International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 10; Issue 05 (D); May 2021; Page No.24502-24508
DOI: http://dx.doi.org/10.24327/ijcar.2021.24508.4862

Research Article

APPROACHES FOR SCALING UP STEM CELLS USED IN THERAPEUTIC PROCESS

Muktha Bharadwaj S

R V College of Engineering

A R T I C L E I N F O

Article History:
Received 13th February, 2021
Received in revised form 11th March, 2021
Accepted 8th April, 2021
Published online 28th May, 2021

Key words:

Current good manufacturing practices (cGMP), human-induced pluripotent stem cells (hiPSCs), human embryonic stem cells (hESCs), computational fluid dynamics (CFD).

A B S T R A C T

Cell therapy is one of the most fastest-growing segments in the life sciences. Involves the delivery of living cells to a patient for the treatment of disease. Cell therapy offers a promising clinical approach in treatment of diseases. Both induced pluripotent stem cells and embryonic stem cells are known for their potential use in drug screening, disease modeling study and cell-based therapy. Induced pluripotent derived stem cells (iPSCs) have been used in a wide variety of small and large animal models to treat many different diseases, as it holds a great potential to generate novel and curative cell therapy products. Human induced pluripotent stem cells (hiPSCs) has a great scope of application in regenerative medicine due to their inherent ability to self-renew and differentiate into cells from three embryonic germ layers. Scalable and standardized culture processes of hiPSCs produced in large quantities are required for clinical applications, but the current methods to generate novel therapies do not meet commercial and clinical demands, lack scalability, requires large footprint and labor-intensive, hence it is essential to develop scalable manufacturing processes that accommodates the generation of high-quality iPSCs derivatives under controlled conditions. The current methods for scaling up cell therapy process is based on empirical and geometry-dependent methods which does not precisely represent the hydrodynamics of 3D bioreactors as it requires multiple iterations of scale up studies that results in increased timeand development cost.

INTRODUCTION

Cell-based therapies have the potential that contribute to disease treatment, where in living cells are introduced as therapeutic agents as regenerative medicine and in biomedical applications. Live cell therapies achieve more complex and integrated functions than drugs resulting in more effective disease treatments. hPSCSsuch as both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) represent a highly promising cell sources for new cell-based therapies, due to their ability to continuously regenerate and differentiate into all cell types of an organism (pluripotency), and other properties such as high proliferationand self-renewing capability. hiPSCsare mostly preferred as they can be derived from individual patients in order to avoid histocompatibility issues for cell-based implants and therapies, which can be used for a variety of applications such as personalized drug testing. hiPSCs hold a great promise for regenerative medicine as the pluripotent nature of these cells allow them to differentiate into virtually any cell type in the body. The generation of hiPSCs from somatic cells became a major leap forward in the field of regenerative medicine.

Corresponding author:*__Muktha Bharadwaj S__
R V College of Engineering

In addition to stem cells, primary immune cells such as genetically-modified T cells, tumor infiltrating-lymphocytes, and dendritic cells showed powerful anti-cancer effects and are quickly adopted for cell therapies (Chen *et al* 2019). Biomaterials are used during cell production to establish a biomimetic microenvironment to promote proliferation and cell adhesion while maintaining target cell phenotype and genotype. The increasing use of automated production methods and auxiliary biomaterials provides an opportunity to increase production efficiency using standardized GMP-compliant procedures and to improve quality control.

Number of cell required for treatment

- The global market for cell manufacturing is estimated to reach $11 billion by 2030 (assuming average growth rate of 14.9%). A single dose of therapeutic cells for transplantation in adults is $10^8 to 10^9$cells (Chen *et al* 2019).
- Development and production of cell used for replacement therapies from iPSCs is carried out in a small scale culture that is not suitable for producing a large number of cells required for therapies. For treating a heart failure it is estimated that 10^9 cells would be required per patient (Mummery *et al*., 2012). The increasing rate of heart failure potentially requires

thousands of doses a year, the number of cells required annually is$10^{11} to 10^{14}$(Shafa*et al* 2019).

