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WHITE PAPER

Single-use technologies for the production of cell therapeutics

Table of Contents

1. Introduction 3
2. Differences to dominating biopharmaceutical production processes 4
3. Suspension cell-based autologous platforms: CAR-T cells as a case-study 6
    3.1. The manufacturing process 6
    3.2. Applicable SUTs 7
4. Manufacturing platforms for the expansion and differentiation of adherent stem cells 9
    4.1. MSC-based products 9
        4.1.1. Process overview 9
        4.1.2. Proven single-use systems 10
    4.2. iPSC-based products 11
        4.2.1. Single-use bioreactors applied towards iPSC expansion 13
        4.2.2. iPSC differentiation and applicable single-use systems 14
        4.2.3. Other similarities to MSC manufacturing 14
5. Process monitoring and quality control in ATMP manufacturing 15
6. Conclusion and outlook 19
7. Acknowledgements 19
8. References 20
9. Appendix 25
10. Authors 25

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1. Introduction

The focus of biomanufacturing is on the consistent production of complex therapeutics derived from biological sources to diagnose, prevent, and treat diseases. Aging global populations are associated with a rising incidence of chronic, difficult-to-treat, and often terminal conditions. Consequently, the field of biomanufacturing has gained considerable attention in recent years, prompting greater investment in research and development to address the growing need for innovative, accessible, and affordable treatment options [1], [2], [3].

Given this outset, it is no surprise that the biopharmaceutical market has remained highly lucrative, with its global value projected to surpass USD 1 trillion by 2030 at a compound annual growth rate of about 14 % [4], fueled by both established recombinant proteins and viral vector-based therapeutics (e.g., Neupogen, Avastin, Hemaagenix) and emerging cell-based therapeutics (e.g., Yescarta, Ryoncil).

While recombinant protein therapeutics continue to dominate the market [5], cell-based therapies offer highly innovative, potentially curative treatment options that can persist for years post-infusion [6]. However, these benefits come at a cost: therapeutic efficacy and overall resilience are closely tied to preserving the cells' mechanism of action and critical quality attributes (CQAs) throughout manufacturing [7], placing individual unit operations at the center of product quality and clinical outcome. Furthermore, as these therapies are often used as last-resort interventions, particular emphasis must be placed on timely and safe production if patient survival is to be ensured.

Undoubtedly, single-use technologies (SUTs) are well positioned to meet these requirements, as they have proven themselves in terms of safety [8], [9], biocompatibilty [10], and processing efficiency [9], [11]. Moreover, regulatory authorities worldwide increasingly recommend SUTs for cell therapy manufacturing [12]. Current SUT platforms span a wide range of applications, including storage, freezing, mixing, bioreactors, separation and filling systems, tubing assemblies, and even single-use needles. Building on their well-established use in monoclonal antibody (mAb) production and on process flow diagrams for selected cell therapies, this paper presents suitable single-use systems and platforms for cell therapy manufacturing. The primary focus lies on autologous chimeric antigen receptor (CAR)-T cell therapies, as well as both autologous and allogeneic stem cell therapies based on mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs).

Aimed at both newcomers and established stakeholders, this white paper also considers aspects of process monitoring and quality control in cell therapy production. The outlook section concludes with emerging trends in the application of SUTs, driven by the continued development and approval of new cell therapies.

2. Differences to dominating biopharmaceutical production processes

Therapeutic proteins (particularly mammalian cell-derived mAbs) have long dominated biopharmaceutical manufacturing. As a result, most commercially available SUTs have been developed and optimized for the production of such biologics, typically using Chinese hamster ovary (CHO) cell lines cultivated as single cells in suspension. These CHO production cell lines have undergone decades of in vitro evolution and now bear little resemblance to their ancestral counterparts. Their recombinant protein productivity has increased substantially, they no longer depend on adhesion, and they tolerate the often harsh hydrodynamic conditions encountered in conventional stirred bioreactors.

In contrast, many emerging cell-based therapies rely on viable primary cells derived from consenting donors, which serve both as the starting material and (sometimes in modified form) as the final therapeutic product. These cells are frequently adherent and exhibit limited proliferative capacity. Prolonged in vitro cultivation typically leads to senescence and may compromise genetic integrity, thereby reducing both product yield and quality. Consequently, the manufacturing of cell-based therapeutics generally requires substantially shorter process durations than mAb production, limiting both achievable scale and the range of applicable SUTs in favor of preserving cell quality and functional integrity.

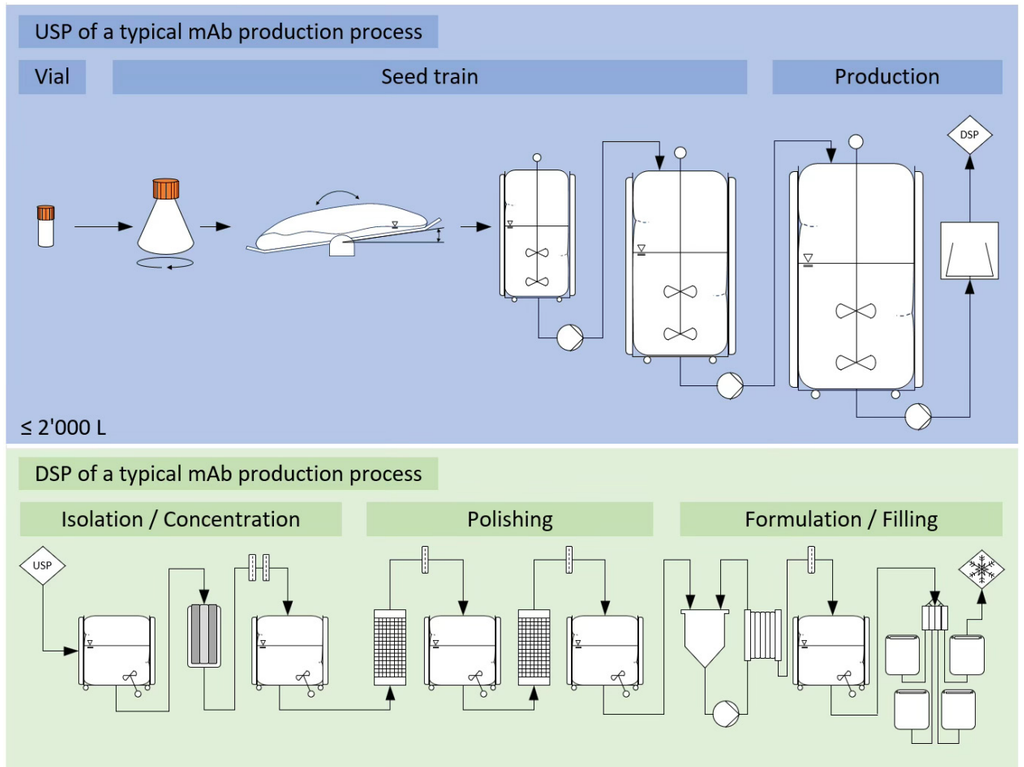

Figure 1: *Typical mAb production process conducted at 2'000 L scale using a single-use production bioreactor (modified after Anderlei et al. [16]). At this scale, the entire sequence of USP and downstream DSP operations, from inoculum preparation in shake flasks to aseptic filling of the drug substance, can be carried out exclusively using SUTs (see also Jossen et al. [15]). Not depicted in this illustration is the preparation and storage of the culture medium and buffers, which are required for both USP, DSP, as well as fill and finish of the drug product.*

To illustrate the differences in scale and manufacturing principle between the therapeutic modalities of mAbs and cellular therapeutics, we consider their manufacturing work flows in some detail starting with that of a mAb (Figure 1).

Conventional mAb production typically begins with thawing cells from a fully characterized working cell bank, which has been previously genetically engineered to express the target recombinant protein. Initial cell expansion may be carried out in shake flasks, followed by sequential transfer to larger-scale single-use wave-mixed or stirred-tank bioreactors within the seed train [9]. These cultivation systems allow for real-time monitoring and control of critical process parameters (such as pH, temperature, and nutrient concentrations) to maintain optimal growth conditions throughout the expansion phase. Alongside such cultivation systems, instrumented orbitally shaken cultivation vessels have also proven effective for pilot-scale cell expansion [9], [13], [14], and are available in working volumes of up to 2.5 m^3.

Regardless of the bioreactor type used, once the desired scale and cell density have been reached, cells are transferred from the seed train to the production bioreactor, where they express the drug substance over a period of 10–14 days [15]. Following the production phase, primary recovery (commonly via centrifugation and occasionally via depth filtration) then removes most cellular debris, marking the end of upstream processing (USP) and preparing the drug substance for subsequent downstream processing (DSP).

While USP focuses on maximizing product titer and separating intact cells from the bulk product, DSP aims to eliminate residual impurities arising from cell lysis, such as host cell protein and nucleic acid. Accordingly, key DSP steps include isolation and concentration of the active pharmaceutical ingredient (typically using protein A affinity chromatography for mAb capture) followed by viral inactivation (commonly achieved through a pH shift), cation and anion exchange chromatography for purification and polishing, and finally, virus filtration [15]. After purification, the drug substance is formulated and sterile filtered prior to aseptic filling into bulk containers. It is then frozen at –80 °C in single-use bags or containers in preparation for transport or further formulation and final filling (also known as fill and finish or fill-finish).

In contrast to mAb production, the manufacture of Advanced Therapy Medicinal Products (ATMPs), examples of which include CAR-T- and stem cell-based therapeutics, involves significantly fewer process steps and unit operations. This is partly due to the smaller USP scale, as batches are often produced for a single patient, and partly due to the absence of chromatographic purification steps, substantially shortening DSP. Furthermore, similar to cell production steps in mAb seed-train workflows that aim to maintain CHO cell viability and functionality prior to recombinant protein expression, cell therapy USP favors operation modes such as repeated-batch and perfusion cultivation, in which the culture medium is periodically or continuously exchanged [17], [18]. However, unlike CHO-based mAb processes, USP for cell therapeutics must also often accommodate adherent cell cultivation (one prominent exception being hematopoietic cells which naturally survive and proliferate in suspension [19]). Adherent cell types include MSCs derived from adipose tissue, bone marrow, or umbilical or placental tissue [20]; iPSCs reprogrammed from somatic tissues [21]; and many of their differentiated progeny [22], [23], [24]. This requirement adds a layer of complexity to the process, as failure to support cell attachment can trigger anoikis, a special form of apoptosis induced by a loss of cell-matrix interaction. This reduces both cell quantity and quality, which can render the product unsuitable for therapeutic use [25], [26]. To prevent anoikis, adherent cells intended for clinical administration should be cultivated on disposable attachment surfaces or scaffolds. Such surfaces include planar plastic substrates [27], polymer membranes [28], or spherical particles (also known as microcarriers) [29], all of which promote cell attachment, migration, and proliferation. This approach, known as scaffold- or carrier-supported cultivation, is especially prevalent in cell manufacturing processes that exceed benchtop-scale working volumes [30], [31], [32], [33], [34].

At smaller scales and under carefully optimized conditions, carrier-free suspension cultivation (which promotes the formation of dense, three-dimensional [3D] aggregates or spheroids) can serve as an alternative to scaffold-based approaches [35], [36], [37]. Here, suspended cells rely on intercellular adhesion and the expression of extracellular matrix proteins to form hydrodynamically resilient aggregates [38]. While this eliminates the need for artificial scaffolds, spheroid formation and size are strongly influenced by vessel

geometry and hydrodynamic profiles [39], often resulting in heterogeneous populations characterized by non-uniform sizes and variable cell numbers. Under suboptimal conditions, spheroids may become tightly packed, with diameters exceeding 300 µm [35], [39]. This exposes the cells contained within to a broad range of mechanical stimuli and restricts the diffusion of nutrients and oxygen. In addition, the accumulation of metabolic waste can result in a sharp decrease in the pH of the cellular microenvironment. Finally, suboptimal conditions can also prevent the spheroids from forming completely, which also significantly impairs cell quality [40]. Consequently, although numerous spheroid-based cultivation strategies have demonstrated success at benchtop scale [35], [41], [42], scaling these approaches to larger working volumes has proven challenging. For this reason, the present position paper places greater emphasis on carrier-based production processes.

3. Suspension cell-based autologous platforms: CAR-T cells as a case-study

Similar to conventional biopharmaceutical manufacturing processes relying on carrier-free cell lines, hematopoietic cells also possess the capacity to proliferate as single cells in suspension. As a result, SUTs originally developed for the production of recombinant proteins using suspension-adapted cell lines can, in principle, be repurposed for the manufacture of peripheral blood mononuclear cell (PBMC)-based therapeutics, such as CAR-T cells, with only minor modifications [18], [45], [46]. However, in contrast to CHO-based mAb processes, the primary goal of such therapies is to genetically modify a patient's own cells to enable long-term immune-mediated control of specific hematological malignancies and cancers [6].

To date, reported manufacturing processes rarely exceed working volumes of 1 L, as each batch is typically designed to treat a single donor or patient [47], [48], [49]. This personalized and autologous production approach significantly increases manufacturing costs [50]. Consequently, there is a growing trend toward designing flexible and functionally closed production platforms for CAR-T cell-based therapies, which have been shown to contribute to cost reductions [51].

