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Chapter

Integrated Biologics Manufacturing in Stirred-Suspension Bioreactor: A Stem Cell Perspective

Suman C. Nath and Derrick E. Rancourt

Abstract

Stem cell therapy is garnering attention as several clinical trials have taken place in the recent years by using human pluripotent stem cells (hPSCs). Hundreds of biotechnological companies are investing to find a permanent cure for difficult-to-treat diseases like age-related macular degeneration, Parkinson's disease, diabetes, etc. by using hPSCs. Therefore, clinical-grade cell manufacturing has become an important issue to make cell therapy products safe and effective. Current manufacturing practices are adopted from conventional antibody or protein production in the pharmaceutical industry where cells are used as a vector for producing the desired products. In cell therapy applications, cells are the products that are sensitive to physicochemical parameters and storage conditions anywhere between isolation to patient administration. Moreover, cell-based product manufacturing consists of multi-step processing, including isolation from patients, genetic modification, derivation, expansion, differentiation, purification, characterization, cryopreservation, etc. This can require long processing times and pose high risk of product contamination as well as high production cost. Herein, we discuss the current methods of biologics manufacturing and its limitations. We also review current practices for integrating and automating cell manufacturing facilities. Finally, we propose how to integrate multi-step cell processing in a single bioreactor to make the cell manufacturing practices more direct.

Keywords: biologics, stem cell therapy, genetic modification, integrated manufacturing, bioreactor

1. Introduction

Based on their self-renewal and differentiation capabilities, human pluripotent stem cells (hPSCs) including embryonic stem cells (ESCs) [1] and induced pluripotent stem cells (iPSCs) [2] are attractive tools in the field of regenerative medicine. After the discovery of hiPSCs in 2007, this field expanded vigorously and hundreds of biotechnological companies were established to use these cells for treating degenerative diseases. The most common degenerative diseases treated by the hESCs are age-related macular degeneration (AMD), type I diabetes mellitus, heart failure, Parkinson's disease, and spinal cord injury [3]. Although hiPSCs are a better source

for autologous cell therapy applications, they are less preferable for clinical trials because of less genetic stability compared to the hESCs. However, a few clinical trials have already been started using the patient-derived hiPSCs. The Takahashi group from the Riken Center for Developmental Biology has recently conducted a clinical trial for treating wet AMD [4]. Similarly, a Takahashi from Kyoto University is conducting a clinical trial for treating Parkinson's disease by using hiPSCs [5]. A few clinical trials are also ongoing in the USA for treating different diseases like β-thalassemia, liver diseases, diabetes, etc. using hiPSCs and their use is expanding worldwide day by day [6].

As stem cell therapy is garnering increasing attention, a lot of clinical trials are ongoing using both hESCs and hiPSCs cells. About 6849 clinical trials and 1415 stem cell-based therapies were found based upon searches we recently performed on clinicaltrials.gov (October, 2018) [7]. However, the percentage of success is not high enough as speculated from the previous clinical trials. Among the 315 clinical trials conducted (26.0% Phase 1, 40.6% Phase 1/2, 22.5% Phase 2, 3.8% Phase 2/3, and 6.7% Phase 3), only 0.3% went to Phase 4 [3]. The low percentage of completion of clinical trials depends on various factors. One of the major factors is manufacturing practices that can provide high safety and efficacy of cell therapy products. Moreover, production cost of multiple doses also hinders the success rate of clinical trials. As cell therapy revenue exceeded multi-million dollars and has been a profitable business in recent years, but much attention is needed to produce high quality cells for treating incurable diseases [8, 9].

The production of stem cell biologics is adapted from the conventional pharmaceutical protein and vaccine production. Conventional biologics production involves the following basic steps: isolation and identification of raw materials, formulation, filling, packaging, and storage, where the total processing stops at the storage of final products.

