Towards biomanufacturing of pluripotent stem cell derived products: scale out and scale up

Pluripotent stem cell-related products represent a new product category from the pharmaceutical and biotechnology industry. The increased demand in disease research, drug screening and toxicity testing motivates the activities for large-scale production of stem cells or their derivatives through scale out and scale up approaches. This article highlights recent advances and the challenges in scale out and scale up of pluripotent stem cell derived products. While most current processes use scale out approaches, emerging technologies are being explored to fulfill the potential of scale up culture systems with the emphasis on the design of bioinspired process for large-scale biomanufacturing of stem cells.

Keywords: biomanufacturing • pluripotent stem cells • scale out • scale up

Market potential of stem cell-based products

In 2011, the market for stem cell therapy and stem cell related products was estimated as US$5.3 billion in the USA and it is expected to expand to US$8.8 billion by 2016 [1]. Among different stem cell types, commercialization of human pluripotent stem cell (hPSC) related products has been a novel path and direction in pharmaceutical and biotechnology industry during the past 10 years. Both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) have extensive proliferation ability and the capability to differentiate into almost all the types of somatic cells [2,3]. HPSCs and their derivatives can be used for reagent development, establishing disease models (e.g., microcephaly) [4], identifying novel drugs [5,6] and treating the diseases [7], opening a new avenue for biotechnology and pharmaceutical industry. In recent years, many new products (culture medium, culture substrates, antibodies, reprogramming kits and so on) related to hPSC cultures become commercially available from companies such as Invitrogen LifeTechnologies (Carlsbad, CA, USA), Stemgent (Cambridge, MA, USA), STEMCELL Technologies (Vancouver, Canada), R&D Systems (Minneapolis, MN, USA), Lonza (Walkersville, MD, USA), GE Healthcare (Little Chalfont, UK), Corning Incorporated (Corning, NY, USA), BD Biosciences (San Jose, CA, USA) and Millipore (Temecula, CA, USA) etc. [8], which provide reagents for academic research and life science sectors in industry. Cellular Dynamic International (CDI, Madison, WI, USA) [9], Axiogenesis [10] (Cologne, Germany, with Sigma® Life Science as the distributor), and ReproCELL (Kanagawa 222-0033, Japan) [11] also supply hiPSC-derived cardiomyocytes, neurons, hepatocytes and several other cell types for disease research, drug screening and toxicity testing. With the increased demand of the stem cell market for research, drug discovery and the potential applications in cell therapy, the expansion and differentiation of hPSCs or their derivatives at large scale have been under development [12,13]. There are generally two strategies for large-scale production of stem cells or their derivatives: scale out and scale up (Figure 1). Scale out approach uses the replication of culture vessels (linearly proportional increase in surface area) with a
Figure 1. Illustration of scale out and scale up of the process. (A) Scale out. The scale is increased by increasing the number of culture vessels. This figure was produced using Servier Medical Art. (B) Scale up. The scale is increased by increasing the culture volume, which usually involves the use of bioreactors. Adapted with permission from [17].
Embryoid body formation [20], the use of low attachment culture vessels still can meet the current expansion need. Controlling the raw materials and using the defined culture reagents to enable robust production (e.g., the peptide surface for hPSC expansion [21], dissociation method, defined medium [22,23]) are probably the main focuses of process development of hPSC culture in scale out strategy. Given the example of the surface coating for hPSC expansion, the commonly used mouse tumor-derived Matrigel® has undefined composition and is subjected to lot-to-lot variation and tedious coating procedure. The single proteins such as laminin 511, the peptide sequence from vitronectin and the polymer surfaces with defined chemistry are identified in recent years to support hPSC expansion [18,24]. Continuing efforts such as reducing the cost of peptide surface (e.g., Corning Synthemax®, US$100 per T75) and developing the commercialized process for polymer surface (e.g., poly[2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide [PMEDSAH]) are still required to further improve the scale out processes [18].