3.1 THE MANUFACTURING PROCESS

Before choosing particular SUTs for the production of CAR-T cell therapeutics in accordance with Good Manufacturing Practice (GMP), a clear understanding of the manufacturing workflow is required. From a technical perspective, the production of autologous CAR-T cell therapeutics typically begins with the enrichment of leukocytes from a patient's or donor's whole blood at a medical facility using a procedure known as leukapheresis. The resulting cell mixture, composed primarily of leukocytes, is then cooled or cryopreserved for transport to the site where the therapeutic product will be manufactured [52].

Upon arrival at the manufacturing facility, if applicable, the cells are thawed and washed, after which a selection step is introduced to isolate the target cell population, which are typically $CD3^+/CD28^+$ or $CD4^+/CD8^+$ T cells (Figure 2). Once isolated, these T cells undergo several key processing steps. Namely, activation, to induce proliferation and maturation, transduction, where the gene encoding the CAR is introduced via viral or non-viral vectors, and expansion, during which the modified T cells are brought to proliferate to clinically relevant numbers. The process concludes with harvest, formulation, cryopreservation, and quality control, after which the final product is transported back to the medical facility for patient administration [50], [53].

3.2 APPLICABLE SUTS

While the clinical application of CAR-T cells is a comparatively recent development [54], the application of SUTs for the collection, processing, storage, and infusion of blood is well established. The first instance of single-use bags being used towards this purpose date back to 1947 and the development of the Thanksgiving Day blood bag by Carl Walter and later Fenwal laboratories [55]. Since then, substantial advances have been made in this area, with many modern devices

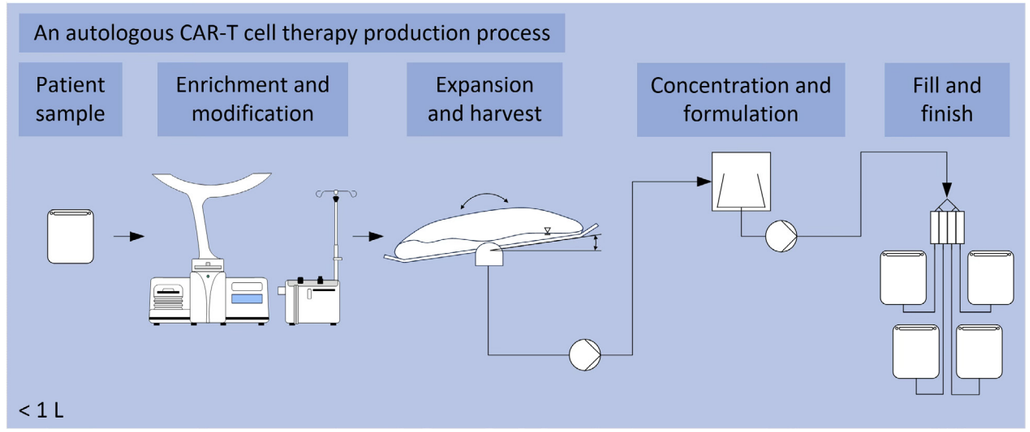

Figure 2: *Simplified overview of an autologous CAR-T cell therapy manufacturing process, typically spanning 7 to 14 days. Owing to the patient-specific nature of these therapies, working volumes rarely exceed 1 L.*

building on this original concept to support a wide range of integrated functions. This trend is particularly evident in autologous CAR-T cell therapy manufacturing, where integrated single-use platforms, such as Miltenyi Biotec's CliniMACS Prodigy® support the entire process, from cell selection to activation, genetic modification, expansion, formulation, and final fill and finish [51], [56]. Some SUTs serve more discrete roles, with a clear trend toward cross-platform compatibility and functionally closed processing.

For example, leukapheresis may be performed using Terumo BCT's COBE® Spectra Optia®, Fresenius Kabi's Amicus™ Apheresis Collection System, or Haemonetics' MCS®+ [57], [58], all of which utilize disposable processing kits. On the other hand, Cytiva's Sepax™ C-Pro cell processing instrument [59] is able to selectively enrich PBMCs via density gradient centrifugation, while more targeted isolation and enrichment of $CD3^+$ T cells is typically achieved through magnetic bead-based selection, using SUTs such as Thermo Fisher Scientific's DynaCellect™ [60], Miltenyi's CliniMACS® Plus [61], and Cytiva's Sefia Select™ [62].

To date, T cell activation has primarily been achieved either through soluble factors or via specially engineered single-use activation beads, the latter apparently inducing more sustained activation and stronger T cell responses [58]. Notably, some modified single-use beads may also serve a dual function, facilitating both T cell enrichment and activation, thereby integrating process steps and shortening the overall manufacturing workflow [60]. Devices which make use of single-use inserts and support the integration of these unit operations through the application of beads include Cytiva's Sefia Select™ system [62], Lonza's Cocoon® [46], and Thermo Fisher Scientific's DynaCellect™ [60].

It might be worth mentioning that T cell activation and transduction may also be performed directly within the cultivation vessels used for subsequent expansion to the target therapeutic dose [6], [63], typically estimated at 10^4 to 10^7 cells per kg body weight [48], [50], [64], thereby enabling integration of these unit operations into a single process step. Nevertheless, the scale and type of cultivation vessel needs to reflect the target cell titer and potency. Generally, the lower the potency of the product or the lower the final cell density, the larger the required working volume of the cultivation vessel. Therefore, depending on the characteristics of the product, this expansion step should be performed under either static or dynamic conditions.

SUTs which have been applied successfully towards the expansion of CAR-T cells include static platforms such as Wilson Wolf's G-Rex [64], Cytiva's Sefia™ expansion system [62], and Miltenyi Biotec's CliniMACS Prodigy® [51], [56], mentioned earlier, while dynamic conditions are provided by rocking or wave-mixed bioreactors, such as Lonza's Cocoon® [46], Sartorius' Biostat® RM TX [65], and Cytiva's Xuri™ cell expansion system W25 [64]. All systems utilize disposable components and offer

working volumes ranging from 5 mL to 5 L. Incidentally, while wave-mixed bioreactors are considered established in commercial CAR-T cell production, scalable single-use stirred-tank and perfusion-compatible bioreactors have also emerged as viable options in a research setting, alongside static and rocking cultivation vessels. Examples include Sartorius' Ambr® 15 [63] and Ambr® 250 [18] and Merck Millipore's Mobius® Breez Microbioreactor [66].

Once harvested, any residual solid components, e.g. activation beads, are removed and the CAR-T cells are concentrated and formulated [58]. During these steps, the cell density is adjusted to match the final dose, typically ranging from 10^6 to 10^9 cells mL^{-1}, and the spent medium is replaced with a suitable cryopreservation solution. This is followed by aseptic filling of the cell suspension into containers or bags, which are subsequently frozen for storage and transport to the site of administration; typically, quality control measures are also conducted at this stage [52]. Given that most cryopreservation formulations still contain dimethyl sulfoxide (DMSO), a cryoprotectant with known cytotoxicity, and that prolonged exposure degrades cell quality, these DSP operations are time-sensitive and should, where possible, be performed under cooled conditions (e.g. on ice) [7], [58]. Alternatively, DMSO exposure can be minimized through the application of comparatively less established DMSO-reduced or -free cryoprotectants or integrated downstream unit operations, which offer additional benefits such as reduced cytotoxicity or manual handling, shorter transition times between steps, and an overall acceleration of the production timeline. Alongside DMSO-free formulations, such as Merck's CRYO-DMSO-F or X-Therma's XT-Thrive® [68], several SUTs have been used to integrate the separation, concentration, and formulation steps by incorporating a continuous centrifugation component. Examples of such technologies include Cytiva's Sefia Select™ and Sepax™ C-Pro cell processing system [59], [62], Thermo Fisher Scientific's CTS™ Rotea™ Counterflow Centrifugation System [69], and Sartorius' kSep® [67].

The final step of CAR-T cell production involves fill and finish, alongside freezing the modified and expanded CAR-T cells away in cryopreservation containers, typically at temperatures below –80 °C [53]. Fill and finish systems used towards this purpose include Xiogenix's Ares™ series, Terumo BCT's Finia® Fill and Finish system [70], Sexton Biotechnologies Signata™ CT-5 system [58], as well as Cytiva's Sefia Select™ and Sepax™ C-Pro, while controlled-rate freezing and subsequent thawing may carried out using systems such as Cytiva's VIA Freeze™ and VIA Thaw™, Thermo Fisher Scientific's CryoMed™ [52], BioLife Solutions' ThawSTAR® series [69], and the Barkey plasmatherm [71].

GMP compliant single-use containers compatible with these systems include both rigid cassettes and flexible bags. Flexible single-use cryopreservation bags, designed to withstand ultra-low temperatures, are typically composed of ethylene vinyl acetate or fluorinated ethylene propylene and are available in filling volumes ranging from 6 to 1000 mL [72]. Common examples include Miltenyi Biotec's CryoMACS® Freezing Bags [73], Corning's Cryopreservation Bags, and Saint-Gobain's KryoSure® Cryopreservation Bags [74]. Alternatively, rigid cryopreservation vials and cartridges may be used instead, with filling volumes ranging from a few microliters to several liters in scale. Representative examples include Fisherbrand™ Cryogenic Storage Vials, Nalgene™ Cryogenic Tubes [53], and the CellSeal® range from Sexton Biotechnologies [72].

The SUTs now available for CAR-T cell manufacturing demonstrate that these products can be manufactured entirely using functionally closed single-use solutions. While this may be considered a substantial step forward, further development in terms of automation, connectivity, and data integration will be necessary in order to fully exploit the economic potential of single-use platforms for autologous CAR-T cell production, as described in the paper by Weber [51].

4. Manufacturing platforms for the expansion and differentiation of adherent stem cells

4.1 MSC-BASED PRODUCTS

Biomanufacturing of MSC-based ATMPs differs from that of CAR-T cells (see Section 3), particularly with regard to cultivation requirements, scalability potential, and cell handling approaches. In contrast to CAR-T cells, MSCs require attachment to a scaffold, matrix, carrier, or substrate for effective in vitro expansion [17], [75]. This growth surface can either be provided by static, two-dimensional (2D) cultivation systems, such well plates, T-flasks, and multi-layered stacked plate systems [76], or microcarriers when targeting higher cell numbers under dynamic conditions in stirred [30], [32] or wave-mixed bioreactors [77], [78], [79]. Another distinction pertains to the intrinsic therapeutic functionality of MSCs.These multipotent cells possess natural immunomodulatory properties, thereby eliminating the need for extensive genetic modification [75]. MSCs secrete a wide array of bioactive molecules involved in tissue repair, immune regulation, and anti-inflammatory responses [80], as well as extracellular matrix. Consequently, the manufacturing focus for MSC-based therapies is primarily on the controlled expansion of cells to achieve clinically relevant doses, rather than on complex cell engineering workflows.

The immunological characteristics of MSCs also facilitate their application in an allogeneic setting, where cells derived from a single healthy donor can be administered to multiple recipients without eliciting significant immune rejection [17], [48]. This allogeneic compatibility has profound implications for production scalability and logistics. Specifically, it facilitates the generation of substantial, well-characterized cell banks from a single donor source, thereby reducing batch-to-batch variability and streamlining regulatory compliance. Furthermore, it facilitates the implementation of large-scale single-use bioprocessing technologies. As indicated by Jossen et al. [81], bioreactors with working volumes reaching 200 L have been utilized for the expansion of MSCs, thereby emphasizing the necessity for industrial-scale infrastructure to facilitate widespread therapeutic implementation.

4.1.1 Process overview

Similar to CAR-T cell therapeutics, the manufacturing of allogeneic MSC-based products under GMP conditions involves a series of carefully controlled steps designed to ensure product quality, safety, and efficacy [81]. The process typically begins with the procurement of tissue (such as human bone marrow, adipose tissue,

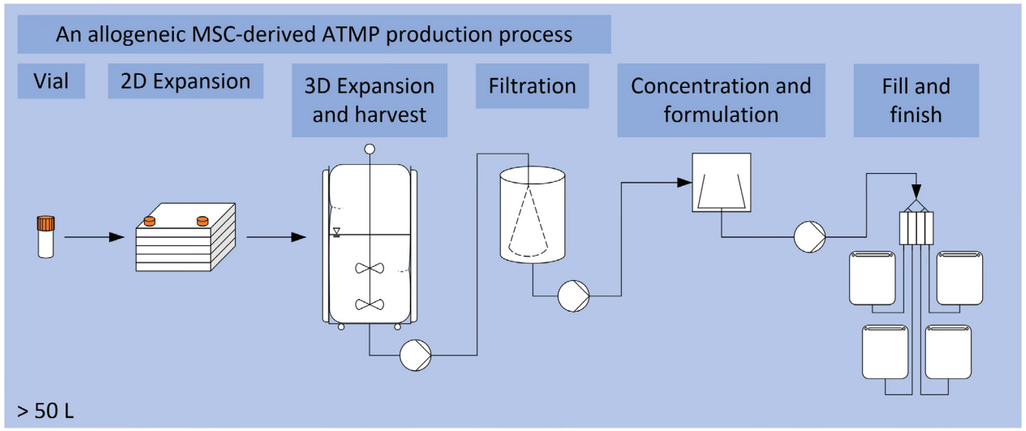

Figure 3: *A simplified workflow for the production of allogeneic cell therapies based in MSCs. Following cell isolation, the manufacturing process is typically divided into (i) expansion and harvest, (ii) filtration, (iii) concentration and formulation, and (iv) fill and finish. Adapted from Jossen et al. [81].*

or umbilical cord, among others [82]), followed by cell isolation, in vitro expansion, harvesting, and the establishment of a well-characterized cell stock, which serves as the foundation for subsequent ATMP production.