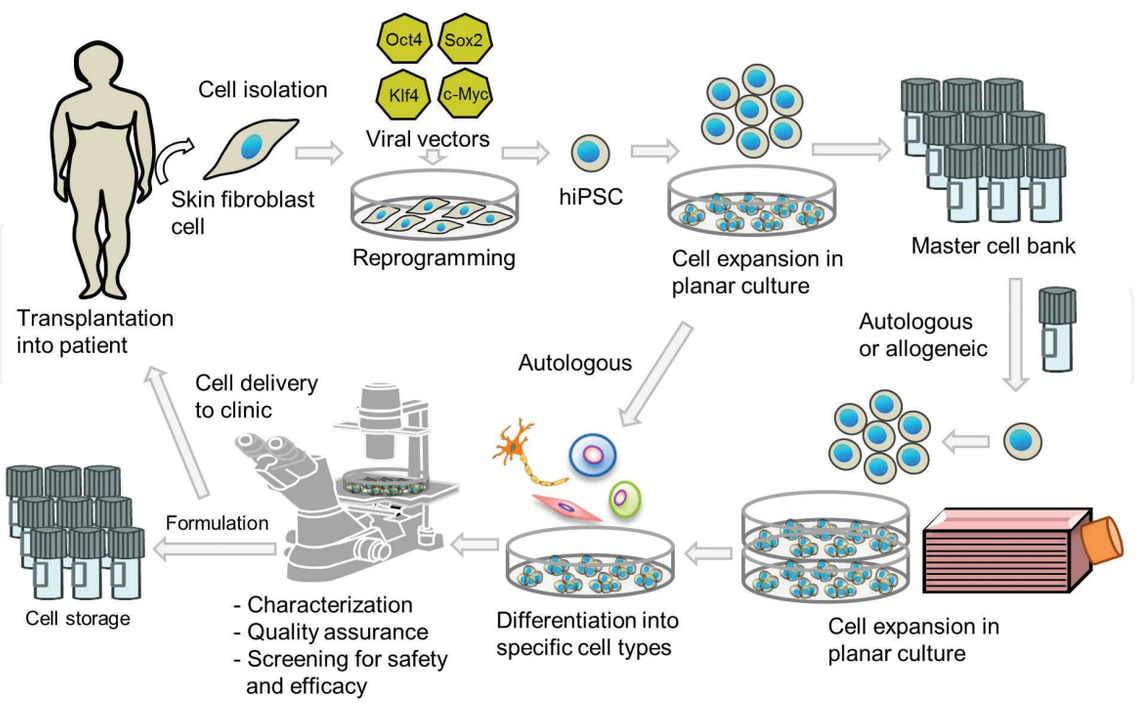

Figure 1.
Schematic illustration of current multi-step cell manufacturing strategies in planar culture for stem cell therapy applications. Skin cells are isolated from the patient and reprogrammed to hiPSCs using viral vectors. After reprogramming, hiPSCs are stored in a master cell bank or differentiated directly in autologous cell therapy applications. In some cases like allogeneic cell therapy applications, cells are expanded in a large amount and then differentiated. After performing characterization, quality assurance, and screening for safety and efficacy, cells are delivered to hospital or stored in a cell bank for future use.

There is a big difference between the production of conventional biologics and cell-based therapy products. For vaccines or pharmaceutical protein production, cells are used as a platform for obtaining desired proteins. After that, cells are discarded. However, in cell-based therapies, cells, which are sensitive to the physical or chemical attributes of the residing environment, are the final products. Therefore, much consideration is needed before translating cell-based products from bench to clinic. This extends to the acquisition of tissue samples and isolation of cells, initial cell purification, selection, activation and transduction, cell expansion in plate or bioreactor culture, differentiation, washing, harvesting and formulation, filling and cryopreservation, and finally, storage and delivery to the clinics (**Figure 1**) [10].

Cumbersome multi-step manufacturing systems can cause batch to batch variability, inefficacy, and low quality of cells for transplantation and need to be simplified and made more direct. In this context, we will discuss current limitations of cell manufacturing strategies and propose how to overcome these by integrating the total process in a single bioreactor to make cell manufacturing straight forward enough to deliver high quality cell therapy products to the clinic. In this review, we will also discuss how to integrate genetic modification—transfection or transduction, reprogramming, differentiation, purification, and formulation of final products in a single bioreactor.

2. Current manufacturing strategies for stem cell therapy

Current manufacturing strategies for cell therapy products are replicated from biologics manufacturing in the pharmaceutical industry. However, the processing of cells is far different from pharmaceutical proteins or vaccines. For pharmaceutical peptide production from microorganisms, the raw materials are extracted from bacteria or fungus [11, 12]. They are then separated, purified, and examined for quality assurance to meet the requirements of regulatory agencies, e.g., Food and Drug Administration (FDA), British Pharmacopeia, etc. The final products are stored or marketed in a dose-dependent manner.

