a more attractive option in terms of cost[75] compared to the autologous manufacturing approach. For a comprehensive list of hMSC-based therapeutics and their indications, readers are referred to the review by Teale *et al*.,[24] which also provides a detailed description of the typical clinical-grade allogeneic hMSC production procedure and the necessary equipment (see also Fig. 6). Compared to autologous CAR-T cell manufacturing, which is addressed in Section 3.7, allogeneic hMSC production is less complex, comprising the following steps, provided that a cell bank with donor cells has already been established: Cell expansion → Harvest and separation → Washing and concentration → Medium exchange → Final formulation for cryopreservation.

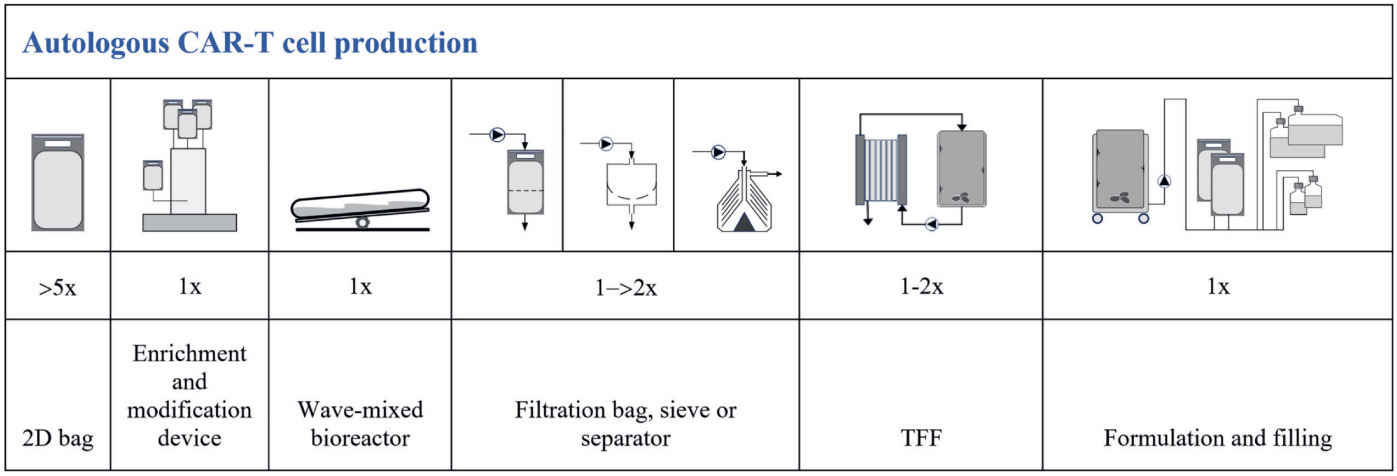

Fig. 5. Typical single-use equipment used in clinical and commercial autologous CAR-T cell production.

In contrast to CAR-T cells, hMSCs must be cultivated adherently to retain their stem cell properties. One option is to use a cultivation vessel which provides a suitable growth surface for the cells to colonize during their expansion (*e.g.* planar plastic surfaces, membranes, or other scaffolds). Alternatively, the growth surface can be provided by microcarriers (beads with diameters around 100 to 250 µm), allowing the cells to attach and expand in a bioreactor designed for mammalian suspension cells. In this context, the PhD thesis by Jossen[76] and the review by Jankovic *et al.*[77] detail commonly used cultivation systems/bioreactors used for hMSC expansion alongside suitable microcarriers. For such processes, SUT use is preferred to minimize batch failures caused by contamination, as the culture medium is at least ten times more expensive than that used for mAb production. Popular single-use cultivation systems/bioreactors applied for hMSC expansion include T-flasks, multi-layer flasks, roller bottles, multiplate bioreactors, hollow-fibre bioreactors, fixed-bed bioreactors, rotating bed bioreactors, and stirred bioreactors. Notably, stirred single-use bioreactors operating with microcarriers are considered the system of choice for GMP-compliant allogeneic hMSC expansion. These systems are closed, scalable, can be operated automatically, and require significantly less cleanroom space.