The further increase in culture scale, however, may require process automation. Scale out strategy triggers the development of automation device to handle the medium exchange and even cell passaging. For example, the CompacT Select automation platform has been tested for the passage of induced pluripotent stem cells (iPSCs) as cell clumps [25]. The same platform was also used to scale out the adherent hiPSC-derived long-term self-renewing neuroepithelial-like stem (It-NES) cells for drug screening, where the medium-throughput screening of 1000 compounds was demonstrated [26]. Other automation devices are also available such as the Cell host® system for adherent stem cell cultures [17,27]. While most systems have evaluated the undifferentiated marker expression (e.g., Oct-4 and Nanog) and the three-germ layer differentiation, the specific lineage differentiation toward the target products (e.g., hPSC-derived neural progenitors) is the more stringent assessment.

### Challenges of scale out strategy

The need to define the biomanufacturing process using scale out strategy motivates the process development activities for characterization of raw materials and culture refinements. Especially, the automation process to increase the lot size still meets several challenges. Current automation devices are designed for a specific protocol and lack of flexibility for procedure change. Modifications need to be made to refine the device for specific cell type as seen in It-NES cell expansion compared with undifferentiated hPSCs [26]. Daily

### Key term

**Microcarriers**: Support matrices at microscale that enable the growth of adherent cells in suspension. While conventional microcarriers are usually spherical, microcarriers can have different shapes such as cylindrical shape.

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**Table 1. A comparison between scale out and scale up strategies.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Scale out</th>
<th>Scale up</th>
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<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Horizontally scaling method</td>
<td>Vertically scaling method</td>
</tr>
<tr>
<td></td>
<td>Replication of the culture vessels in many batches</td>
<td>Increasing culture volume in a one or a few vessels (usually bioreactors)</td>
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<tr>
<td><strong>Advantages</strong></td>
<td>Simplified scalable process</td>
<td>Unlimited scalability (e.g., $10^{9}$–$10^{12}$ cells per batch)</td>
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<td></td>
<td>Minimal impact on cell phenotype</td>
<td>Cost effective</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Time-consuming and labor-intensive</td>
<td>Culture environment change may affect cell phenotype</td>
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<td></td>
<td>Exist batch-to-batch variations</td>
<td></td>
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<tr>
<td></td>
<td>Limited scalability (up to $10^{9}$–$10^{10}$ cells per batch)</td>
<td></td>
</tr>
<tr>
<td><strong>Recent Advancements</strong></td>
<td>Defined medium development; defined substrate development</td>
<td>Microcarrier-based culture systems and aggregate-based culture systems</td>
</tr>
<tr>
<td><strong>Challenges</strong></td>
<td>Cost reduction for culture media and substrates, among others, process automation</td>
<td>Few systems for the differentiated cells, process robustness, reducing the cost, process integration</td>
</tr>
<tr>
<td><strong>Applications</strong></td>
<td>Most current cGMP processes for stem cell derived products; autologous cell therapy; early stage of processes that provide a small number of cells</td>
<td>Cell therapy and drug screening that require a large quantity of the cells, for example, pluripotent stem cell-derived cardiomyocytes</td>
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</table>

**cGMP**: Current GMP.
microscopic evaluation of the cultures (e.g., checking confluency, low-grade contamination and plating efficiency) is also difficult and needs the incorporation of additional part (e.g., microscope with digital platform) in the automation platform [28]. Incorporation of a centrifuge machine and the sampling device in the automation platform is usually required as well. In addition, most current automation devices are designed for adherent cells (e.g., undifferentiated stem cells) and few devices have been reported for suspension cells (more often seen during differentiation of hPSCs). With the discovery of iPSC technology, automation of iPSC reprogramming process is also necessary because generating a large amount of iPSC lines is very tedious [29]. Overall, increasing the process capacity during scale out has been the main goal for process improvement.

**Scale up strategy**

Various scale up systems have been developed for hPSC culture recently, especially microcarrier cultures and aggregate cultures in suspension [12,30–32]. The recent advances in suspension culture systems of hPSCs have been elegantly reviewed in several articles [12,33–36]. While the proof-of-concept study of using stirred bioreactors to produce microcarrier-expanded and aggregate-expanded stem cells have been demonstrated, establishing a robust process based on suspension bioreactors is still a challenging task. Here several more recent developments in microcarrier- and aggregate-based culture systems are highlighted.