Cell processing is more intensified when the pharmaceutical proteins are produced by using human, animal, or plant cells as a by-product. In this case, high quality products depend on the maintenance of high quality cells, and maintaining a sterile condition is very important. Therefore, good bioprocessing is required to optimize the production of desired proteins. After inoculating from a master cell bank, the cells are cultured for a specific period of time [13, 14]. The supernatant is then collected and the desired proteins are separated, purified, and concentrated. The isolated products then go through quality assurance to meet the criteria of the regulatory agency. Finally, the products are stored and marketed in a dose-dependent manner.

The manufacturing of stem-cell based products is not as straight forward as the production of pharmaceutical proteins or vaccines. This is because cells are the final product in stem cell therapy and are vulnerable to physical or chemical operations from isolation to delivery to patients. Cell manufacturing strategies also vary from source to source and depend on autologous or allogeneic transplantation (**Figure 1**). The major general steps are the acquisition of tissue samples and isolation of cells, initial cell purification, selection, activation and transduction, cell expansion, differentiation, washing, harvesting and formulation, filling and cryopreservation, and finally, storage and delivery to the clinics [10].

For stem-cell based products, cells are isolated from specific tissues of patients, e.g., blood, skin, etc. for autologous transplantation or can be used from cell banks for allogeneic transplantation. Heterogeneity of final products may arise from the cell isolation step because patients' tissues contain various undesired

subpopulations. For example, in chimeric antigen receptor T-cell (CAR-T) therapy, cells are isolated from patients' blood tissue, which contains abnormal levels of inhibitory factors and regulatory cells [15, 16] because patients are treated with chemo- and radiotherapies. As a result, heterogeneity occurs in the final products, which need much attention during the cell isolation step. Cells isolated from patients need to be purified by centrifugation, magnetic-activated cell sorting (MACS), or fluorescent-activated cell sorting (FACS). Then, initial cell culture is done for selection, activation, or transduction of specific interest.

After purification, cells are expanded in plate culture or bioreactor. Based on demand, large-scale expansion is required in a sterile condition, which also requires intensive consideration because it is the rate-limiting step for commercialization of cell therapy products. The most important considerations for large-scale expansions are: operational, economic, quality and safety.

Operational design for culture systems (2D or 3D) with manual or automatic (desirable) operation is important before large-scale expansion [17]. Bioreactors are superior to plate culture for obtaining a large number of cells. Online monitoring and control of process parameters (pH, DO, pCO_2, etc.) and considering the shortest possible culture time are also important parameters for operational consideration. A prediction model for medium consumption (glucose and glutamine) and toxic material production (lactic acid and ammonium) is very useful for determining medium feeding regimen. A dedicated single-use vessel is also a big operational consideration before large-scale expansion of cell-based products.

As cell-based products are costlier, economic considerations for medium, efficient cell lines and other indirect utilities are important. However, the most important consideration in large-scale expansion is product quality and safety. For this purpose, dedicated cell manufacturing facilities are required to maintain current manufacturing practices (cGMP) for high product purity and safety.

After large-scale expansion, cells are harvested by detaching them from the culture substrate using enzymatic treatment. Non-enzymatic detachment is also available by changing temperature or pH [18–20]. Aggregate culture in bioreactors may not necessarily need a detachment step for harvesting [21–25]. Next steps are washing and volume reduction, which can be done by centrifugation or tangential flow filtration on a large scale by using automated commercial devices (kSep systems and Terumo BCT).

Purified cells are formulated in a dose-dependent manner and checked for quality assurance. Quality assurance is done in three different stages: microbial contamination, chemical contamination, and quality or potency assurance. Microbial contamination is checked for bacterial, fungal, or viral contamination by sterility tests with various methods [26, 27]. The most commonly used sterility test is a 14-day incubation of cell products for bacterial and fungal contamination [28, 29]. Chemical testing includes checking for molecules accompanying the culture medium or other factors used during isolation, expansion, and storage. One commonly used chemical test is the LAL test for bacterial endotoxin. There is now an automated 15 min test for determining endotoxin in cell therapy products, which was developed following FDA regulations [30]. Other chemical testing concerns are checking for residual proteins of different origins, serum, and other harmful particles originated from cell processing.