Initial isolation of MSCs typically involves enzymatic digestion, mechanical dissociation, or a combination of both to release cells from their tissue matrix [83]. Following isolation, MSCs are cultivated under xeno-free conditions, commonly based on human platelet lysate or, ideally, fully chemically defined medium formulations to eliminate the use of animal-derived components [17]. While single-use cryovials are frequently used for generating early-stage cell banks, cryopreservation bags, such as those applied in CAR-T cell workflows, may also be employed, particularly where scale or integration into closed systems is a priority.

Once appropriate cell stocks have been established, production of an MSC-based ATMP may begin in earnest. In line with CAR-T cell manufacturing, MSC production follows a structured workflow that typically starts with cell thawing, followed by 2D expansion, often carried out in T-flasks or multilayer stacked plate culture vessels [84], as illustrated in Figure 3. This initial expansion step supports rapid cell recovery and proliferation in a controlled adherent environment. Subsequently, cultures may be transferred into 3D single-use bioreactors for large-scale expansion, enabling production volumes exceeding 50 L [81]. These systems commonly employ polymer-based microcarriers, which act as a surface to which the cells attach during expansion but must be removed prior to or during DSP. Detachment of MSCs from microcarriers is typically achieved enzymatically or mechanically, often directly within the bioreactor [85], followed by filtration-based separation to remove intact carriers and residual debris during harvest. Microcarriers composed of biological polymers such as gelatin or dextran may offer advantages in this context, as they support robust MSC growth and can be enzymatically digested, thereby simplifying subsequent DSP [83].

DSP then continues with washing, concentration, and formulation steps, commonly performed using filtration systems or closed centrifugation platforms. These technologies enable buffer exchange and volume adjustment while preserving cell viability and structural integrity [86], [87], [88], [89]. As with other cell therapy products, the penultimate step, fill and finish, involves the aseptic dispensing of the formulated cell product into final containers, which are subsequently cryopreserved for transport and clinical administration.

Similar to CAR-T cell therapies, those administering MSCs cannot be terminally sterilized. Therefore, the entire manufacturing workflow, from source material handling through to final container closure, must be conducted aseptically. Furthermore, in contrast to CHO-based production systems, the limited proliferative capacity and inherent heterogeneity of MSCs preclude the routine generation of classical master and working cell banks. This limitation is acknowledged in the relevant European regulatory guidance for ATMPs, which instead refers to the use of cell stocks or cell preparations.

4.1.2 Proven single-use systems

Similar to CAR-T cell therapy manufacturing, MSC production processes often begin with cell revival from cryopreserved vials or bags, conducted under controlled and aseptic conditions using temperature-regulated thawing equipment, e.g. BioLife Solutions' ThawSTAR® system for cryovials or Cytiva's VIA Thaw platform for cryopreservation bags (see Section 3.2).

For the initial 2D expansion phase, multilayered cell culture systems such as Corning's CellSTACK® and Thermo Scientific's Nunc™ EasyFill™ Cell Factory™ are commonly utilized [90]. These disposable culture vessels are particularly well-suited for early-stage expansion due to their scalable surface area and compatibility with closed manifold tubing sets for both manual and automated media exchange and harvest operations. Automated platforms that integrate these disposable vessels as inserts include Sinfonia Technology's CellQualia™ and VivaBioCell's NANT XL System, both of which have been applied in clinical-grade MSC manufacturing [84], [91].

For clinical MSC production beyond 10 L scale, a range of single-use bioreactor systems have already demonstrated their technical suitability and have been successfully implemented in various manufacturing workflows [32], [92], [93], [94], [95] in addition to static multilayered cell culture systems. These include stirred-tank bioreactors such as Sartorius' BIOSTAT® STR, Eppendorf's BioBLU®, Merck's Mobius® CellReady, and Cytiva's Allegro™ STR; wave-mixed bioreactors such as the Sartorius BIOSTAT® RM and Cytiva Xuri™ cell expansion system W25; and vertical-wheel bioreactors from PBS Biotech®. From a process engineering perspective, microcarrier properties are particularly important for ensuring that hydrodynamic shear stress remains within acceptable limits while maintaining sufficient mixing and mass transfer throughout the cultivation process. In the Mobius® CellReady 50 L bioreactor, MSCs were successfully expanded on microcarriers, demonstrating robust proliferation while preserving key CQAs, including high viability, phenotypic identity, and trilineage differentiation capacity [32]. Similar outcomes were reported for the BIOSTAT® STR 50 L bioreactor (Sartorius), where microcarrier-based MSC expansion under serum-reduced conditions resulted in a 40-fold increase in cell number, yielding approximately 1×10^{10} cells with a viability exceeding 98% [30]. Beyond the cylindrical geometries of the Mobius® CellReady and BIOSTAT® STR systems, successful MSC expansion has also been demonstrated in the cubic-shaped Allegro™ STR bioreactor (former Pall), highlighting the suitability of microcarrier-based processes across different single-use vessel configurations [96]. In a comparative study, the wave-mixed Xuri™ cell expansion system W25 (Cytiva) supported efficient MSC expansion while maintaining phenotypic marker expression ($CD73^+$, $CD90^+$, $CD105^+$) and trilineage differentiation potential [78], [79]. Furthermore, the PBS Biotech® vertical-wheel bioreactor has been shown to support high-yield MSC expansion under low shear conditions, enabling the production of clinically relevant cell doses (10^8–10^9 cells) while maintaining excellent viability and functional properties [97], [98].

As stated in Section 4.1.1, MSCs need to be separated from microcarriers following (enzymatic) cell detachment. One example of a system designed for this purpose is the Harvestainer™ BioProcess Container from ThermoFisher Scientific, which features a bag-in-bag design: microcarriers are retained in the inner bag via a mesh-like filter, while the detached cells pass through into the outer chamber [81]. Alternative approaches for cell separation include the use of single-use normal flow and membrane filtration systems, such as Millipore's OptiCap® or Sartorius' Sartopore® filters, which are available with customizable pore sizes. For instance, Cunha et al. [94] employed OptiCap XL 1 capsules with a 75 µm pore size and a flow rate of 0.3 L min^{-1} to effectively separate cells from microcarriers. However, at larger scales and with higher microcarrier and cell densities, more advanced harvest solutions may be required to avoid membrane fouling. In such cases, alternating tangential flow or ATF filtration, as offered by Repligen, presents a compelling solution. This SUT has been shown to effectively reduce membrane fouling by maintaining continuous crossflow over the membrane surface - an approach recently demonstrated to be effective for the harvest of MSCs following microcarrier-based expansion [99]. Alternatively, continuous flow centrifugation systems, such as Sartorius' kSep® [100] and Thermo Fisher Scientific's CTS™ Rotea™ Counterflow Centrifugation System [101], may also be used for both clinical and commercial manufacturing. Notably, both alternating tangential flow filtration and counterflow centrifugation systems can be seamlessly integrated into DSP workflows for subsequent washing, concentration, and buffer exchange steps [101]. In addition, tangential flow filtration or TFF platforms such as Repligen's KrosFlo® offer precise control of shear stress, enabling the preservation of high cell viability during these critical processing stages [86].

The final stage of the manufacturing process involves aseptic dispensing of the cell suspension into final containers, typically performed using single-use filling systems. Notable examples include the Single Use Supports's RoSS.FILL CGT, Comecer's ValueCell FILL, Watson-Marlow's Cellefill™, Merck's Mobius® Final Fill Solutions, and Terumo BCT's FINIA® Fill and Finish System. These platforms support accurate volumetric filling of single-use vials, intermediate containers, or final product bags, while ensuring closed-system processing, minimal human intervention, and sterility assurance.

4.2 IPSC-BASED PRODUCTS

Similar to MSC-based therapies, ATMPs derived from iPSCs are increasingly being applied in clinical settings. However, iPSC-based manufacturing workflows differ from those of MSC-based products in several important respects, reflecting both the unique biological properties of the starting material and the inherently multistage nature of iPSC-based production.

Fundamentally, iPSCs do not themselves constitute therapeutic products. Rather, they are generated from somatic cells via the Yamanaka reprogramming protocol or its derivatives [102], [103]. This reprogramming, preferably achieved using non-integrating vectors [104], [105], restores pluripotency and confers an apparently

unlimited proliferative capacity, similar to embryonic stem cells (ESCs). In contrast to ESCs, however, iPSCs are derived from adult tissue and are therefore widely regarded as ethically non-contentious. These properties make iPSCs attractive enabling intermediates from which a broad range of therapeutic cell types can be derived.

While their proliferative capacity may facilitate the creation of well-characterized master and working cell banks and support manufacturing at scale, it also introduces a critical safety challenge. Undifferentiated iPSCs can form teratomas if administered, with as few as 130–630 residual cells reported to induce tumor formation [106], [107]. Consequently, iPSC-based manufacturing processes must incorporate robust safeguards to guide differentiation toward the intended lineage and to ensure the effective elimination of undifferentiated cells. Given these considerations, iPSC-based manufacturing can be structured into two distinct phases: (i) an expansion phase, analogous to a seed train, during which sufficient iPSC starting material is generated; and (ii) a directed differentiation or production phase, in which the iPSCs are differentiated to confer functionality and yield the therapeutic cell product. This second phase may also incorporate DSP elements or purification strategies aimed at removing residual undifferentiated iPSCs to ensure patient safety. Finally, given the importance of verifying iPSC quality prior to initiating the production phase and the fact that one-step or direct inoculation from cryopreserved iPSCs has been successfully demonstrated [100], [108], [109], this two-phase manufacturing workflow may be strategically decoupled through the establishment of an intermediate, high-density iPSC cell bank (see Figure 4), thereby reducing manufacturing complexity and the overall SUT footprint.

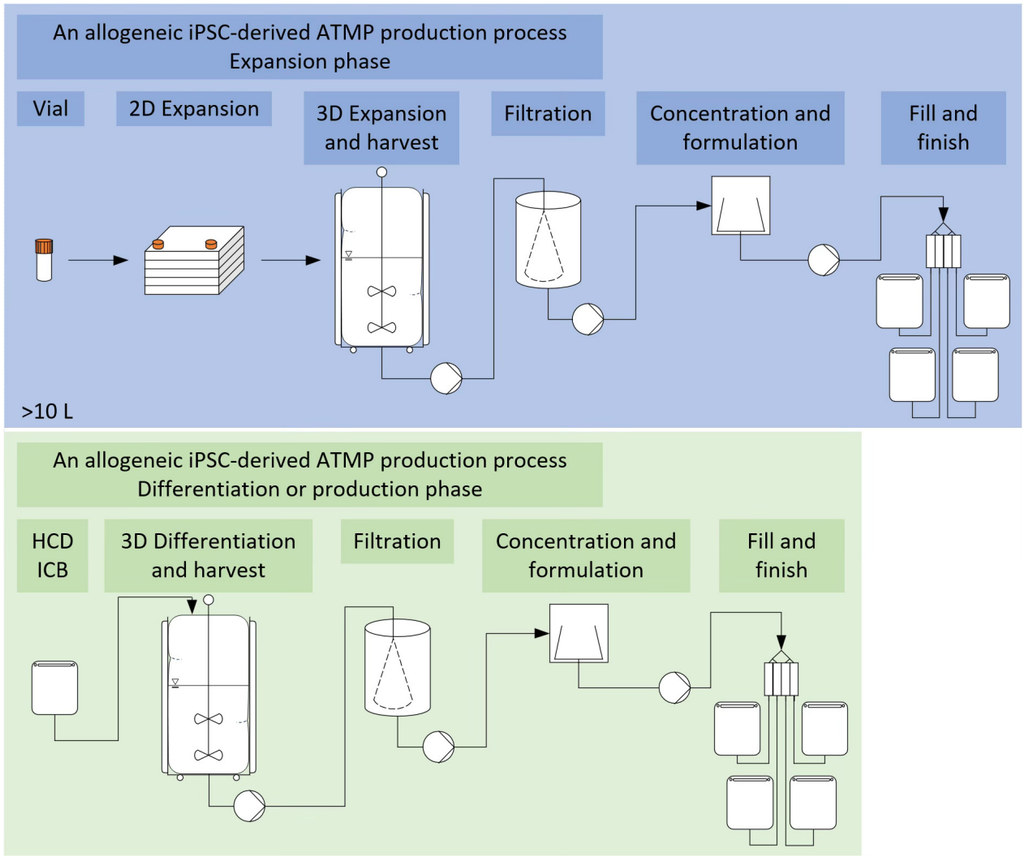

Figure 4:A simplified workflow for the production of an iPSC-based cell therapy product. Following cell isolation/ selection and reprogramming, the manufacturing process is typically divided into (i) expansion and harvest, (ii) filtration, (iii) concentration and formulation, and (iv) fill and finish. Given that iPSCs do not constitute a final cell therapy product, the same process steps must again be applied to their differentiation, ideally following cell inoculation from a high cell density intermediate cell bank (ICB) generated towards the end the expansion phase. Adapted from various sources [48], [110].