For the expansion of allogeneic hMSCs, stirred single-use bioreactors such as the Biostat STR® (Sartorius) and the Mobius® Bioreactor (Merck Millipore), initially developed for mammalian cell-based recombinant protein production, have been shown to successfully produce cell densities exceeding 0.5 Mio cells mL^{-1} within 5 to 9 days[76,78,79] when operated (in repeated batch or feeding mode) at maximum working volumes of up to 50 L. Assuming a cell density of 0.5 Mio. cells mL^{-1} and a batch size of 100 Bio cells, sufficient for 95% of the indications requiring hMSC treatments, a 200 L bioreactor would meet the requirements for phase III trials (go-to-market phase) and a 2,000 L bioreactor would be sufficient for commercial manufacturing, according to calculations made by RoosterBio.[80]

Single-use equipment is also available for the processing steps following hMSC expansion (Fig. 6). The cells, which are typically enzymatically detached from the microcarriers after harvest, must be separated using size exclusion filtration. Here, filter systems with a pore size greater than 75 µm, such as the Harvestainer BPC systems from Thermo Fisher Scientific, have proven effective.[75,78,81] In preparation for the final cryoformulation, the cells must then be washed and concentrated. At pilot scale, this process is typically carried out in parallel using TFF (*e.g.* Cytiva's Ready-ToProcess™ HF microfiltration cartridges or Merck Millipore's Pellicon® XL Cassettes)[82,83] and counterflow centrifugation (*e.g.* Sartorius' Ksep® System).[75] The final step of post-harvest DSP in allogeneic hMSC manufacturing requires exchanging the medium, which is typically executed using diafiltration devices of various suppliers.[84]

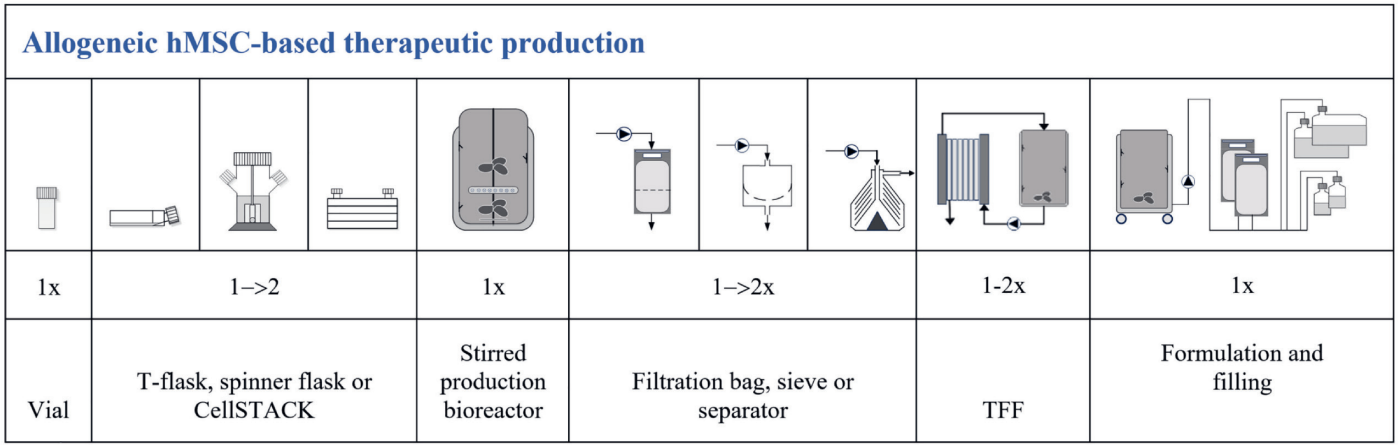

Fig. 6. Typical single-use equipment used in clinical allogeneic hMSC-based therapeutic production.