In cell therapy products, quality is the major concern, especially because cell growth is a requirement. For that reason, a cell viability assay is done to determine live or dead cells in the product using a variety of staining methods. Colony forming unit (CFU) is also useful for determining biological activity of cell therapy products [31, 32]. Product potency is an important criterion to meet before releasing the product. For example, if a cell therapy product is applied for the chimeric antigen

receptor T (CART)-related cancer therapy, it needs to be examined for the secretion of cytotoxic cytokines (IFN-γ) and killing of target cells [33]. However, for hPSCs, the final products are differentiated cells, wherein potency should be checked via transplantation into disease models.

For hPSC-derived products, strict quality control is imperative before transplantation to the patients because there is high risk of oncogene transfer to patients. A clinical trial was halted in 2015 in Japan while treating AMD by autologous hiPSC-derived retinal pigmented epithelial cells because of genetic abnormality [34]. Since genetic abnormalities occur in hiPSC-derived products from reprogramming to finally differentiated cells [35], cells should be strictly screened for epigenetic signatures, karyotyping, telomerase activity, mitochondrial remodeling, etc. [36–38]. Rohani et al. summarized possible molecular cytogenetics for quality control that should be checked before releasing the final products [39]. Some of the proposed quality testings are whole-genome sequencing, single-cell genome sequencing, epigenomic analysis, and mitochondrial DNA integrity testing for maximizing the patient safety.

After passing the product quality assurance, cells need to be delivered to clinics immediately or stored for future use. Cells are shipped generally to the clinics on dry ice (−78°C) or in liquid nitrogen dry shippers (−160°C) if the cells are vitrified. The mostly used technique for cell storage is cryopreservation in liquid nitrogen at −196°C which is adapted from the conventional stem cell banking [40, 41]. For cryopreservation, dimethyl sulfoxide (DMSO), glycerol, sugars, or other polymers are used. Among them, clinical grade DMSO is widely used although it is detrimental and can cause harmful effects to cells [42, 43]. Therefore, removing it from cryopreservation protocols or lowering the concentration is important. However, developing appropriate protocols for freezing and thawing is also important for high recovery of cells. Generally, slow-freezing and quick thawing is highly applicable for better recovery of cryopreserved cells [44, 45]. Since intracellular ice crystal formation is a big obstacle in cryopreservation, using ice recrystallization inhibitors is also an effective process for cryopreservation of clinical cell therapy products [46, 47].

Product delivery is also an important step to consider before administration to the patients. Since the products are carried in an environment where temperature is extreme, the container should be made with such materials that can withstand extreme low temperature and do not cause any leakage compromising the product quality [48]. For autologous cell therapy applications especially for CAR-T cell therapy, a dedicated vessel, which can withstand extreme low temperature, is needed [49].

3. Integrated biologics manufacturing in bioreactors

The conventional production of pharmaceutical proteins or other biologics consists of multiple steps from raw materials to finished products. As biologics need to maintain stringent quality control, multiple steps in production facilities compromise the product quality significantly. They also reduce productivity and become prone to human errors, which decrease product efficacy and safety. Moreover, multiple steps in cell processing consume a lot of time, which indirectly increases production cost. To overcome these drawbacks, integrated pharmaceutical production has been attempted by various pharmaceutical companies. One of the significant attempts was made by the Novartis-MIT Center for Continuous Manufacturing of pharmaceutical products to fully integrate the cell processing system [50, 51]. Another attempt was taken by Genzyme™ for continuous production of pharmaceutical recombinant protein in bioreactors, where cell culture to product isolation and purification was integrated in a single flow [52]. By using this system,

they respectively reported successful production of monoclonal antibody as well as highly complex, less stable pharmaceutical protein with consistent product quality, high product output, and low cost. Process integrity is necessary for reducing cumbersome production steps and cutting cost significantly. One such integrated system developed by Johnson & Johnson has recently got FDA approval for large-scale HIV drug production [53] that reduces time and cost by one third compared to the conventional batch processing.