- For spinal cord injury, the number of cells required per treatment ranges from $2 \times 106\ to\ 20 \times 106$ (Kwok *et al* 2017).
- Other general estimates of the cell numbers required for cell based therapies are $5 \times 104\ to\ 1 \times 1010$ (Oh *et al* 2013). This range of cell numbers is used for many other applications of hiPSCs such as in bio-artificial organs, disease modeling (Mekhoubad*et al* 2012), and in high throughput drug screening.
- CAR T cells are genetically engineered to initiate an immune response against cancer cells and to target human CD19. CAR T cells have shown clinical efficacy against malignant tumors including non-Hodgkin lymphoma and acute lymphoblastic leukemia (ALL). CAR T-cell therapy for ALL was approved by FDAin the year 2017. The first allogeneic CAR T-cell therapy using non-gene editing methods was approved by FDA in 2018 (Chen *et al* 2019).
- Treating patients using cell therapy for diabetes with allogeneic Hescderived cells is in progress (clinicaltrials.gov), which requires about 1 billion cells for a single application (Kwok *et al* 2017).
- 50 million people suffering due to Alzheimer's disease, 18.1 million cancer cases along with 9.6 million cancer deaths worldwide was recorded in the year 2018. Cell-based therapy offers a new technique for treating these diseases along with other intractable diseases. In 2007, the first HIV cure was achieved by transplanting allogeneic human stem cells and 10 years later, a second HIV cure was achievedusing blood stem cell transplant, thus demonstrating the tremendous potential of stem cell therapy. In 2009, the world's first human embryonic stem cell clinical trial was approved by the FDA (Li *et al* 2019).

Problems faced during scaling up

- Therapeutic applications of hiPSCs and their derivatives require large quantities of cells that will be difficult to achieve using traditional two dimensional cultures.Current technologies for pluripotent stem cell expansion does not fulfil the demands in the need of clinical usage and regulatory boards, since they lack stringent quality control, do not meet GMP standardsand low cost efficiency. Hence, development ofscalable and standardized method for pluripotent stem cell expansion in compatible with automation and in a cost effective manner is of great interest. Particularly forthe stem cells groups that are robust, used at the laboratory scale, cost efficient adaptation to billions of cell-scale that represents a major challenge i.e, significant obstacle to clinical translation. Production of hiPSCin large quantities are used for cell based therapies and cell loaded implants through standard adherent culture can be hardly achieved as it lacks process scalability.
- Number of challenges faced during industrializing manufacture of iPSCsand their derivatives including:

a. Development of scalable bioprocessing platforms.
b. Access to acceptable starting material such as iPSCs generated under current cGMP.
c. Development of optimized and qualified analytical methods for assessing cellular characteristics and function. The use of cGMP acceptable starting material is mainly required for manufacturing clinical product, which includes reagents, consumables, and media used in the process along with the starting cell population.

Strategies for Scalable Expansion

***Commercial strategiesfor scale up cell therapy process* development**(https://www.bioprocessonline.com): This minimize risks through optimization, quality control, assay development, scaling, closing and automation. To achieve commercial success in cell therapy scale-up strategies, the process must be suitable for manufacturing environment and it should be scalable. The scale up strategy depends on the type of cell therapy (allogenic and autologous).

- **Allogeneic therapies** are used to treat many patients. The scaling up strategies of traditional bioprocessing, such as monoclonal antibody therapy processes which is common and is a seed-train approach, where scale up is across systems and progressively increasing in size. Scaling up within the same system is desirable in order to minimize handling risks and sterility in more patient derived cells. This can be achieved with low cell volumes, starting with batch feeding followed by ramping up feed and waste production rates with perfusion to progressively expand cells. Scaling up allogeneic processes can be optimized from the range of 100 mL to 2 L, and volume up to 25 L can be achieved in Cytiva'sXuri rocking bioreactors, which canaccommodate a very large batch up to 2000 L in Cytiva'sXuri rocking stirred-tank bioreactors.
- **Autologous cell therapies** are personalized for each patient, where the batch sizes are small. This method uses scale-out strategy, which uses additional small scale bioreactors to increase production throughput.For this process, 0.5 to 5 L volume range is usually enough. Large-scale bioreactors can be used for other immunotherapy platforms which require stimulatory cell lines or feeder layers.