4.2.1 Single-use bioreactors applied towards iPSC expansion

As adherent cells, iPSCs share certain cultivation requirements with MSCs, allowing many of the SUTs established for MSC cultivation to be directly to the expansion of iPSCs (see Table 1). One requirement particular to iPSCs is that of a more stringent need for cell-cell contact. To date, reported working volumes for iPSC expansion range from 15 mL to 50 L [43], [111], with PBS Biotech's Vertical Wheel™ PBS-80 recently demonstrating the ability to produce more than 2×10^{11} cells within 7 days.

At these scales, iPSCs are typically cultured under carrier-free conditions, i.e. as cell clusters or spheroids, with perfusion systems employed to accommodate continuous medium exchange [41], [43], [100], [112].

Table 1: *Instrumented SU bioreactors used for the expansion of iPSCs. Listed are approximate maximum cell yields per cultivation, the expansion factor achieved and cultivation duration. The expansion factor was calculated as the ratio between the maximum number of cells reached and the number used for inoculation. Adapted from Teale et al. [17]*

Working principle	V_L range	Bioreactor	Vendor	Cultivation mode	Cultivation approach	X_{Max} [×10⁶ cells]	EF (Day)	Reference
Mechanically-driven: Stirred	15–250 mL	ambr® 15	Sartorius	Not specified	Carrier-free	Not specified	- (4)	[111]
		BioBLU® 0.3c SU	Eppendorf	Perfusion	Carrier-free	713	5.7 (7)	[113]
					Carrier-dependent	1 600	123.5 (6)	Own data
		Vertical-Wheel™ PBS-0.1	PBS Biotech	Repeated batch	Carrier-free	187.5	93.8 (5)	[37]
		BioBLU® 1c SU	Eppendorf	Perfusion	Carrier-dependent	3 250	25.5 (5)	[112]
	0.4–10 L	BioBLU® 3c SU	Eppendorf	Perfusion	Carrier-dependent	5 640	62.6 (9)	[100]
		Mobius® 3L SU	Merck	Repeated batch	Carrier-free	5 970	10 (7)	[114]
		Vertical-Wheel™ PBS-3	PBS Biotech	Repeated batch	Carrier-free	472*	47.2 (6)*	[115]
		Xcellerex™ XDR-10	Cytiva	Perfusion	Carrier-free	10 200	6.7 (6)*	[41]
	15–50 L	Vertical-Wheel™ PBS-80	PBS Biotech	Perfusion	Carrier-free	200 000*	80 (7)*	[43]
Mechanically-driven: Oscillating	0.5–1 L	Xuri™ Cellbag™	Cytiva	Not specified	Carrier-free	-	-	[116]
Hydraulically-driven: Multiplate, Hollow-fiber, and fixed-bed	0.5–3.5 L	Xpansion® 10	Cytiva	Perfusion	Scaffold-dependent	4 100	35.3 (5)	[117]
		Quantum® Cell Expansion System	Terumo BCT	Perfusion	Scaffold-dependent	690	14 (6.5)	[21]
		Ascent 1 m²	Corning	Perfusion	Scaffold-dependent	4 600	19.5 (5)	[117]

* Values approximated from publication figures. Abbreviations: Expansion factor (EF), working volume (V_L), maximum reported cell yield (X_{Max})

Perfusion rates applied to iPSC expansion are comparable to N-1 perfusion processes used in CHO-based mAb production, reaching dilution rates of up to 7 vvd [35], [118]. However, iPSC cultures have typically been kept at cell densities $< 3.5 \times 10^7$ cells mL^{-1} to preserve viability and biological function. It is also worth noting that operation in perfusion mode is not confined solely to stirred systems. Scalable hydraulically driven bioreactor designs capable of operation in perfusion mode have also demonstrated promise when applied towards iPSC expansion, such as Cytiva's Xpansion® multiplate bioreactor (offering up to 12.2 m² surface area) and Corning's Ascent® fixed-bed bioreactor (currently available with a 100 m² fixed-bed, with a 1000 m² model in development) [117], [119].

4.2.2 iPSC differentiation and applicable single-use systems

In contrast to expansion processes, reported differentiation workflows have remained largely confined to mL-scale [120], [121], [122], with only a few reports describing L-scale operation [23]. This limitation stems largely from the high cost of differentiation media and the extended cultivation times required to achieve complete lineage commitment [123]. Single-use systems currently used for small-scale differentiation include orbitally shaken systems (e.g., shake flasks, CERO 3D) and stirred systems such as the Vertical Wheel™ PBS-0.1 and BioBLU® 0.3c [124], [125]. Although larger-scale counterparts of these devices are commercially available, their suitability for robust and efficient differentiation across diverse iPSC lineages has yet to be fully demonstrated.

As with MSC-based processes, both mechanical and biological cues during cultivation play a decisive role in determining iPSC fate and product quality. Shear stress, in particular, has been shown to influence differentiation efficiency and lineage specification [126] suggesting that bioreactor design and operational parameters may need to be optimized for each target product individually. Other mechanical stimuli known to impact cell fate include surface stiffness [127]. Given that iPSC differentiation has been demonstrated for processes operated with carriers or scaffolds, such as for the generation of MSCs or red blood cells [128], [129], as well as under scaffold- or carrier-free conditions, for the production of cardiomyocytes and macrophages [124], [130], this aspect will likely prove equally important when developing scalable differentiation processes.

Alongside mechanical stimuli, biological cues are also leveraged to direct iPSC differentiation. This is typically achieved by supplementing the culture medium with specific inhibitors or activators, with the composition dynamically adjusted as cells progress through defined developmental stages [23]. Medium composition may also be used to apply selective pressure and eliminate undifferentiated iPSCs in the bioreactor prior to harvest [27]. However, while complete medium exchange is relatively straightforward at mL-scale, it becomes technically challenging at L-scale and beyond, making perfusion-based operation an attractive alternative. Single-use perfusion technologies originally developed for CHO cell culture [131] and successfully applied to CAR-T cell and MSC processes [49], [99], such as Repligen's XCell™ ATF and KrosFlo® TFDF® systems, may likewise prove valuable for facilitating continuous medium exchange during directed differentiation.

4.2.3 Other similarities to MSC manufacturing

Adequate DSP remains a critical component in ensuring product purity and patient safety. This is particularly important for iPSC-based cell therapies, where the removal of residual undifferentiated cells is essential to mitigate the risk of teratoma formation. Although purification may already begin within inside differentiation bioreactor through the application of selective pressures, such as cell type-specific biological pathways, surface coatings, media composition, or differences in aggregation behavior, dedicated SUTs capable of selective purification (comparable to the chromatography or filtration technologies established in mAb production) are still notably absent in DSP. Consequently, this stage of manufacturing is characterized by considerable developmental potential.

For the remaining stages of iPSC-based manufacturing, many of the SUTs applied to MSC workflows continue to be applicable. Similar to MSC products, iPSCs have been concentrated to cell densities exceeding 1×10^8 cells mL^{-1}, washed, and formulated using counterflow centrifugation in fluidized-bed systems such as Sartorius' kSep platform prior to cryopreservation [100]. Such processing is not only well suited for the long-term storage of intermediate products but has also been shown to support subsequent intensified iPSC expansion in carrier-based processes [108]. The same applies to aseptic filling operations. Strategies established for MSC-based products are equally relevant for iPSC-derived therapies [132]. However, it

has been shown that for iPSCs, filling should ideally be performed at 4 °C and in the presence of antioxidants such as N-acetylcysteine to maintain cell quality [110]. A more detailed description of appropriate single-use filling systems is given in Section 4.1.2.

5. Process monitoring and quality control in ATMP manufacturing

At the center of every biopharmaceutical manufacturing process lies the quality target product profile or QTPP, which serves as the foundation for ensuring product quality through process design. For ATMPs, where the cells themselves constitute the product, this principle also applies. However, unlike conventional biopharmaceuticals, where the CQAs are largely defined by protein structure and purity, the therapeutic entity in ATMPs is a living and extraordinarily complex cell. As a result, complete product characterization is not possible, and thus the quality target product profile often remains only partially defined [47]. Moreover, these cellular products are highly sensitive to variations in the culture environment, demanding precise control of physical, chemical, and biological parameters throughout the manufacturing cycle.

To address this complexity, process analytical technologies (PAT) and on-line monitoring tools are increasingly being deployed to facilitate process automation and provide real-time insight into critical process parameters or CPPs. Their integration reduces reliance on off-line sampling, lowers contamination risk, and facilitates the development of closed and automated bioprocesses that are required for safe, consistent, and GMP-compliant manufacturing. For ATMPs, these capabilities are particularly desirable, as small production scales, short shelf lives, and patient-specific batches leave little margin for error. A single batch failure can have severe, potentially fatal consequences, given the long manufacturing lead times and the often fragile condition of the patients awaiting treatment. A key enabler of PAT implementation in ATMP manufacturing has therefore been the development of single-use sensors. Today, devices for monitoring critical parameters such as pH, dissolved oxygen (pO_2), dissolved carbon dioxide (pCO_2), and biomass (via capacitance) are available in single-use formats, typically supplied pre-calibrated and gamma-sterilized together with the bioreactor [133]. Commercial solutions include pH, pO_2, and pCO_2 sensors from Presens, Hamilton, Mettler, Broadley James, Polestar, and Pyroscience, as well as capacitance- or turbidity-based biomass monitoring systems from ABER Instruments and Hamilton.

Table 2 summarizes the range of available sensors and analytical techniques used to monitor physical, chemical, and biological process parameters. Physical variables such as temperature, stirring, and pressure are routinely measured with robust sensors including thermistors, torque meters, and pressure transducers, while foam and liquid levels can be tracked using capacitance, optical, or ultrasonic probes. Chemical monitoring commonly relies on electrochemical or optical sensors for pH, pO_2, and pCO_2, and is further supported by spectroscopy (NIR, MIR, Raman, UV-VIS) and enzymatic biosensors to track nutrients and metabolites. Together, these tools enable direct and indirect monitoring of critical substrates such as glucose, lactate, and ammonia, which significantly impact cell growth and product quality [40].

By contrast, biological monitoring remains an area with significant potential for development. While single-use capacitance (dielectric spectroscopy) and optical methods already provide real-time information on CQAs such as viability and cell quantity, advanced analytical platforms, including those based on flow cytometry, imaging, immunoassays, and sequencing, are still required to assess identity, potency, genomic integrity, purity, sterility, and stability (see Table 3) [75], [117], [134]. These methods are predominantly applied at- or off-line, with single-use alternatives remaining largely unavailable. This may be attributed to the fact that integrating such analytical systems into closed single-use processes remains technically challenging.

Table 2: Overview of available sensors and techniques for process monitoring and control. (*) Technologies are available as SUT.