3.9 *Production of Cellular Agricultural Products*

Cellular agriculture products are also being developed and manufactured using SUT, although its frequency of use is still relatively low compared to the production of biotherapeutics and cell/gene therapy products. The most well-known application is the production of plant cell culture extracts for the cosmetics industry, which incorporates single-use flasks, wave-mixed bioreactors, and orbitally shaken bioreactors for the mass propagation of plant suspension cells.[85,86] Prominent examples of such processes include the platform established by the Swiss company Mibelle Biochemistry which employs several wave-mixed bags (one degree of freedom motion, 50 L *wv*) for the commercial manufacturing of PhytoCellTec™ Malus Domestica. This liquid extract, derived from apple suspension cells, is used by various cosmetics companies in high-end anti-aging products.[29] Although wave-mixed bioreactors with one degree of freedom motion (*e.g.* Sartorius' Biostat® RM or Cytiva's ReadyToProcess Wave™) may reach operational limits as the viscosity of the plant cell culture-based broth increases over the cultivation period (exhibiting non-Newtonian flow behaviour), such systems offer a simplified DSP workflow. This is because wave-mixed systems eliminate the need for antifoam agents due to their enhanced ability to reduce foam formation, unlike stirred, bubble column, or airlift bioreactors.

Both Eleva Biologics and Mosa Meat have leveraged these advantages for pharma and food manufacturing. While Eleva Biologics[30,87] uses wave-mixed (one degree of freedom), as well as stirred single-use bioreactors for the production of high-value proteins with its liver moss cell platform, Mosa Meat uses wave-mixed single-use bioreactors (two degree of freedom) for the propagation of fat and muscle precursor cells and *in vitro* meat production.[88] Scientists of the company Mosa Meat have even successfully cultivated both cell types in the CELL-tainer® from BIONET.[89] As such, the additional degree of freedom in motion enables improved specific power input and oxygen transfer in the CELL-tainer®, an advantage wave-mixed systems with one degree of freedom do not have.

4. Single-use Facility Types and their Layout

Generally speaking, in single-use production facilities, the conversion, transportation and storage of starting materials, intermediates and end products is carried out largely or entirely in single-use systems. Consequently, three types of single-use production facilities can be distinguished: (1) the hybrid facilities already mentioned in Section 3.1, (2) single-use facilities of the closed type, and (3) single-use facilities operating in stations.[90] As such, hybrid facilities (traditional glass or steel production facilities in which single-use systems are integrated) represent the state of the art in biopharmaceutical production. It is estimated that approximately 75% of all biopharmaceutical production facilities use hybrid solutions.[91] The ratio of glass or stainless steel to plastic is determined by various factors such as the size of the facility, the process parameters and aspects of GMP, as well as safety and environmental considerations. Solutions and recommendations for the efficient and safe design and operation of hybrid facilities are described by Kiesewetter and Platas Barradas[92] and Whitford and Szafir.[93]

In contrast, single-use production facilities of the closed type and those operating in stations are fully equipped with single-use systems. While all materials in a closed-type single-use facility are transported from one process step to the next through pre-assembled and coupled single-use systems through free flow or pressure gradients, they are moved from one process step to the next using transportable containers in a single-use facility operating in stations.[90] Consequently, the single-use facility operating in stations can be considered the most flexible solution of the single-use facility types described.

The most common layouts for single-use biopharmaceutical production facilities described in the literature are currently the 'ballroom' and 'dance floor' concepts. The 'ballroom' concept[94] serves as a flexible room concept and uses large rooms of a low cleanroom classification with minimal segregation. The equipment is not fixed in place and can be changed depending on the process. This facility layout is seen more as a concept for large, established biotherapeutic manufacturers. However, it is considered less practical for contract development and manufacturing organizations.[95] The 'dance floor' concept is a modified 'ballroom' concept and allows the application of the 'ballroom' concept in existing facilities with minimal changes. In contrast to the 'ballroom' concept, it is smaller and more defined, spatially speaking. The 'dance floor' concept likewise has no permanently installed equipment and minimal segregation. The arrangement of the media and buffer preparation facilities are in close proximity to the manufacturing processes, and liquid transport usually takes place between rooms *via* a mousehole in the wall.