Scalable Expansion strategies for Pluripotent Stem Cells (Netaet al. 2018)

The diversity in potential cell sources for cell therapy such as hiPSCs, adult stem cells, progenitor stem cells, hESCs etc., final therapeutic product includingsingle cells, encapsulated micro-tissues, secreted molecules and aggregates,desired cell yield and culturing methodologies has led to the development of many cellculture platforms with extensive research on the optimization of cell culture conditions for each purpose.

The following basic features are generally required for the production of clinically suitable cell therapy product which are homogeneous, scalable and reproducible:

1) **Automatic control and online monitoring** helps in tracking culture conditions at all times which is essential for better scalability system and optimization.In addition to it an automated feedback looped system reduces the heterogeneity that can arise from manual systems that might suffer from human error.
2) **Increasing robustness and homogeneity** of the process is performed by, Scaling up method by moving to a larger vessel, this is preferred over scaling out by using

multiple smaller vessels, as it minimizes the risk of contamination. The transition from small scale to large scale without anydramatic changes inculture conditions are ideally supported by scalable system, which enables optimizations to be carried out in a costefficient manner and in small volumes.

3) **Dynamic culturing** allows improved mass transfer to ensure optimized circulation of homogenous distribution of cells, nutrients and oxygen. The mixing technique used should be optimized for the desired product. Current dynamic culture techniques use air-based mixing, impeller-based rocking and stirring, rotating platforms. The main difference between these systems is application of shear force on the cells.
4) **Continuous media change** is important to remove waste products,supply cells with fresh nutrients and maintain the stability of the culture conditions.Sharp changes in growth factor such as nutrients which are limiting factors in the proliferation of hPSCs, pH, oxygen levels and concentration. Cell culture platforms whichsupports continuous media change along with appropriate cell retention apparatus is important for optimization of a large-scale system for cell therapy products.
5) **Cost effectiveness** of the process is affected by many parameters, the cost to yield ratio should be evaluated for each system. Optimized expansion processes will allow differentiation to the cell type required as the final product, allows high-rate proliferation, keep the cells genetic stability and pluripotent.

Approaches for scaling up

- Using appropriate methods to properly characterize the cell therapy manufacturing process is very important. The process of iPSCs differentiation to clinically relevant cells involves passing through a number of different stages and is necessary to understand the extent of differentiation through each stage. For an example, during differentiation of iPSCs to cardiomyocytes, the cells start from pluripotency to mesoderm to cardiac progenitors and finally mature cardiomyocytes.
- The development of a scalable system is needed to facilitate the large scale manufacture of iPSCs and their derivatives. The most commonly applied format for scaling up is stirred tank bioreactors due to their ability to accommodate a large range in volume (up to $2{,}000\ L$) and has relatively simpler numerical calculations for estimation of hydrodynamics between different size vessels and approximate scale up. However, the equations typically used rely mainly on the geometry of the bioreactors, impeller design, and would be quite inaccurate when geometries are not similar between small and large scale vessels. The hydrodynamics also have an impact on the phenotype and differentiation of iPSCs, and the inaccuracy present in these equations hinder the ability to properly scale from lower to higher volumes. Hence to address this issues CFD modeling has been developed to precisely calculate different hydrodynamic properties in fluids which are used for scaling up of bioreactor cultures (Borys*et al* 2018).
- CFD modeling is areliable and innovative methodology for scaling up of iPSC cultures in 3D bioreactors. Shafa*et al* 2019 utilized a spinner flask scale up protocol for differentiating iPSCs to cardiomyocytes and then translated it using a cGMP-derived hiPSC line to scale up in a 3 L computer controlled bioreactor.
- The stirred suspension culture technique is the most promising methodology for the large-scale bioprocessing of pluripotent stem cells and maintaining their pluripotency (Amit *et al* 2011). The aim of their study was to demonstrate the scalability of the technique in a single use stirred tank bioreactor (Kaiser *et al* 2011), and to develop a protocol for expansion of billions of human pluripotency stem cells using stirred suspension cell culture vessels, by maintaining hiPSCs properties such as, undifferentiated, self-renewing state and pluripotency of those cells.
- They (Oh *et al*.) first developed a method for expanding human embryonic stem cells in suspension culture on micro-carriers. This approach was later applied to the expansion of hiPSCs(Meng*et al* 2017).