Variable type	Bioprocess parameter	Technique
Physical	Temperature (*)	» Thermistor / RTD probes » Wireless capsule sensors
	Power input / Stirring	» Torque measurement » Tachometer / Revolution-counter
	Pressure (*)	» Strain gauge transducers » Capacitive sensors » Piezoresistive MEMS sensors » Wireless capsule sensors
	Foam (*)	» Conductivity / capacitance probes » Optical (IR / laser) » Ultrasonic / acoustic » Camera-based (R&D)
	Level / Volume (*)	» Load cells » Hydrostatic pressure » Ultrasonic
Chemical	pH (*)	» Electrochemical (glass / ISFET) » Optical (fluorescent patches) » Wireless capsule
	Dissolved Oxygen (pO2) (*)	» Amperometric (Clark-type) » Optical (luminescent quenching)
	Dissolved CO2 (pCO2) (*)	» Severinghaus-type (electrochemical) » Optical absorbance » Optical fluorescence quenching
	Glucose (*)	» Enzymatic biosensors » UV-VIS, NIR, MIR, Raman spectroscopy
	Lactate (*)	» Enzymatic biosensors » UV-VIS, NIR, MIR, Raman spectroscopy
	Ammonia	» Ion-selective electrodes » NIR, MIR, Raman spectroscopy » Fluorescence spectroscopy
	Other metabolites (Amino acids, glutamine, glutamate)	» Raman / NIR with chemometrics » At-line HPLC/LC-MS
Biological	Cell count / morphology (Cell density and viability, viable cell volume) (*)	» Dielectric spectroscopy (capacitance) » Light scattering (turbidity) » Absorption (turbidity) » Combined light scattering / absorption (turbidity) » At-line fluorescence probes » At-line flow cytometry / imaging
	Proteins (e.g., LDH)	» MIR, Raman spectroscopy » At-line immunoassays
	Contaminants (microbial)	» Raman fingerprinting

Table 3: *List of CQAs for CAR-T cells, MSCs and iPSCs designated for cell therapy application, including acceptance criteria and how quantification may be realized. Adapted from various sources [58], [117], [135], [136], [137], [138], [139], [140]*

Property	Assay	Method	Acceptance criteria		
			CAR-T cells	**MSCs**	**iPSCs**
Quantity	Cell count	Microscopy, staining, or capacitance			
Viability	Live/dead cell ratio	Staining	> 70 % based on post-revival datasets	≥ 70–80 % depending on the jurisdiction	> 60 %
		Viability assay kit			
		Flow cytometry			
Identity	Morphology	Microscopy	Spherical	Fibroblast-like	Epithelial-like
			5 distinct morphologies identified, namely regular, activated, apoptotic, bi-nucleated, and mitotic	Spindle- or slightly star-shaped	High nucleus-to-cytoplasm ratio
			Grow as single cells in suspension	Large cell nuclei with large, dark granules	Underdeveloped mitochondria
				Adhere to plastic	
				Grow adherently	Grow adherently or as spheroids
			Display motility	Display motility	Do not display significant motility
	Marker profile	Flow cytometry	CAR^+, $CD3^+$, $CD4^+$ $CD8^+$, $HLA\text{-}DR^+$ $PD1^-$, $LAG3^-$, $TIM3^-$, $CTLA4^-$, $CD25^-$, $CD57^-$, $CD69^-$	$CD73^+$, $CD90^+$, $CD105^+$ (≥ 95 %) $CD45^+$, $CD34^+$, $CD14^+/CD11b^+$, $CD79\alpha^+/CD19^+$ and $HLA\text{-}DR^+$ (≤ 2 %)	Any positive intra- and extracellular marker (> 70 %) SSEA-1 (< 5 %)
	Genotype	Single tandem repeat genotyping	Identical to donor	Identical to donor	Identical to donor
	Differentiation capacity	Staining or flow cytometry	Unipotent. Restricted to the T cell lineage.	Multipotent. Differentiate into adipocytes, chondroblasts, and osteoblasts	Pluripotent. Differentiate into endo-, meso-, and ectodermal tissues

This table makes no claim to be complete and only highlights CQAs frequently associated with CAR-T cells, MSCs, and iPSCs. iPSCs do not constitute a final therapeutic product but rather function as enabling intermediates for a broader portfolio of " off-the-shelf" cell therapies, such as MSCs and T cells. For this reason, their CQAs are also listed. Abbreviations: Cluster of differentiation, chimeric antigen receptor (CAR) T cell, (CD) critical quality attribute (CQA), induced pluripotent stem cell (iPSC), mesenchymal stem cell (MSC), Limulus amebocyte lysate (LAL), polymerase chain reaction (PCR), reverse transcriptase (RT).

Table 3: Continued

Property	Assay	Method	Acceptance criteria		
			CAR-T cells	**MSCs**	**iPSCs**
Potency	Cytotoxicity*	LDH release, cytokine release, or microscopy	Target-directed cytotoxic activity and target-induced IFN-γ secretion		
	Paracine secretion**	High-pressure liquid chromatography or enzyme-linked immunosorbent assay		Significant cytokine expression or kynurenine production following the induction of IDO pathway with IFN-γ	
	Capacity to differentiate to all germ layers***	Embryonic body formation or directed differentiation assay			Demonstrate the presence of all three layers.
Genomic integrity	Karyotyping	G-banding	Absence of chromosomal abnormalities	Absence of chromosomal abnormalities	Absence of chromosomal abnormalities
	Residual vector testing*	RT-qPCR	Target vector copy number		≤ 1 plasmid copy per 100 cells
	Single nucleotide polymorphism*	Single nucleotide polymorphism genotyping			High-resolution alternative to karyotyping
	Other cancer-associated panels*	Whole genome or whole exome sequencing			No cancer-specific mutations
Purity	Endotoxin detection	LAL Assay	No activity (clotting, discoloration, etc.)	No activity (clotting, discoloration, etc.)	No activity (clotting, discoloration, etc.)
Sterility	Microbial-related	Direct inoculation method	Negative (No increase in turbidity)	Negative (No increase in turbidity)	Negative (No increase in turbidity)
	Mycoplasma-related	Real-time PCR	Negative	Negative	Negative
	Virus-related	RT-qPCR	Negative	Negative	Negative
Stability	Storage-period related	Change in all of the above over time	No change	No change	No change

* Applies to CAR-T cells only. ** Applies to MSCs only. *** Applies to iPSCs only

This table makes no claim to be complete and only highlights CQAs frequently associated with CAR-T cells, MSCs, and iPSCs. iPSCs do not constitute a final therapeutic product but rather function as enabling intermediates for a broader portfolio of " off-the-shelf" cell therapies, such as MSCs and T cells. For this reason, their CQAs are also listed. Abbreviations: Cluster of differentiation, chimeric antigen receptor (CAR) T cell, (CD) critical quality attribute (CQA), induced pluripotent stem cell (iPSC), mesenchymal stem cell (MSC), Limulus amebocyte lysate (LAL), polymerase chain reaction (PCR), reverse transcriptase (RT).

Finally, for effective PAT implementation, these analytical measures must be embedded within an automation framework, if manual intervention is to be minimized and continuous process control is to be ensured. Ideally, each unit operation (such as those illustrated in Figure 4) should operate in a fully automated and continuously monitored manner to safeguard the quality of the final product. This level of automation is already achievable for individual process units; e.g., commercially available single-use bioreactor systems suitable for cell therapy manufacturing (see Table 1).

However, achieving a fully automated end-to-end workflow from cell expansion to final fill and finish, including processing steps such as routine medium exchange, cell concentration, and formulation, requires the interconnection of these individual units operations. Such integration would not only enable seamless communication across the entire manufacturing chain, but also closed and sterile processing and the uninterrupted flow of product, improving product quality control and enhancing data integrity for batch release.

The primary challenge to interconnected process automation lies in the fact that different units often rely on different operating systems and communication interfaces. Several approaches exist to address this issue: (i) integrated suppliers, who offer pre-connected, unified process streams; (ii) signal standardization, where devices communicate using basic interfaces such as I/O or 4–20 mA signals, though with limited data transfer capacity; and (iii) protocol standardization, the most advanced yet most demanding solution, in which integration is achieved through unified communication protocols, such as the Open Platform Communications Unified Architecture (OPC UA), enabling flexible manufacturing and true interoperability between the various operating units.

By combining SUTs with multi-parameter on-line monitoring and advanced data analytics which implement such standardized protocols, manufacturers can move toward real-time release testing and predictive control strategies [141]. Early implementations of such approaches are already emerging, including the use of Raman fingerprinting for the rapid detection of microbial contaminants and impurities (enabled in some cases by in-situ measurements using optical interfaces, such as the SCHOTT ViewCell™), stem cell differentiation monitoring, and extracellular vesicle purification [142], [143]. Consequently, such integration has been shown to strengthen process robustness, scalability, and cost-effectiveness, all prerequisites for ensuring the commercial viability and broader accessibility of ATMPs.

6. Conclusion and outlook

Cell therapies have emerged as a major driver of innovation in biopharmaceutical manufacturing, even though mAbs continue to represent the leading product class. SUTs are increasingly important in mAb and cell therapy manufacturing, shaping both R&D and commercial production. However, with respect to production scale, commercially available single-use systems are better matched to the manufacturing of autologous and allogeneic cell therapies than mAbs.

It is therefore unsurprising that our analyses show that developers and manufacturers of cell therapies already make extensive use of SUTs; however, manual operations, such as encountered when using stackable tray systems, still frequently dominate current processes. Meanwhile, the pressure to increase efficiency and reduce costs is accelerating the adoption of allogeneic approaches and intensified upstream processes in both CAR-T cell and stem cell-based therapies. This has given rise to a clear trend toward modular, automated, and closed systems that enable perfusion, as well as real-time monitoring and process control.

We wish to emphasize that the current state of the field already represents a remarkable technological achievement, enabling the cost-effective manufacture of therapies that, until recently, would have been considered unattainable. Despite these advances, important technological gaps remain. Single-use sensor technologies (e.g., those used to monitor cell density and viability) require further development, and new solutions are needed for single-use equipment in cell purification, which relies on fundamentally different technologies than those established for mAb manufacturing. A further open question concerns whether existing single-use centrifugation technologies, perfusion devices, and disposable fluid-management components are sufficient to meet the demands of large-scale allogeneic cell therapy production. Nevertheless, we anticipate that continued innovation in the coming years will address these challenges and enable the manufacture of increasingly complex and transformative therapeutic modalities.

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8. References

[1] I. Soerjomataram and F. Bray, 'Planning for tomorrow: global cancer incidence and the role of prevention 2020–2070', Nat. Rev. Clin. Oncol., vol. 18, no. 10, pp. 663–672, Oct. 2021, doi: 10.1038/s41571-021-00514-z.

[2] J. Zhu et al., 'Temporal trends in the prevalence of Parkinson's disease from 1980 to 2023: a systematic review and meta-analysis', Lancet Healthy Longev., vol. 5, no. 7, pp. e464–e479, Jul. 2024, doi: 10.1016/S2666-7568(24)00094-1.

[3] M. S. Khan, I. Shahid, A. Bennis, A. Rakisheva, M. Metra, and J. Butler, 'Global epidemiology of heart failure', Nat. Rev. Cardiol., vol. 21, no. 10, pp. 717–734, Oct. 2024, doi: 10.1038/s41569-024-01046-6.

[4] D. Pandey, 'Biopharmaceuticals Market Size to Hit USD 1796.21 Billion by 2034', Precedence Research. Accessed: Feb. 18, 2025. [Online]. Available: https://www.precedenceresearch.com/biopharmaceutical-market

[5] D. Clarke, K. Cordovado, R. Jacquemart, P. Priebe, and S. R. Schmidt, '22nd Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production', Bioplan Associates, 22, 2025. Accessed: Sep. 28, 2025. [Online]. Available: https://www.bioplanassociates.com/22nd/

[6] G. López-Cantillo, C. Urueña, B. A. Camacho, and C. Ramírez-Segura, 'CAR-T Cell Performance: How to Improve Their Persistence?', Front. Immunol., vol. 13, Apr. 2022, doi: 10.3389/fimmu.2022.878209.

[7] C. Cottle et al., 'Impact of Cryopreservation and Freeze-Thawing on Therapeutic Properties of Mesenchymal Stromal/Stem Cells and Other Common Cellular Therapeutics', Curr. Stem Cell Rep., vol. 8, no. 2, pp. 72–92, Apr. 2022, doi: 10.1007/s40778-022-00212-1.

[8] M. Ottinger, I. Wenk, J. Carvalho Pereira, G. John, and S. Junne, 'Single-Use Technology in the Biopharmaceutical Industry and Sustainability: A Contradiction?', Chem. Ing. Tech., vol. 94, no. 12, pp. 1883–1891, 2022, doi: 10.1002/cite.202200105.

[9] D. R. Schmidhalter, D. Eibl, and R. Eibl, 'Single-Use Technology Today – A Cornucopia of Applications', CHIMIA, vol. 79, no. 5, Art. no. 5, May 2025, doi: 10.2533/chimia.2025.312.

[10] I. Pahl et al., 'Assessing biologic/toxicologic effects of extractables from plastic contact materials for advanced therapy manufacturing using cell painting assay and cytotoxicity screening', Sci. Rep., vol. 14, no. 1, p. 5933, Mar. 2024, doi: 10.1038/s41598-024-55952-3.

[11] D. R. Schmidhalter, A. C. Schmidt, S. Beck, and S. A. Morgenstern-Egli, 'A Strategic Approach to the Management of Single-Use Technology', Chem. Ing. Tech., vol. 94, no. 12, pp. 1902–1913, 2022, doi: 10.1002/cite.202200146.

[12] D. E. S. Nogueira, J. M. S. Cabral, and C. A. V. Rodrigues, 'Single-Use Bioreactors for Human Pluripotent and Adult Stem Cells: Towards Regenerative Medicine Applications', Bioengineering, vol. 8, no. 5, Art. no. 5, May 2021, doi: 10.3390/bioengineering8050068.

[13] X. Zhang et al., 'Efficient oxygen transfer by surface aeration in shaken cylindrical containers for mammalian cell cultivation at volumetric scales up to 1000 L', Biochem. Eng. J., vol. 45, no. 1, pp. 41–47, Jun. 2009, doi: 10.1016/j.bej.2009.02.003.

[14] X. Zhang et al., 'Use of Orbital Shaken Disposable Bioreactors for Mammalian Cell Cultures from the Milliliter-Scale to the 1,000-Liter Scale', in Disposable Bioreactors, R. Eibl and D. Eibl, Eds., Berlin, Heidelberg: Springer, 2010, pp. 33–53. doi: 10.1007/10_2008_18.