Since biologics production for cell therapies require multiple steps, integration of all of the steps will give high product quality and safety, as well as help overcome stringent regulatory requirements. In this context, we will discuss how to integrate some important basic steps of cell manufacturing especially genetic modification, cellular reprogramming, expansion, and differentiation in bioreactors to promote a single-step approach for cell-based therapies (**Figure 2**).

3.1 Genetic modifications in bioreactor

Genetic modification is one of the biggest steps in producing cell therapy products. In biologics manufacturing, it has been practiced for many years for producing antibodies, proteins, or other biotechnological drugs. It has also been used extensively in the cell therapy industry as various cell-based products have been applied for treating multiple incurable genetic diseases in recent years. Some genetic modifications affect patients directly and some indirectly. For example, in adrenoleukodystrophy (ALD), a neurological disorder occurs due to malfunction of oligodendrocytes and microglia where genetic modification can affect a patient directly. To recover from it, a corrected gene is inserted into the patient-derived hPSCs and transplanted into the

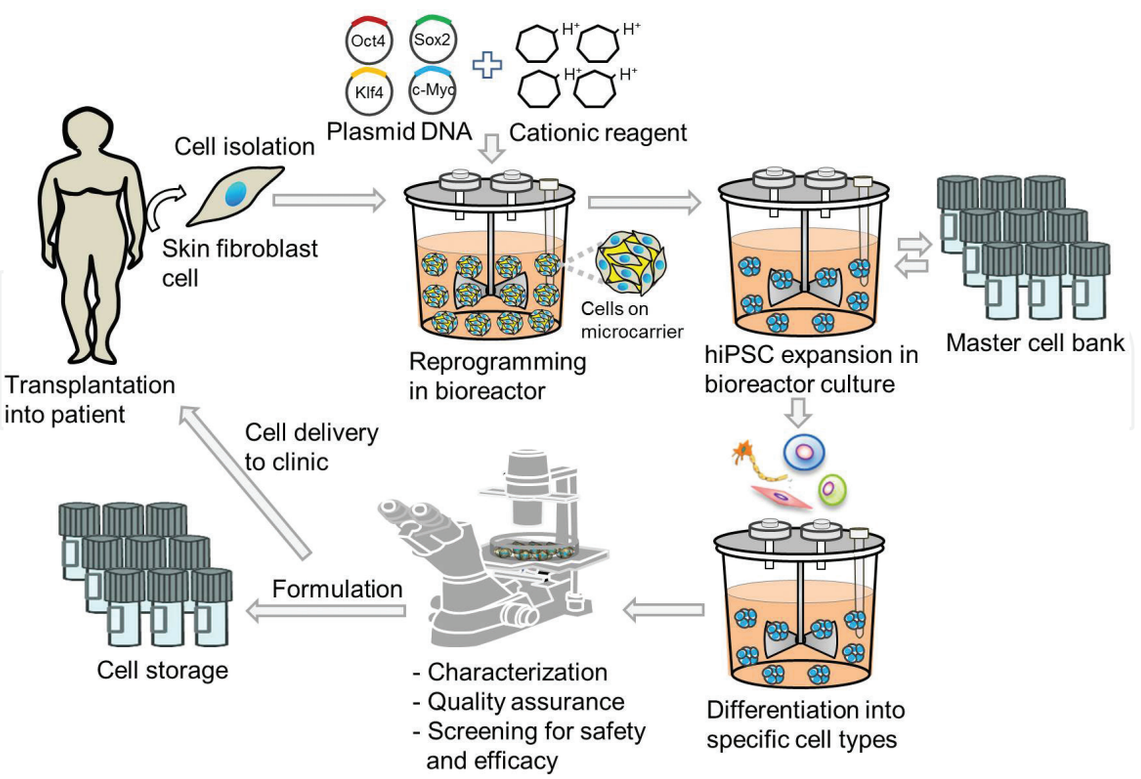

Figure 2.
Schematic illustrations of integrated single-step cell manufacturing strategies in bioreactor culture for stem cell therapy applications. Skin cells are isolated from the patient and reprogrammed to hiPSCs on microcarriers using a nonviral approach. After expansion as aggregates, hiPSCs are stored in a master cell bank or differentiated directly in bioreactor. After performing characterization, quality assurance, and screening for safety and efficacy, cells are delivered to hospital or stored in a cell bank for future use.

patient's brain, which is differentiated into microglia to promote production of myelin in the patient's brain that recovered the ALD [54].