Surface coating of microcarriers with extracellular matrix proteins and the design of new microcarriers for microcarrier-based culture systems are required. HPSCs usually need special substrate for cell attachment and self-renewal. So the commercially available microcarriers have been coated with extracellular matrix proteins such as Matrigel, laminin and vitronectin for hPSC expansion and differentiation [33]. Microcarriers with synthetic peptide surface eliminate the need for coating as the new development compared with conventional microcarriers, while the cost still needs to be reduced [18,37]. Another challenge is the dynamic seeding of hPSCs onto the microcarriers and the control of hPSC/microcarrier aggregate evolution for efficient expansion. Recent finding used cationic surface charges (poly-L-lysine) and laminin to enable the seeding and aggregate evolution under constant agitation [38]. The agitation speed of microcarrier culture ranges from 25 to 70 rpm in general [35]. Especially, the flow stress in cardiomyocyte differentiation of hPSCs in stirred cultures was found to reduce the differentiation efficiency and thus the low shear stress using wave flow motion and intermittent agitation were used to improve the differentiation (from 23 to 65%) [39]. Microcarrier culture may result in the small particulates due to the bead crushing or agitation, which are usually purified along with the cells [40]. Such contamination of foreign materials with the cells is not acceptable for cell injection. The microcarrier design includes size (from 10 to 250 μm), shape (e.g., sphere or cylinder), materials composition (e.g., collagens) and surface properties (e.g., positive or negative charge) etc. [35,41]. The impact of microcarrier properties on stem cell culture has been well discussed elsewhere [33]. Taken together, the design and manufacturing of novel microcarriers are still required for the use of microcarriers in the scale up process.

Aggregate-based expansion of hPSCs has been refined during the past several years since the initial demonstration of growing hPSC aggregates in suspension [42–44]. The main advantage of aggregate-based culture is the elimination of adherent substrate in the system. The aggregate cultures may result in heterogeneous cell population due to the diffusion limitation [45]. Therefore the control of aggregate size (in the range of 100–200 μm) is a critical consideration in the design of large-scale culture system [46]. In addition, the expanded cell numbers achieved in aggregate-based cultures has been lower than microcarrier-based cultures (1–2 × 10^6 vs 3–6 × 10^6 cells/ml) possibly due to the diffusion limitation [33,47]. So understanding the hydrodynamic environment in stirred bioreactors has been important for aggregate culture design [46]. The evaluations of different culture medium, splitting intervals, agitation speeds and the control of aggregate size have also been performed with stepwise experimental designs [48,49]. Recently, factorial experiment design with two parameters (inoculation density and agitation rate) and three levels (3^2 = 9) was demonstrated in bioreactor runs to reveal the interactions between culture variables [50]. However, it will be difficult in running more complex factorial design experiments (e.g., 2^2 = 32) in bioreactors and thus developing scale down models is necessary.

Another scale up system that has been reported is based on the packed bed cultures, while the published results for packed bed bioreactors are very limited. Compared to suspension cultures, packed bed bioreactors allow high cell density (e.g., 1 × 10^8 cells/ml vs 5 × 10^6 cells/ml) and small culture volumes (1–40 l vs 300 l) [19]. Hollow fiber-based 3D perfusion bioreactor is also designed with interwoven four-compartment capillary membrane technology for the expansion of undifferentiated ESCs [51]. This system can achieve about 5 × 10^9 cells, which replaces up to 800 of 100-mm Petri dishes. However, the cell harvesting may become a challenging step in these systems. Novel strategies to ease the cell harvesting and the transition from expansion stage to differentiation stages have been explored
as shown in the development and characterization of different types of hydrogels [52,53], while the implementation of hydrogel systems in the industrial process is yet to be demonstrated. To avoid the agitation, culture media containing polymers such as methylcellulose and low-acyl gellan gum have been evaluated to prevent hPSC sedimentation in static culture [54]. The scale up potential of this system has been demonstrated in gas-permeable culture bags.