[15] V. Jossen, R. Eibl, G. Broccard, and D. Eibl, 'Single-Use Systems in Biopharmaceutical Manufacture: State of the Art and Recent Trends', in Biopharmaceutical Manufacturing: Progress, Trends and Challenges, R. Pörtner, Ed., in Cell Engineering. , Cham: Springer International Publishing, 2023, pp. 3–38. doi: 10.1007/978-3-031-45669-5_1.

[16] T. Anderlei et al., 'Status paper: Facility of the Future', DECHEMA. Accessed: Dec. 19, 2025. [Online]. Available: https://dechema.de/en/FoF-path-123212,124930.html

[17] M. A. Teale, S. Schneider, D. Eibl, C. van den Bos, P. Neubauer, and R. Eibl, 'Mesenchymal and induced pluripotent stem cell–based therapeutics: a comparison', Appl. Microbiol. Biotechnol., May 2023, doi: 10.1007/s00253-023-12583-4.

[18] T. Hood et al., 'Establishing a scalable perfusion strategy for the manufacture of CAR-T cells in stirred-tank bioreactors using a quality-by-design approach', Bioeng. Transl. Med., vol. n/a, no. n/a, p. e10753, 2025, doi: 10.1002/btm2.10753.

[19] L. K. Nielsen, 'Bioreactors for Hematopoietic Cell Culture', Annu. Rev. Biomed. Eng., vol. 1, no. 1, pp. 129–152, Aug. 1999, doi: 10.1146/annurev.bioeng.1.1.129.

[20] C. Mennan, J. Garcia, S. Roberts, C. Hulme, and K. Wright, 'A comprehensive characterisation of large-scale expanded human bone marrow and umbilical cord mesenchymal stem cells', Stem Cell Res. Ther., vol. 10, no. 1, p. 99, Mar. 2019, doi: 10.1186/s13287-019-1202-4.

[21] F. C. Paccola Mesquita, C. Hochman-Mendez, J. Morrissey, L. C. Sampaio, and D. A. Taylor, 'Laminin as a Potent Substrate for Large-Scale Expansion of Human Induced Pluripotent Stem Cells in a Closed Cell Expansion System', Stem Cells Int., vol. 2019, p. e9704945, Jan. 2019, doi: 10.1155/2019/9704945.

[22] A. Sahabian et al., 'Chemically-Defined, Xeno-Free, Scalable Production of hPSC-Derived Definitive Endoderm Aggregates with Multi-Lineage Differentiation Potential', Cells, vol. 8, no. 12, Art. no. 12, Dec. 2019, doi: 10.3390/cells8121571.

[23] F. Laco et al., 'Selection of human induced pluripotent stem cells lines optimization of cardiomyocytes differentiation in an integrated suspension microcarrier bioreactor', Stem Cell Res. Ther., vol. 11, no. 1, p. 118, Mar. 2020, doi: 10.1186/s13287-020-01618-6.

[24] E. F. Jacobson, Z. Chen, D. M. Stoukides, G. G. Nair, M. Hebrok, and E. S. Tzanakakis, 'Non-xenogeneic expansion and definitive endoderm differentiation of human pluripotent stem cells in an automated bioreactor', Biotechnol. Bioeng., vol. 118, no. 2, pp. 979–991, 2021, doi: 10.1002/bit.27629.

[25] S. M. Frisch and R. A. Screaton, 'Anoikis mechanisms', Curr. Opin. Cell Biol., vol. 13, no. 5, pp. 555–562, Oct. 2001, doi: 10.1016/S0955-0674(00)00251-9.

[26] K. Watanabe et al., 'A ROCK inhibitor permits survival of dissociated human embryonic stem cells', Nat. Biotechnol., vol. 25, no. 6, Art. no. 6, Jun. 2007, doi: 10.1038/nbt1310.

[27] S. Tohyama et al., 'Efficient Large-Scale 2D Culture System for Human Induced Pluripotent Stem Cells and Differentiated Cardiomyocytes', Stem Cell Rep., vol. 9, no. 5, pp. 1406–1414, Nov. 2017, doi: 10.1016/j.stemcr.2017.08.025.

[28] C. H. Hulme et al., 'A comprehensive review of quantum bioreactor cell manufacture: Research and clinical applications', Cytotherapy, vol. 25, no. 10, pp. 1017–1026, Oct. 2023, doi: 10.1016/j.jcyt.2023.04.004.

[29] S. Yu et al., 'Selection of O-negative induced pluripotent stem cell clones for high-density red blood cell production in a scalable perfusion bioreactor system', Cell Prolif., vol. 55, no. 8, p. e13218, 2022, doi: 10.1111/cpr.13218.

[30] C. Schirmaier et al., 'Scale-up of adipose tissue-derived mesenchymal stem cell production in stirred single-use bioreactors under low-serum conditions', Eng. Life Sci., vol. 14, no. 3, pp. 292–303, May 2014, doi: 10.1002/elsc.201300134.

[31] V. Jossen et al., 'Mass Production of Mesenchymal Stem Cells — Impact of Bioreactor Design and Flow Conditions on Proliferation and Differentiation', in Cells and Biomaterials in Regenerative Medicine, IntechOpen, 2014. doi: 10.5772/59385.

[32] T. Lawson et al., 'Process development for expansion of human mesenchymal stromal cells in a 50L single-use stirred tank bioreactor', Biochem. Eng. J., vol. 120, pp. 49–62, Apr. 2017, doi: 10.1016/j.bej.2016.11.020.

[33] H. P. Lesch, P. Valonen, and M. Karhinen, 'Evaluation of the Single-Use Fixed-Bed Bioreactors in Scalable Virus Production', Biotechnol. J., vol. 16, no. 1, p. 2000020, 2021, doi: 10.1002/biot.202000020.

[34] V. N. Goral et al., 'Innovative fixed bed bioreactor platform: Enabling linearly scalable adherent cell biomanufacturing with real-time biomass prediction from nutrient consumption', Biotechnol. J., vol. 19, no. 8, p. 2300635, 2024, doi: 10.1002/biot.202300635.

[35] F. Manstein et al., 'High density bioprocessing of human pluripotent stem cells by metabolic control and in silico modeling', STEM CELLS Transl. Med., vol. 10, no. 7, pp. 1063–1080, 2021, doi: 10.1002/sctm.20-0453.

[36] F. Petry and D. Salzig, 'Large-Scale Production of Size-Adjusted β-Cell Spheroids in a Fully Controlled Stirred-Tank Reactor', Processes, vol. 10, no. 5, Art. no. 5, May 2022, doi: 10.3390/pr10050861.

[37] N. Cuesta-Gomez et al., 'Suspension culture improves iPSC expansion and pluripotency phenotype', Stem Cell Res. Ther., vol. 14, no. 1, p. 154, Jun. 2023, doi: 10.1186/s13287-023-03382-9.

[38] M.-H. Kim and M. Kino-oka, 'Bioengineering Considerations for a Nurturing Way to Enhance Scalable Expansion of Human Pluripotent Stem Cells', Biotechnol. J., vol. 15, no. 4, p. 1900314, 2020, doi: 10.1002/biot.201900314.

[39] D. G. Iworima, R. K. Baker, J. M. Piret, and T. J. Kieffer, 'Analysis of the effects of bench-scale cell culture platforms and inoculum cell concentrations on PSC aggregate formation and culture', Front. Bioeng. Biotechnol., vol. 11, Dec. 2023, doi: 10.3389/fbioe.2023.1267007.

[40] T. Darwish, N. T. Swaidan, and M. M. Emara, 'Stress Factors as Possible Regulators of Pluripotent Stem Cell Survival and Differentiation', Biology, vol. 12, no. 8, Art. no. 8, Aug. 2023, doi: 10.3390/biology12081119.

[41] S. Huang et al., 'Process development and scale-up of pluripotent stem cell manufacturing', Cell Gene Ther. Insights, vol. 6, no. 9, pp. 1277–1298, Oct. 2020, doi: 10.18609/cgti.2020.141.

[42] B. S. Borys et al., 'Overcoming bioprocess bottlenecks in the large-scale expansion of high-quality hiPSC aggregates in vertical-wheel stirred suspension bioreactors', Stem Cell Res. Ther., vol. 12, no. 1, p. 55, Jan. 2021, doi: 10.1186/s13287-020-02109-4.

[43] H. Worden, 'Record >200 Billion iPSCs Harvested from One Vertical-Wheel Bioreactor Run', presented at the 4th Annual iPSC Drug Development & Manufacturing Summit, Boston, MA, Oct. 02, 2024. Accessed: May 18, 2025. [Online]. Available: https://ipsc-therapies-summit.com/

[44] F. Petry and D. Salzig, 'Impact of Bioreactor Geometry on Mesenchymal Stem Cell Production in Stirred-Tank Bioreactors', Chem. Ing. Tech., vol. 93, no. 10, pp. 1537–1554, 2021, doi: 10.1002/cite.202100041.

[45] E. Costariol et al., 'Demonstrating the Manufacture of Human CAR-T Cells in an Automated Stirred-Tank Bioreactor', Biotechnol. J., vol. 15, no. 9, p. 2000177, 2020, doi: 10.1002/biot.202000177.

[46] N. Trainor et al., 'Automated production of gene-modified chimeric antigen receptor T cells using the Cocoon Platform', Cytotherapy, vol. 25, no. 12, pp. 1349–1360, Dec. 2023, doi: 10.1016/j.jcyt.2023.07.012.

[47] C. van den Bos, R. Keefe, C. Schirmaier, and M. McCaman, 'Pillars of Regenerative Medicine: Therapeutic Human Cells and Their Manufacture', in Industrial Scale Suspension Culture of Living Cells, John Wiley & Sons, Ltd, 2014, pp. 464–502. doi: 10.1002/9783527683321.ch13.

[48] E. Scibona and M. Morbidelli, 'Expansion processes for cell-based therapies', Biotechnol. Adv., vol. 37, no. 8, p. 107455, Dec. 2019, doi: 10.1016/j.biotechadv.2019.107455.

[49] P. Springuel et al., 'Optimising and adapting perfusion feeds in serum-free medium to intensify CAR-T cell expansion in stirred-tank bioreactors', Front. Bioeng. Biotechnol., vol. 13, Jun. 2025, doi: 10.3389/fbioe.2025.1593895.

[50] P. Vormittag, R. Gunn, S. Ghorashian, and F. S. Veraitch, 'A guide to manufacturing CAR T cell therapies', Curr. Opin. Biotechnol., vol. 53, pp. 164–181, Oct. 2018, doi: 10.1016/j.copbio.2018.01.025.

[51] S. Weber, 'Integrierte Herstellplattformen - Industrialisierung der Produktion von autologen Zell- und Gentherapien', Tech4Pharma, vol. 15, no. 3, pp. 140–149, 2025.

[52] J. Wei et al., 'Cryopreserved leukapheresis material can be transferred from controlled rate freezers to ultracold storage at warmer temperatures without affecting downstream CAR-T cell culture performance and in-vitro functionality', Cryobiology, vol. 115, p. 104889, Jun. 2024, doi: 10.1016/j.cryobiol.2024.104889.

[53] K. Brezinger-Dayan et al., 'Impact of cryopreservation on CAR T production and clinical response', Front. Oncol., vol. 12, Oct. 2022, doi: 10.3389/fonc.2022.1024362.

[54] A. Mitra, A. Barua, L. Huang, S. Ganguly, Q. Feng, and B. He, 'From bench to bedside: the history and progress of CAR T cell therapy', Front. Immunol., vol. 14, May 2023, doi: 10.3389/fimmu.2023.1188049.

[55] C. W. Walter, 'Invention and Development of the Blood Bag', Vox Sang., vol. 47, no. 4, pp. 318–324, Oct. 1984, doi: 10.1111/j.1423-0410.1984.tb01603.x.

[56] V. Vucinic, A. Quaiser, P. Lückemeier, S. Fricke, U. Platzbecker, and U. Koehl, 'Production and Application of CAR T Cells: Current and Future Role of Europe', Front. Med., vol. 8, Aug. 2021, doi: 10.3389/fmed.2021.713401.

[57] F. Wu et al., 'Comparing peripheral blood stem cell collection using the COBE Spectra, Haemonetics MCS+, and Baxter Amicus', Transfus. Apher. Sci., vol. 47, no. 3, pp. 345–350, Dec. 2012, doi: 10.1016/j.transci.2012.07.015.

[58] J. Dias, J. Garcia, G. Agliardi, and C. Roddie, 'CAR-T cell manufacturing landscape—Lessons from the past decade and considerations for early clinical development', Mol. Ther. Methods Clin. Dev., vol. 32, no. 2, Jun. 2024, doi: 10.1016/j.omtm.2024.101250.