In some gene therapy applications, patients are exposed indirectly to genetic modification. For example, in thalassemia, patient blood cells are extracted from the body and the cells are modified and enriched in *ex vivo* to target the specific antigens of patients' body [55]. Other indirect genetic modifications used for treating CAR or T-cell receptor (TCR) genes to T-cells [56], expression of CD40 ligand in dendritic cells [57], adenosine-deaminase severe-combined immunodeficiency [58], and beta-thalassemia [59], as well as deletion or insertion of desired genes in a specific genomic location. Among them, CAR-T cell therapy has got much attention for treating cancer-related diseases. These genetically modified T-cells can specifically target the antigens and kill the cancer cells efficiently [60]. CARs and TCRs are the mostly used receptors which are engineered to activate the T-cells [61]. Nowadays, a lot of CAR-T cell-based therapies are being established for treating advanced-stage lymphoma [62] and B-cell lymphoma [63] as well as other autoimmune diseases [64].

Viral vectors are commonly used to deliver genetic cargo to cells (**Figure 1**). This involves a two-step process: preparation for viral vectors and transduction for modifying the cells to express desired property. Lentiviral and gamma-retroviral are widely-used for their superior transduction efficiency but their transgenes are integrated with the host genome [65]. Another choice for viral transduction is adenovirus where viral transgenes are not integrated into the host genome but less efficient than lenti- and retro-virus. The major drawbacks in viral vector mediated transduction are concerns for safety of the products [66]. Viral vectors are widely used for reprogramming hiPSCs from skin fibroblasts cells [2].

Other methods for cellular transduction use nonviral approaches, including nucleofection or electroporation, or liposome-mediated delivery of DNA or RNA into cells. Although DNA vectors are easy to scale-up, carry large-size DNA with less immunotoxicity, this process is less efficient than the viral transduction. There are some other methods for skipping the use of viral vectors which are also efficient in doing the transgene expression [67–69]. Hsu et al. reported successful transfection by using commercially available nonviral cationic reagents, for example, TransIT-3D, TransIT-2020, XtremeGENE 9, XtremeGENE HP, JetPrime, Lipofectamine 3000, and Effectene and compared their transfection efficiency [70]. Warren et al. reported efficient reprogramming of hiPSCs from various cell sources by using mRNA and differentiated the cells into three germ layers [71]. hiPSCs were also reprogrammed by using recombinant protein that also maintained all the three germ layers [72].

Since transgene possesses high risk of cancer-causing agents; therefore, removal after transduction is highly desired. There are a few methods developed for the removal of these vectors. One of the methods is the piggyBac transposon system, which has been used to remove tandem Yamanaka reprograming genes Oct4, Sox2, Klf4, and c-Myc from iPSCs following reprogramming [73]. Removal of transgenes after incorporating CAR into T-cells used another transposon system called Sleeping Beauty, which successfully removed any genetic scar from the transduced cells [74, 75]. Likewise, transgene-free iPSCs have also been produced by Cre excision of reprogramming genes via loxP sites [76]. Integration-deficient viral vectors are also good candidates for producing transgene-free cell therapy products by mutating viral integrase [77]. Another approach is to use site-directed integration using targeting nucleases [78–80].

Various genome engineering technologies have been explored for gene addition, deletion, or correction in the cell therapy industry and are increasing day by day [81]. The most widely used targeting nucleases are zinc-finger nucleases (ZFNs), clustered regulatory interspaced short palindromic repeats (CRISPR)/Cas

endonucleases, or transcription-activator like effector nucleases (TALENs) [82]. Although the CRISPR/Cas system has recently received much attention due to broad use in genome engineering of patient cells [83], ZFNs are also popular for treating graft-versus-host disease in T-cell therapy [84].

Recently, a nuclease dead variant of Cas9 bearing a transcriptional trans-activator has recently been used in cellular reprogramming by activating the transcription factors Oct4 and Sox2, which maintained pluripotency and expressed the markers for the three germ layers [85].