**Challenges of scale up strategy**

Implementation of a large-scale bioreactor in manufacturing facility still has several challenges. The robustness of the process is probably the main concern because it may impose a higher risk compared with scale out process due to the large batch size. A variety of different prototypes of bioreactor operations with different culture parameters have been developed [12,47,55–57], while the standardized process is lacking. Most systems have focused on undifferentiated hPSCs and the expansion of lineage-specific differentiated cells is less reported. Especially, the biological functions of the bioreactor-produced cells may be different compared with T-flask culture and the implications of potential changes need to be evaluated even if the product attribute appears to be positive (e.g., higher purity of the desired cells). The reduced lineage-specific differentiation (e.g., cardiomyocytes) in spinner microcarrier cultures and the preservation of pluripotent cells in aggregate-based bioreactor cultures have also been observed due to the possible impact of shear stress on the signaling pathways (e.g., TGF-β) [58,59]. In general, the datasets of the large-scale bioreactors are not large enough compared with the datasets from the T-flask culture in order to demonstrate the process robustness. While bioreactors allow the online monitoring and feedback control of the process, the use of such system in stem cell production (especially hPSCs) is still at early stage of development.

The strategies of scale out or scale up depend on the type of the differentiated cells from hPSCs. For the treatments that only need a low dose of the cells (e.g., $5 \times 10^4$ cells per patient for dry age-related macular degeneration) [60], a scale out process is sufficient. For the applications that need high doses of the cells (e.g., $10^8$ cells per patient for myocardial infarction), a scale up process become critical toward the potential clinical trials. Both types of processes need the careful process development efforts to reduce variability and to increase the successful rate of the production runs.

**Reducing the variability of stem cell biomanufacturing**

For both the scale out and scale up processes, the importance of the process is indicated by the statement ‘the process is the product’ [40]. One big challenge of stem cell-based processes is the large variability of the produced cells, especially for the differentiated cell types. Significant work on data analysis (e.g., trending analysis) needs to be performed to analyze and identify the sources of variability. Understanding and reducing process variability is the basic responsibility of process scientists and engineers during the course of scale out and scale up of the current processes. Examples of some common sources of variability in stem cell production are discussed as follows.

**Raw materials**

The critical raw materials in the culture system, which include culture medium, growth factors, harvesting enzymes and extracellular matrix substrates, are very likely to contribute to the process variability. Hence, comparability study for the critical raw materials (e.g., different vendors, lots, preparation methods) needs to be performed even just for the understanding of lot-to-lot variability. Usually, the most critical raw material in stem cell bioprocessing is the starting cell source, which exhibits donor-to-donor variability, cell line-to-cell line variability, passage number variability, cell bank variability, and so on. Thus, characterization of the starting cell source for cell production is the priority in process development. The correlations between the starting cell source and the final cell products will be helpful for process design, while establishing such correlations, if exist, needs tremendous work.

**Operational procedure**

Examples of culture parameters in operational procedures are seeding density, enzyme incubation time and culture medium volume etc. The standard operational procedures should provide a range of operation parameters instead of an exact value to allow the flexibility of the production, while the defined operational range should cause the minimal process variations. One consideration is that the manufacturing usually takes longer time for each step compared with the research lab procedure, and hence the process limits need to be well defined for the critical step (e.g., the time to load cryopreservation buffer for 200 vials compared with five vials), which can be evaluated using a quality-by-design approach [61].

**Operators**

The hPSC expansion and differentiation process needs to be robust enough so that any trained operator can follow the procedure and make the desired products. The variations of operators are usually due to the variations of the process and procedure. For example, the cell scraping during hPSC passaging by different
operators results in cell clumps of variable size. So the single-cell dissociation would be preferred for cell passing to minimize variability from operators. In general, standardizing the procedure for all the operators is required for biomanufacturing.