[59] R. Traynor et al., 'Efficient manufacturing of CAR-T cells from whole blood: a scalable approach to reduce costs and enhance accessibility in cancer therapy', Cytotherapy, vol. 27, no. 3, pp. 400–409, Mar. 2025, doi: 10.1016/j.jcyt.2024.11.013.
[60] H. Meås et al., 'An Automated Solution for Efficient Isolation and Gentle Activation of T Cells Using CTS Detachable Dynabeads CD4, CD8 and CD3/CD28 for CAR-T Manufacturing', Cytotherapy, vol. 27, no. 5, Supplement, pp. S213–S214, May 2025, doi: 10.1016/j.jcyt.2025.03.436.
[61] T. Bikkani et al., 'Robust Cell Selections and Depletions across Various Hematopoietic Cell Fractions on the Clinimacs Plus Instrument', Blood, vol. 132, no. Supplement 1, p. 3818, Nov. 2018, doi: 10.1182/blood-2018-99-118582.
[62] C. F. Reyes et al., 'End-to-end automated manufacturing of autologous CAR T cell therapies with the new SefiaTM cell therapy manufacturing platform', Cytotherapy, vol. 26, no. 6, Supplement, pp. S166–S167, Jun. 2024, doi: 10.1016/j.jcyt.2024.03.325.
[63] M. S. Costa et al., 'Controlled activation modulates T-cell expansion and phenotype in stirred-tank bioreactors', Cytotherapy, vol. 27, no. 6, pp. 774–781, Jun. 2025, doi: 10.1016/j.jcyt.2025.02.003.
[64] H. W. Song et al., 'CAR-T cell expansion platforms yield distinct T cell differentiation states', Cytotherapy, vol. 26, no. 7, pp. 757–768, Jul. 2024, doi: 10.1016/j.jcyt.2024.03.003.
[65] J. Hupfeld, K. Heinrichs, M. Kraft, and A. Tappe, 'Comparison of primary T cells cultured in static conditions versus rocking motion bioreactors', Cytotherapy, vol. 22, no. 5, Supplement, p. S147, May 2020, doi: 10.1016/j.jcyt.2020.03.298.
[66] W.-X. Sin et al., 'A high-density microfluidic bioreactor for the automated manufacturing of CAR T cells', Nat. Biomed. Eng., pp. 1–21, Jun. 2024, doi: 10.1038/s41551-024-01219-1.
[67] P. Springuel et al., 'Perfusion-powered production of over 100 CAR-T doses in a 2-litre stirred-tank bioreactor with automated product harvest, wash and concentration', Cytotherapy, vol. 27, no. 5, Supplement, pp. S18–S19, May 2025, doi: 10.1016/j.jcyt.2025.03.023.
[68] R. Gilfanova et al., 'A bioinspired and chemically defined alternative to dimethyl sulfoxide for the cryopreservation of human hematopoietic stem cells', Bone Marrow Transplant., vol. 56, no. 11, pp. 2644–2650, Nov. 2021, doi: 10.1038/s41409-021-01368-w.
[69] J. Isaacson et al., 'Enhancing CAR T-cell therapy manufacturing efficiency through semi-automated bioprocessing', Clin. Transl. Immunol., vol. 14, no. 6, p. e70025, 2025, doi: 10.1002/cti2.70025.
[70] M. Park et al., 'Automation preserves product consistency and quality for the formulation, fill, and finish of T cell-based therapies', Cytotherapy, vol. 26, no. 12, pp. 1566–1570, Dec. 2024, doi: 10.1016/j.jcyt.2024.07.006.
[71] L. Caballero, M. McMillan, and A. Leavitt, 'Thawing cryopreserved apheresis products: comparison of barkey plasmatherm dry heating device and 370C water bath', Cytotherapy, vol. 22, no. 5, Supplement, pp. S140–S141, May 2020, doi: 10.1016/j.jcyt.2020.03.281.
[72] C. F. van der Walle, S. Godbert, G. Saito, and Z. Azhari, 'Formulation Considerations for Autologous T Cell Drug Products', Pharmaceutics, vol. 13, no. 8, Art. no. 8, Aug. 2021, doi: 10.3390/pharmaceutics13081317.
[73] S. Akel et al., 'Preparation of cryopreserved chimeric antigen receptor T cells for the locoreogional delivery to the neural axis', Cytotherapy, vol. 25, no. 11, pp. 1149–1154, Nov. 2023, doi: 10.1016/j.jcyt.2023.08.004.
[74] S. R. Panch et al., 'Effect of Cryopreservation on Autologous Chimeric Antigen Receptor T Cell Characteristics', Mol. Ther., vol. 27, no. 7, pp. 1275–1285, Jul. 2019, doi: 10.1016/j.ymthe.2019.05.015.
[75] S. Viswanathan et al., 'Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature', Cytotherapy, vol. 21, no. 10, pp. 1019–1024, Oct. 2019, doi: 10.1016/j.jcyt.2019.08.002.
[76] T. Lambrechts et al., 'Evaluation of a monitored multiplate bioreactor for large-scale expansion of human periosteum derived stem cells for bone tissue engineering applications', Biochem. Eng. J., vol. 108, pp. 58–68, Apr. 2016, doi: 10.1016/j.bej.2015.07.015.
[77] V. Jossen et al., 'Theoretical and Practical Issues That Are Relevant When Scaling Up hMSC Microcarrier Production Processes', Stem Cells Int., vol. 2016, no. 1, p. 4760414, 2016, doi: 10.1155/2016/4760414.
[78] J. de Sá da Silva et al., 'Improving wave-induced motion bioreactor performance for human mesenchymal stromal cell expansion', Process Biochem., vol. 84, pp. 143–152, Sep. 2019, doi: 10.1016/j.procbio.2019.06.004.
[79] J. de Sá da Silva et al., 'Mesenchymal stromal cells maintain the major quality attributes when expanded in different bioreactor systems', Biochem. Eng. J., vol. 161, p. 107693, Sep. 2020, doi: 10.1016/j.bej.2020.107693.
[80] M. Jeyaraman et al., 'Fostering mesenchymal stem cell therapy to halt cytokine storm in COVID-19', Biochim. Biophys. Acta Mol. Basis Dis., vol. 1867, no. 2, p. 166014, Feb. 2021, doi: 10.1016/j.bbadis.2020.166014.
[81] V. Jossen, C. van den Bos, R. Eibl, and D. Eibl, 'Manufacturing human mesenchymal stem cells at clinical scale: process and regulatory challenges', Appl. Microbiol. Biotechnol., vol. 102, no. 9, pp. 3981–3994, May 2018, doi: 10.1007/s00253-018-8912-x.
[82] C. Brown et al., 'Mesenchymal stem cells: Cell therapy and regeneration potential', J. Tissue Eng. Regen. Med., vol. 13, no. 9, pp. 1738–1755, 2019, doi: 10.1002/term.2914.
[83] I. Mawji, E. L. Roberts, T. Dang, B. Abraham, and M. S. Kallos, 'Challenges and opportunities in downstream separation processes for mesenchymal stromal cells cultured in microcarrier-based stirred suspension bioreactors', Biotechnol. Bioeng., vol. 119, no. 11, pp. 3062–3078, 2022, doi: 10.1002/bit.28210.
[84] M. Strecanska, T. Sekelova, V. Smolinska, M. Kuniakova, and A. Nicodemou, 'Automated Manufacturing Processes and Platforms for Large-scale Production of Clinical-grade Mesenchymal Stem/ Stromal Cells', Stem Cell Rev. Rep., Nov. 2024, doi: 10.1007/s12015-024-10812-5.
[85] A. W. Nienow, Q. A. Rafiq, K. Coopman, and C. J. Hewitt, 'A potentially scalable method for the harvesting of hMSCs from microcarriers', Biochem. Eng. J., vol. 85, pp. 79–88, Apr. 2014, doi: 10.1016/j.bej.2014.02.005.
[86] B. Cunha, C. Peixoto, M. M. Silva, M. J. T. Carrondo, M. Serra, and P. M. Alves, 'Filtration methodologies for the clarification and concentration of human mesenchymal stem cells', J. Membr. Sci., vol. 478, pp. 117–129, Mar. 2015, doi: 10.1016/j.memsci.2014.12.041.
[87] B. Cunha et al., 'Exploring continuous and integrated strategies for the up- and downstream processing of human mesenchymal stem cells', J. Biotechnol., vol. 213, pp. 97–108, Nov. 2015, doi: 10.1016/j.jbiotec.2015.02.023.
[88] A. Li, G. Kusuma, D. James, and R. Lim, 'Design of Experiment (DoE) approach to identify critical parameters in a counterflow centrifugation system', Cytotherapy, vol. 22, no. 5, Supplement, pp. S151–S152, May 2020, doi: 10.1016/j.jcyt.2020.03.313.
[89] A. T. L. Lam et al., 'Human Mesenchymal Stem Cell Processing for Clinical Applications Using a Closed Semi-Automated Workflow', J. Vis. Exp. JoVE, no. 193, p. e64707, Mar. 2023, doi: 10.3791/64707.
[90] H. Rouard et al., 'Assessment of Pall's Xpansion® bioreactor for the production of mesenchymal stromal cells for therapeutic use', Cytotherapy, vol. 22, no. 5, Supplement, pp. S99–S100, May 2020, doi: 10.1016/j.jcyt.2020.03.175.
[91] J. C. Fitzgerald et al., 'GMP-Compliant Production of Autologous Adipose-Derived Stromal Cells in the NANT 001 Closed Automated Bioreactor', Front. Bioeng. Biotechnol., vol. 10, Mar. 2022, doi: 10.3389/fbioe.2022.834267.
[92] N. e. Timmins et al., 'Closed system isolation and scalable expansion of human placental mesenchymal stem cells', Biotechnol. Bioeng., vol. 109, no. 7, pp. 1817–1826, 2012, doi: 10.1002/bit.24425.
[93] Q. A. Rafiq et al., 'Process development of human multipotent stromal cell microcarrier culture using an automated high-throughput microbioreactor', Biotechnol. Bioeng., vol. 114, no. 10, pp. 2253–2266, 2017, doi: 10.1002/bit.26359.
[94] B. Cunha et al., 'Bioprocess integration for human mesenchymal stem cells: From up to downstream processing scale-up to cell proteome characterization', J. Biotechnol., vol. 248, pp. 87–98, Apr. 2017, doi: 10.1016/j.jbiotec.2017.01.014.
[95] M. Teale, V. Jossen, D. Eibl, and R. Eibl, 'Chemically Defined, Xeno-Free Expansion of Human Mesenchymal Stem Cells (hMSCs) on Benchtop-Scale Using a Stirred Single-Use Bioreactor', in Bioreactors in Stem Cell Biology, vol. 2436, K. Turksen, Ed., in Methods in Molecular Biology, vol. 2436. , New York, NY: Springer US, 2021, pp. 83–111. doi: 10.1007/7651_2021_426.
[96] Pall Biotech, 'Growth of Human Mesenchymal Stem/Stromal Cells on Collagen Microcarriers in the AllegroTM STR 50 L Bioreactor', Pall Corporation, Application Note USD3330a, 2020. Accessed: Jul. 09, 2022. [Online]. Available: https://www.pall.com/content/dam/pall/biopharm/lit-library/non-gated/application-notes/20-07583-usd3330-a-growth-mensenchymal-cells-collagen-microcarriers-allegro-bioreactor-an-en.PDF
[97] R. Wagey et al., 'The validation of an animal component-free suspension culture for human mesenchymal stromal cells in horizontal and vertical wheel culture systems', Cytotherapy, vol. 26, no. 6, Supplement, pp. S69–S70, Jun. 2024, doi: 10.1016/j.jcyt.2024.03.124.
[98] A. Krull et al., 'Efficient Clinical-Grade Expansion of Adipose-Derived Mesenchymal Stromal Cells Using a Vertical Wheel Bioreactor in an Academic Medical Center', Cytotherapy, vol. 27, no. 5, Supplement, pp. S64–S65, May 2025, doi: 10.1016/j.jcyt.2025.03.114.
[99] S. L. Schneider, A. Gopalakrishnan, M. A. Teale, and R. Eibl, 'Cell retention in scalable, perfusion-based mesenchymal stem cell expansion processes: a proof of concept', Front. Bioeng. Biotechnol., vol. 13, Jul. 2025, doi: 10.3389/fbioe.2025.1611703.
[100] P. R. Pandey et al., 'End-to-End Platform for Human Pluripotent Stem Cell Manufacturing', Int. J. Mol. Sci., vol. 21, no. 1, Art. no. 1, Jan. 2020, doi: 10.3390/ijms21010089.
[101] A. Li et al., 'Automated Counterflow Centrifugal System for Small-Scale Cell Processing', J. Vis. Exp., no. 154, p. 60423, Dec. 2019, doi: 10.3791/60423.
[102] K. Tanabe, K. Takahashi, and S. Yamanaka, 'Induction of pluripotency by defined factors', Proc. Jpn. Acad. Ser. B, vol. 90, no. 3, pp. 83–96, 2014, doi: 10.2183/pjab.90.83.
[103] S. Yamanaka, 'Pluripotent Stem Cell-Based Cell Therapy—Promise and Challenges', Cell Stem Cell, vol. 27, no. 4, pp. 523–531, Oct. 2020, doi: 10.1016/j.stem.2020.09.014.
[104] B. A. Baghbaderani et al., 'cGMP-Manufactured Human Induced Pluripotent Stem Cells Are Available for Pre-clinical and Clinical Applications', Stem Cell Rep., vol. 5, no. 4, pp. 647–659, Oct. 2015, doi: 10.1016/j.stemcr.2015.08.015.
[105] B. A. Baghbaderani et al., 'Detailed Characterization of Human Induced Pluripotent Stem Cells Manufactured for Therapeutic Applications', Stem Cell Rev. Rep., vol. 12, no. 4, pp. 394–420, Aug. 2016, doi: 10.1007/s12015-016-9662-8.
[106] H. Kanemura et al., 'Tumorigenicity Studies of Induced Pluripotent Stem Cell (iPSC)-Derived Retinal Pigment Epithelium (RPE) for the Treatment of Age-Related Macular Degeneration', PLOS ONE, vol. 9, no. 1, p. e85336, Jan. 2014, doi: 10.1371/journal.pone.0085336.
[107] S. Yasuda et al., 'Tumorigenicity-associated characteristics of human iPS cell lines', PLOS ONE, vol. 13, no. 10, p. e0205022, Oct. 2018, doi: 10.1371/journal.pone.0205022.
[108] M. A. Teale, S. L. Schneider, D. Eibl, and R. Eibl, 'Process Intensification in Human Pluripotent Stem Cell Expansion with Microcarriers', Processes, vol. 12, no. 3, Art. no. 3, Mar. 2024, doi: 10.3390/pr12030426.
[109] K. Ullmann et al., 'Matrix-free human pluripotent stem cell manufacturing by seed train approach and intermediate cryopreservation', Stem Cell Res. Ther., vol. 15, no. 1, p. 89, Mar. 2024, doi: 10.1186/s13287-024-03699-z.
[110] H. Sugiyama, M. Shiokaramatsu, M. Kagihiro, K. Fukumori, I. Horiguchi, and M. Kino-oka, 'Apoptosis-based method for determining lot sizes in the filling of human-induced pluripotent stem cells', J. Tissue Eng. Regen. Med., vol. 14, no. 11, pp. 1641–1651, 2020, doi: 10.1002/term.3127.
[111] L. Chan, A. Prowse, P. W. Zandstra, and N. Timmins, 'Aggregate-based human pluripotent stem cell manufacturing with the ambr®15 platform', Cytotherapy, vol. 17, no. 6, pp. S69–S70, Jun. 2015, doi: 10.1016/j.jcyt.2015.03.544.
[112] S. L. Schneider et al., 'Expansion of induced pluripotent stem cells under consideration of bioengineering aspects: part 1', Appl. Microbiol. Biotechnol., vol. 109, no. 1, p. 37, Feb. 2025, doi: 10.1007/s00253-024-13372-3.
[113] C. Kropp et al., 'Impact of Feeding Strategies on the Scalable Expansion of Human Pluripotent Stem Cells in Single-Use Stirred Tank Bioreactors', STEM CELLS Transl. Med., vol. 5, no. 10, pp. 1289–1301, 2016, doi: https://doi.org/10.5966/sctm.2015-0253.
[114] C. K. Kwok et al., 'Scalable stirred suspension culture for the generation of billions of human induced pluripotent stem cells using single-use bioreactors', J. Tissue Eng. Regen. Med., vol. 12, no. 2, pp. e1076–e1087, 2018, doi: https://doi.org/10.1002/term.2435.
[115] T. Dang et al., 'Computational fluid dynamic characterization of vertical-wheel bioreactors used for effective scale-up of human induced pluripotent stem cell aggregate culture', Can. J. Chem. Eng., vol. 99, no. 11, pp. 2536–2553, 2021, doi: 10.1002/cjce.24253.
[116] B. M. Davis, E. R. Loghin, K. R. Conway, and X. Zhang, 'Automated Closed-System Expansion of Pluripotent Stem Cell Aggregates in a Rocking-Motion Bioreactor', SLAS Technol. Transl. Life Sci. Innov., vol. 23, no. 4, pp. 364–373, Aug. 2018, doi: 10.1177/2472630318760745.
[117] M. A. Teale, 'The expansion of human induced pluripotent stem cells towards allogeneic cell therapies', 2025, doi: 10.14279/DEPOSITONCE-24083.
[118] J. Müller, V. Ott, D. Eibl, and R. Eibl, 'Seed Train Intensification Using an Ultra-High Cell Density Cell Banking Process', Processes, vol. 10, no. 5, Art. no. 5, May 2022, doi: 10.3390/pr10050911.