Comparison between 2D and 3D cultures for scaling up of cells used in cell therapy (Netaet al 2018)

A. **2D cultures:** 2D cell cultures are being used since the early 1900s. In this systemscells are grown on flat dishes typically made of plastic. The cells are put on to coated surfaces where they spread and adhere.

2D Static T-Flask-Based Culture traditionally hPSCs are grown as a monolayer of cells in colonies, on either a extracellular matrix (ECM) or on fibroblast feeder layer that supports expansion and growth. This method of scaling up is scalable to some extent by scaling out using multiple vessels and by increasing the surface area of the vessels. The 2D static T-flask-based system meets the requirements for products that require personalized hiPSC-based products and small number of cells, such as autologous cell therapies. T-flask based systems are scaled out to create multilayered vessels for large-scale expansion for adherent cell-culture. Multilayered 2D static vessels available on the market include Hyperflasks(Corning), Cell-Factory(Thermo scientific) and Cell-STACK(Corning). Controlled 2D systems that are available in the mark*et al*lows automatic control of pH, monitoring media circulation and oxygen levels. This system applies lower shear forces compared to stirred-tank bioreactors.

B. **3D cultures:**
In late 1990s, 3D cell culture technique has gained more attention from scientists, as it provides more accurate models of tissues.

Aggregate-Based 3D Suspension Culture

This model first showed that dissociated human pluripotent stem cells in non-adherent conditions are able to form 3D spheres called embryoid bodies that supports spontaneous differentiation of undifferentiated human embryonic stem cells into the three established germ layers. It is shown that hPSCs can maintain their proliferative capacity and pluripotency over sequential passages in a matrix-free environment, as hpscs has the ability to aggregate in dynamic and static platforms.

Some different aggregate-based culture platforms are discussed below.

- **3D suspension culture platforms** which are based on orbital shaker. This method is easiest to establish

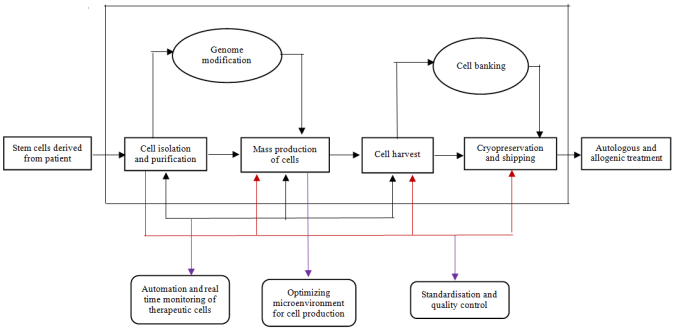
process development and small-scale expansion. Erlenmeyer flasks and low-attachment tissue culture multiwell plates kept on orbital shakers allows the testing of cell concentrations and several growth conditions in volumes of less than millilitre to a few hundred millilitres. It was demonstrated that human embryonic and induced pluripotent stem cells cultured in six-well suspension plates placed on an orbital shaker increases sixfold in number within 4 days. With the use of a defined media, pluripotency was maintained for up to 17 passages. These systems do not permit continuous media change and are not monitored, but the transition from shaker-based platforms to other 3D aggregate-based bioreactors is a common practice.

- **Spinner flask vessels**

These are useful in scaling up process to hundreds of millilitres. In this vessel dynamic conditions are acquired by rotating impellers similar to that in stirred-tank bioreactors. They are not automatically controlled and monitored hence, reproducibility is hard.