There is no doubt that the approval of a Shire facility in the year 2014 for the commercial production of veraglucerase alfa (VPRIV®) for the treatment of type 1 Gaucher disease using single-use bioreactors marked a turning point for the global acceptance of SUT.[96] However, in the first decade of the 21st century SUT was opportunistically used, *i.e.* single-use systems replacing existing solutions in existing facilities. Since 2015, multiple new biopharmaceutical facilities have been commissioned, which, in many cases, facilitate end-to-end production with SUT up to the aforementioned scales.[97–100] Most of these single-use facilities are based on the previously discussed layouts, while some others are based on modular constructions such as Cytiva's KUBio™ turn-key or G-CON's POD solutions.[91,101,102] As such, the design of a facility, *i.e.* the segregation of the different production areas, reflects a company's preferred mode of operation, whether it pursues a multiproduct or mono-product strategy, or clinical versus commercial production. Advantages and caveats of 'ballroom', 'dance floor' and room concepts with higher segregation have already been extensively discussed.[91,103–105]

5. Summary and Outlook

Single-use systems have become an integral part of modern biopharmaceutical production processes. They have proven their suitability for the production of pre- and clinical samples of biotherapeutics, with numerous suppliers offering solutions for USP, DSP through to fill & finish. Nevertheless, wherever there is a technological fit and the volume requirement is satisfactory, SUT is also utilized for commercial manufacturing. Although the largest selection of USP single-use equipment is designed for mammalian cell culture-based processes, which produce therapeutic monoclonal antibodies (mAbs), other therapeutic proteins, vaccines, and cell and gene therapies, single-use systems are also utilized, albeit less frequently, for the cultivation of microorganisms, plant cells, and insect cells. They are also the first choice whenever a product (typically a high-value product) needs to be produced quickly and safely in a small and medium volume range. Furthermore, there is an emerging trend toward the increased use of single-use systems for intensified and continuous production. In this context, specially designed equipment (*e.g.* bioreactors) has already been launched on the market to support targeted ultra-high cell densities and high product titers.

Swiss companies and universities have been pioneers in both the development and implementation of SUT. The first wave-mixed bioreactor, Kühner's OrbShake technology (developed in collaboration with EPFL Lausanne), TPP's TubeSpin® Bioreactors, and the first single-use centrifugal pump (Levitronix AG) were all designed and built in Switzerland. Today, all major biopharmaceutical companies based in Switzerland use SUT, not least to strengthen their leading positions in Europe and globally.

Some of these companies are even involved in the DECHEMA expert group 'Single-Use Technology for Biobased Applications.' In academia, the Zurich University of Applied Sciences has pioneered programs to train students on SUT alongside manufacturers of single-use systems, organizations such as BioPhorum, and conference organizers like ECI (Engineering Conferences International) and Concept Heidelberg. These organizations share the goal of familiarizing future users and professionals with the portfolio of single-use systems, their potential, their current limitations, and their efficient implementation in flexible production facilities. The ongoing improvement of single-use equipment (*e.g.* more robust films, increased standardization) and integration with digitalization, along with introducing non-pharma-grade solutions, will no doubt drive the adoption of SUT beyond the biopharmaceutical sector, while sustainability remains a key focus of scrutiny and development.

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Idea, D.R.S.; Conceptualization, D.R.S., D.E., and R.E.; investigation, D.R.S, D.E., and R.E.; writing—original draft preparation, D.R.S, R.E. and D.E.; writing—review and editing, D.R.S, R.E., and D.E.; visualization, D.E., and D.R.S.

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