**Facilities**

The facilities need to be monitored to understand the instrument variations and operational variations. For example, the CO\(_2\) level and the temperature of the incubator fluctuate due to the frequent opening of the incubator door, which should be minimized. Cell and reagent storage facilities also need to be well monitored.

**Conclusion & future perspective**

Design and establishing scale out and scale up processes have been an active field in stem cell engineering in recent years (Table 2). With most activities focusing on undifferentiated cell expansion, more efforts should be made to produce the differentiated cells. The differentiation of hPSCs into specific cell types requires different protocols (e.g., adherent vs suspension, specific growth factor cocktails etc.), which would result in a variety of scale out and scale up processes for bioinspired differentiations compared with the expansion of undifferentiated cells [62]. The bioinspired process relies on the temporal modulation of signaling pathways (such as Wnt) at the right differentiation stage [63]. The increased differentiation efficiency would reduce the process cost and ease the scale up process. Current scale out processes still need refinements for cost-effective production (e.g., reduce the cost of peptide surface). While scale up processes have been demonstrated in various proof-of-concept studies, the processes still are not robust enough to be implemented in cGMP facility and thus need more detailed investigations on process parameters (e.g., effect of shear stress). During scale up process, the environmental factors (e.g., shear stress) may shift the differentiation paradigm and cell phenotype by affecting the signaling pathways [57]. The influence of these factors needs to be better elucidated. While current scale out and scale up approaches mainly refer to upstream cell production processing, the production capacity of downstream separation or purification and cell storage also need to increase at large scale, which more likely become the bottleneck of the process in the near future [64]. Ultimately, the integration of multiple stages of upstream, downstream and cell storage processes will be required during large-scale stem cell biomanufacturing.

**Financial & competing interests disclosure**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

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**Table 2. Examples of scale out processes and scale up processes.**

<table>
<thead>
<tr>
<th>Process</th>
<th>Applications</th>
<th>Study (year)</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Scale out:</td>
<td></td>
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<tr>
<td>– Adherent cell expansion and the embryoid body formation</td>
<td>hPSC-derived retinal pigment epithelium for treatment on Stargardt’s macular dystrophy, and dry age-related macular degeneration; low-dose study: 5 x 10^4 cells per patient</td>
<td>Schwartz et al. (2012)</td>
<td>[60]</td>
</tr>
<tr>
<td>– Adherent cell expansion and the aggregate formation</td>
<td>Manufacturing process for hPSC-derived pancreatic progenitors; early stage of process with the use of T-flask, well plates and Cell Factories</td>
<td>Schulz et al. (2012)</td>
<td>[15]</td>
</tr>
<tr>
<td>Scale up:</td>
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<tr>
<td>– Microcarrier-based culture</td>
<td>hPSC-derived cardiomyocytes in bioreactors and the culture was sensitive to shear stress. Use Cytodex 1 microcarriers coated with Matrigel, intermittent agitation and rocker movements</td>
<td>Ting et al. (2014)</td>
<td>[39]</td>
</tr>
<tr>
<td>– Aggregate-based culture</td>
<td>Undifferentiated hPSCs expanded in stirred bioreactors for making cell bands. Optimization of medium, seeding density and splitting schedule. Differentiation in cardiomyocytes was also evaluated</td>
<td>Chen et al. (2012)</td>
<td>[49]</td>
</tr>
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</table>

hPSC: Human pluripotent stem cell.

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**Key term**

**Quality-by-design:** an approach that enables the design of processes to supply products that meet the predefined quality levels, based on science and risk management.
Executive summary

**Market potential of stem cell-based products**
- Stem cell-derived products can be used for basic research, drug screening, disease modeling and cell therapy.

**Scale out strategy**
- Scale out: modifications and improvements are required to meet current market demands.

**Scale up strategy**
- Scale up: microcarrier- and aggregate-based bioreactor systems are being developed to meet increased cell number requirements.

**Future perspective**
- Continuing efforts need to be made for making differentiated cell products through process integration.

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• of interest; •• of considerable interest

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**A complete summary of microcarrier-based system in stem cell culture.**

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