Although genetic modification is a rate-limiting step in the cell manufacturing industry, the conventional methods make it more complicated because it is a multi-step process. Conventional genetic modification in planar culture is also costly, labor-intensive, and time-consuming. The bioreactor is a better platform for producing large-scale genetically modified cells for commercial purposes because cell expansion is possible in the same vessel which makes the process straightforward (**Figure 2**). For genetic modification in bioreactor, Hsu et al. recently reported how to transfect reprogramming factors in bioreactor where they tried eliminating viral vectors for gene delivery by using cationic reagents [78]. Generally, transfection of reprogramming factors for generating induced pluripotent stem cells (iPSCs) is done in adherent culture and then cells are expanded in 2D or 3D which is a two-step process. By integrating the genetic modification step in bioreactor, it is possible to establish a single-step process which enables cell manufacturing in automated and closed bioreactor system.

Genetic modification is also a challenging step in CAR-T cell therapy-based products. In CAR-T cell therapy, generally cells are isolated from patients' blood sample and then the cells are expanded after selection and activation. Finally, the cells need to be transduced with the CAR or any other antigens depending on target diseases. Conventional methods for genetic transduction are based on planar culture where every step is performed in open culture system. Recently, a few steps are integrated in bag culture system where selection, activation, and expansion can be done in a single step using DynaMag™ CTS™ [86], whereas the Xuri cell expansion System developed by GE Healthcare can expand cells in large numbers [87–89].

Although washing and concentrating the final product are integrated by the COBE® 2991 system developed by Terumo BCT [90], the transduction step is still not integrated in any of the above systems. Integrating the transduction step with the expansion and formulation will make the CAR-T cell therapy straightforward and performing these steps in bioreactor is a good platform since the physiological parameters as well as automated operation is possible in bioreactor culture. Miltenyi Biotec developed a device named CliniMACS Prodigy™ which is based on bag culture for CAR-T cell therapy. This device integrated major steps especially cell preparation, selection, activation, expansion, transduction, washing, and formulation in an automated system [91–93]. Such integration in the bioreactor will pave a straightforward method for producing cell-based products in a closed and automatic method.

3.2 Integrated system for large-scale expansion and differentiation in bioreactor

Current manufacturing practices for stem cell-based products are multi-step: derivation, expansion, and differentiation. In this process, patient-derived skin fibroblast cells are transduced with reprogramming factors in the planar culture. After deriving hiPSCs, cells are expanded in planar or bioreactor culture to obtain a large number of cells. Then cells are differentiated to target cells of interest. The differentiated cells are characterized and transplanted to the patient in a dose-dependent manner. As this process is complicated with multiple steps, it poses high risk of contamination to the final products. Moreover, maintaining cGMP culture

platform is also mandatory for cell-therapy products [94–96], which makes the cell manufacturing process more complicated. Therefore, developing an integrated system that can combine all these steps from derivation to final products is required. Here, the bioreactor may be a good platform for doing this (**Figure 2**).

The bioreactor platform is widely used for the large-scale expansion of hPSC-based cell therapy production because bioreactor is easy to operate in an automated mode where various physicochemical parameters can be regulated in a closed-system. Two groups have demonstrated that the bioreactor is conducive to cellular reprogramming [97, 98]. Shafa et al. reported a significantly higher reprogramming efficiency in the bioreactor compared to the planar culture [97]. Since mesenchymal-epithelial transition (MET) is an important early step in cellular reprogramming [99], transformed fibroblasts that are moved into the bioreactor will form aggregates that are efficiently expanded in the bioreactor. Indeed because fibroblasts are substrate-dependent, bioreactor culture may be promoting aggregate formation and therefore cellular reprogramming.

Unfortunately, bioreactor reprogramming methods require genetic modification (retroviral, piggyBAC) prior to bioreactor expansion. It is theoretically possible to pursue cellular reprograming fully and completely in the bioreactor. Recently, for example, Hsu et al. has demonstrated that it is possible to transfect human fibroblasts directly on microcarriers [70]. Reprogrammed cells in theory will leave the microcarrier to form aggregates in the bioreactor via MET.

Following bioreactor derivation of hPSCs, the next big steps are expansion and differentiation. Generally, a large number of cells are required for an effective cell therapy application, which is ranging from 10^8 to 10^{10} cells per 70 kg patient [100]. In the conventional process, cell expansion is performed in planar culture. However, it has many drawbacks and limits the cell expansion in various ways. Planar culture is unable to provide enough growth surfaces for the unlimited expansion.