[119] M. A. Teale et al., ‘Expansion of induced pluripotent stem cells under consideration of bioengineering aspects: part 2’, Appl. Microbiol. Biotechnol., vol. 109, no. 1, p. 38, Feb. 2025, doi: 10.1007/s00253-024-13373-2.
[120] T. S. Winston, K. Suddhapas, C. Wang, R. Ramos, P. Soman, and Z. Ma, ‘Serum-Free Manufacturing of Mesenchymal Stem Cell Tissue Rings Using Human-Induced Pluripotent Stem Cells’, Stem Cells Int., vol. 2019, p. 5654324, Jan. 2019, doi: 10.1155/2019/5654324.
[121] A. Sahabian, J. Dahlmann, U. Martin, and R. Olmer, ‘Production and cryopreservation of definitive endoderm from human pluripotent stem cells under defined and scalable culture conditions’, Nat. Protoc., vol. 16, no. 3, Art. no. 3, Mar. 2021, doi: 10.1038/s41596-020-00470-5.
[122] C. K. Kwok et al., ‘Scalable expansion of iPSC and their derivatives across multiple lineages’, Reprod. Toxicol., vol. 112, pp. 23–35, Sep. 2022, doi: 10.1016/j.reprotox.2022.05.007.
[123] Z. Zhao et al., ‘Chemically defined and growth factor-free system for highly efficient endoderm induction of human pluripotent stem cells’, Stem Cell Rep., vol. 20, no. 1, p. 102382, Jan. 2025, doi: 10.1016/j.stemcr.2024.11.012.
[124] Y. Jiang et al., ‘Controlled, Large-Scale Manufacturing of hiPSC-Derived Cardiomyocytes in Stirred-Tank Bioreactors’, Eppendorf AG, Leiden, NL, Application Note 409, Apr. 2019. Accessed: Feb. 09, 2025. [Online]. Available: https://www.eppendorf.com/product-media/doc/en/763613/Fermentors-Bioreactors_Application-Note_409_DASbox_BioFlo-320_Controlled_Large-Scale-Manufacturing-hiPSC-Derived-Cardiomyocytes-Stirred-Tank-Bioreactors.pdf
[125] T. P. Silva et al., ‘Transcriptome profiling of human pluripotent stem cell-derived cerebellar organoids reveals faster commitment under dynamic conditions’, Biotechnol. Bioeng., vol. n/a, no. n/a, 2021, doi: 10.1002/bit.27797.
[126] Y. Huang, J.-Y. Qian, H. Cheng, and X.-M. Li, ‘Effects of shear stress on differentiation of stem cells into endothelial cells’, World J. Stem Cells, vol. 13, no. 7, pp. 894–913, Jul. 2021, doi: 10.4252/wjsc.v13.i7.894.
[127] T. Kaitsuka and F. Hakim, ‘Response of Pluripotent Stem Cells to Environmental Stress and Its Application for Directed Differentiation’, Biology, vol. 10, no. 2, Art. no. 2, Feb. 2021, doi: 10.3390/biology10020084.
[128] R. Goetzke et al., ‘Differentiation of Induced Pluripotent Stem Cells towards Mesenchymal Stromal Cells is Hampered by Culture in 3D Hydrogels’, Sci. Rep., vol. 9, no. 1, Art. no. 1, Oct. 2019, doi: 10.1038/s41598-019-51911-5.
[129] J. Sivalingam et al., ‘A Scalable Suspension Platform for Generating High-Density Cultures of Universal Red Blood Cells from Human Induced Pluripotent Stem Cells’, Stem Cell Rep., vol. 16, no. 1, pp. 182–197, Jan. 2021, doi: 10.1016/j.stemcr.2020.11.008.
[130] M. Ackermann et al., ‘Bioreactor-based mass production of human iPSC-derived macrophages enables immunotherapies against bacterial airway infections’, Nat. Commun., vol. 9, no. 1, p. 5088, Nov. 2018, doi: 10.1038/s41467-018-07570-7.
[131] V. Ott, J. Ott, D. Eibl, and R. Eibl, ‘Scaling Fed-Batch and Perfusion Antibody Production Processes in Geometrically Dissimilar Stirred Bioreactors’, Processes, vol. 12, no. 4, Art. no. 4, Apr. 2024, doi: 10.3390/pr12040806.
[132] M. Kagihiro et al., ‘Kinetic analysis of cell decay during the filling process: Application to lot size determination in manufacturing systems for human induced pluripotent and mesenchymal stem cells’, Biochem. Eng. J., vol. 131, pp. 31–38, Mar. 2018, doi: 10.1016/j.bej.2017.11.019.
[133] S. J. Reyes, Y. Durocher, P. L. Pham, and O. Henry, ‘Modern Sensor Tools and Techniques for Monitoring, Controlling, and Improving Cell Culture Processes’, Processes, vol. 10, no. 2, p. 189, Jan. 2022, doi: 10.3390/pr10020189.
[134] S. Sullivan et al., ‘Quality Control Guidelines for Clinical-Grade Human Induced Pluripotent Stem Cell Lines’, Regen. Med., vol. 13, no. 7, pp. 859–866, Oct. 2018, doi: 10.2217/rme-2018-0095.
[135] M. Dominici et al., ‘Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement’, Cytotherapy, vol. 8, no. 4, pp. 315–317, 2006, doi: 10.1080/14653240600855905.
[136] S. Sullivan et al., ‘Quality control guidelines for clinical-grade human induced pluripotent stem cell lines’, Regen. Med., vol. 13, no. 7, pp. 859–866, Oct. 2018, doi: 10.2217/rme-2018-0095.
[137] P. J. Hanley, ‘Fresh versus Frozen: Effects of Cryopreservation on CAR T Cells’, Mol. Ther., vol. 27, no. 7, pp. 1213–1214, Jul. 2019, doi: 10.1016/j.ymthe.2019.06.001.
[138] C. Maillot, C. Sion, N. De Isla, D. Toye, and E. Olmos, ‘Quality by design to define critical process parameters for mesenchymal stem cell expansion’, Biotechnol. Adv., vol. 50, p. 107765, Sep. 2021, doi: 10.1016/j.biotechadv.2021.107765.
[139] G. Fridberg, B.-Z. Katz, D. Benisty, C. Perry, R. Ram, and I. Avivi, ‘P1183: CAR-T Morphological Characteristics and Dynamics in Peripheral Blood Evaluated by a Novel Microscopy Platform’, HemaSphere, vol. 6, pp. 1069–1070, Jun. 2022, doi: 10.1097/01.HS9.0000847596.39537.8d.
[140] M. Madrid et al., ‘Considerations for the development of iPSC-derived cell therapies: a review of key challenges by the JSRM-ISCT iPSC Committee’, Cytotherapy, vol. 26, no. 11, pp. 1382–1399, Nov. 2024, doi: 10.1016/j.jcyt.2024.05.022.
[141] R. Singh, H. E. Orimi, P. K. R. Pedabaliyarasimhuni, C. A. Hoesli, and M. Chioua, ‘AI- Driven Quality Monitoring and Control in Stem Cell Cultures: A Comprehensive Review’, Biotechnol. J., vol. 20, no. 8, p. e70100, Aug. 2025, doi: 10.1002/biot.70100.
[142] F. Ravera, E. Efeoglu, and H. J. Byrne, ‘Monitoring stem cell differentiation using Raman microspectroscopy: chondrogenic differentiation, towards cartilage formation’, Analyst, vol. 146, no. 1, pp. 322–337, Jan. 2021, doi: 10.1039/D0AN01983F.
[143] H. Saari, H. Marttila, M. M. Poranen, H. M. Oksanen, J. Zini, and S. Laitinen, ‘Inline Raman Spectroscopy Provides Versatile Molecular Monitoring for Platelet Extracellular Vesicle Purification with Anion-Exchange Chromatography’, Int. J. Mol. Sci., vol. 25, no. 15, p. 8130, Jan. 2024, doi: 10.3390/ijms25158130.

9. Appendix

Abbreviations

2D	Two-dimensional
iPSC	Induced pluripotent stem cell
3D	Three-dimensional
mAb	Monoclonal antibody
ATMP	Advanced Therapy Medicinal Products
MSC	Mesenchymal stem cell
CAR	Chimeric antigen receptor
PAT	Process analytical technologies
CHO	Chinese hamster ovary
PBMC	Peripheral blood mononuclear cell
CQA	Critical quality attribute
pCO_2	Dissolved carbon dioxide
DMSO	Dimethylsulfoxide
pO_2	Dissolved oxygen
DSP	Downstream processing
SUT	Single-use technologies
GMP	Good Manufacturing Practice
USP	Upstream processing
ICB	Intermediate cell bank

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