Another major drawback is surface coating. Extracellular matrix (ECM) is needed for surface coating which is initially derived from animal sources, which poses high risk in clinical-grade manufacturing. Currently, recombinant ECM has been discovered, which can be used efficiently for clinical applications [101]. The advancement in cell coating also stimulated the advancement of integration and automation of cell expansion in adherent culture.

Automated planar culture systems have been established for the expansion of hPSCs for clinical-grade cell manufacturing. One of the notable automated systems for cell manufacturing is CompacT SelecT™ developed by the TAP Biosystems. This system is based on T-flask where 90 T175 flasks can be accommodated for large-scale expansion of cells. All the cell culture steps, cell counting, seeding, medium change, passaging, and plating as well as transient transfection can be done automatically by using this robotic system. However, such systems are not used for differentiation since differentiation is a complicated process, which needs several components to add in the culture medium. As a result, the expansion and differentiation process in planar culture is mostly disintegrated.

Cell expansion in bioreactors need not require surface coating except for microcarrier culture. Bioreactor also provides enough growth surface availability. Generally, a single bioreactor (100 mL working volume) is enough for providing clinically relevant number of cells for autologous cell therapy applications. Several types of bioreactors are employed for the expansion of hPSCs [102]. For anchorage-dependent expansion of hPSCs, microcarriers need to be coated with ECM for cell attachment in the bioreactor [100, 103–105].

After large-scale expansion, cells are harvested by detaching them from the microcarrier using enzymatic treatment. Nonenzymatic detachment is also available by changing temperature or pH [18–20]. Bioreactor expansion of hPSCs on

microcarrier is troublesome for clinical application because it needs an extra step for microcarrier separation from the final cell harvest. On the other hand, aggregate culture in bioreactors may not necessarily need a detachment step for harvesting [21–25] and clinically relevant numbers of cells can be produced in a single bioreactor as aggregate [21, 106–108].

A major drawback in aggregate culture is the size limitation. With the increase in aggregate size, the growth potential decreases in the large size aggregate due to diffusion limitation of oxygen and nutrients [109]. Therefore, maintaining aggregate size is an important issue to maintain high growth rate as well as high quality for cell therapy applications [21].

After expansion, cells can be differentiated in the same vessel which makes bioreactor culture a unique choice for integrated biologics manufacturing. Bioreactors were used for differentiation of hPSCs into various cell types, especially for cardiac [110–112], hepatic [113, 114], and neural [115] lineages. To provide straightforward methods for clinical applications, integration of expansion and differentiation is important and there are several reports published recently where expansion and differentiation were integrated [108, 116–118]. However, the integration of derivation with expansion and differentiation is still facing complications and there are a very few reports available.

Steiner et al. reported integration of derivation, propagation and differentiation of hESCs in suspension culture where hESCs were isolated from the inner cell mass in suspension culture that did not involve feeder cells or microcarriers [119]. However, the integration of derivation, expansion, and differentiation is not still realized for personalized medicine especially for autologous or allogenic cell therapy applications. Such integration is needed for overcoming the multi-step cell processing, which will reduce the risk of contamination and save cell processing time as well as reduce manufacturing costs for cell therapy manufacturing.

4. Concluding remarks and future directions

Cell therapy applications utilizing stem cells are increasing day by day and several clinical trials are ongoing to treat incurable diseases. With the growing need for cell-based products, the manufacturing facilities should be compatible for fulfilling the market demand by supplying safe and effective cell-based products. Since the current manufacturing systems are stuck with several drawbacks, especially multi-step processing which poses high risk of contamination as well as long processing time which contributes to increase culture cost, a more straightforward system is required. Bioreactor-based cell manufacturing system can provide a single-step and straightforward processing of cell-based products. Integration of different steps, especially genetic modifications, derivation, and expansion as well as differentiation in bioreactor will pave the future of manufacturing cell-based products. The integrated biologics manufacturing in stirred suspension culture will significantly reduce the risk of contamination of final products, increase product efficacy, and reduce cell processing time and provide a cost-effective platform for cell manufacturing for cell therapy applications.

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SCN conceptualized, designed, and wrote the manuscript. DER conceptualized and revised the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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