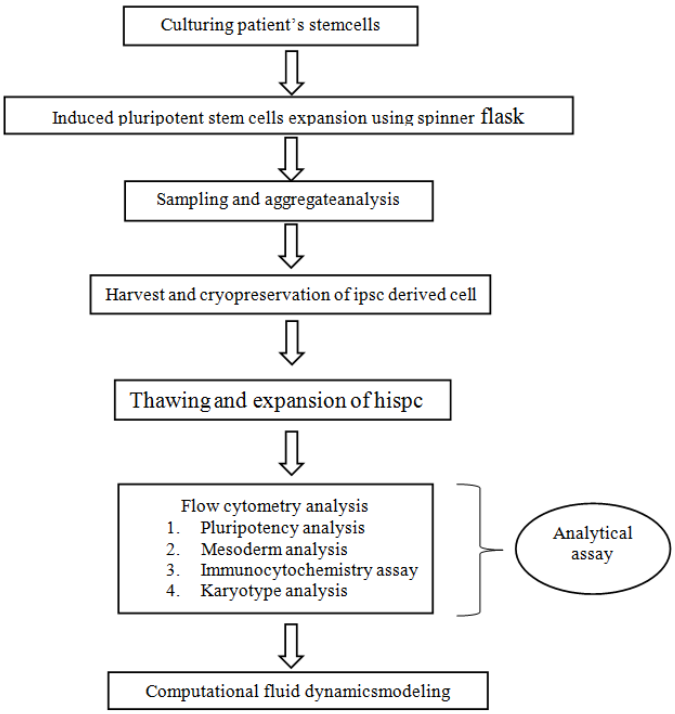
- **Automatic control of culture conditions** such as (impeller design, oxygen concertation, temperature, nutrient concentration, mixing speed and pH) and real-time monitoring can facilitate the development of reproducible culture process in an efficient and homogeneous manner. Followed by scalable expansion of hPSCs in bioreactors equipped with various controlling software and sensors.
- **Rotary cell culture systems** have rotating 3D vessels such as CELLON (Synthecon). These systems provide low-shear stressed environment, dynamic, good mass transfer,controlled oxygenation and continuous media change, to support pluripotent stem cell differentiation and expansion. But the size of the system is limited up to 150 mL and hence scale-up is not easy.
- **Cell culture bags** is another option for large-scale expansion of mammalian cells. Current culture bag systems enable control of pH, gassing and temperature. Scaling-up is easy and cells are subject to relatively low shear forces. The system is suitable for murine iPSC differentiation to cardiomyocytes in suspension culture and mesenchymal stem cell expansion on micro-carriers. Wave Bioreactor is a disposable alternative for large-scale expansion of human pluripotent stem cells. The single-use vessels do not require batch validation or cleaning.
- **Stirred tank bioreactors** are considered as the best platform for 3D culture of hPSCs. This bioreactor is fully controlled and simple to scale, enabling commercial viability of large-scale production of cell therapy products and cell-specific optimization of culture parameters. Stirred-tank bioreactors have a basic design consisting of a glass or single-use plastic vessel with a designated rotating impeller and head plate, probes for monitoring dissolved oxygen, temperature and pH. Such systems are very robust which enables to achieve reproducible results once optimized for their initial culture conditions, culture media, stirring technique and cell density. When the correct parameters for cultivation in small-scale bioreactors are found, scale-up of the method will be quite straight forward and allow a volume increase up to several hundred liters. Parameters such as impeller design, gassing technique, sensor/probe height inside the vessel and perfusion rate may require adjustment when moving to larger volumes to enable the homogenous distribution of cells and accurate online monitoring of culture parameters.

Biomaterial-assisted scalable cell production for cell therapy



Ruoyu Chen *et al* 2019

Computational fluid dynamic approach



Shafa*et al* 2019

The core of computational fluid dynamics modeling is mainly centred on the Navier-Stokes equations. It is a transport equation that represents the transport of momentum and mass through a viscous fluid. The following equations represent momentum and mass transfer:

$$\frac{\partial p}{\partial t} + \nabla.(\rho \mathbf{u}) = 0$$

$$\frac{\partial(\rho \mathbf{u})}{\partial t} + \nabla(\rho \mathbf{u}\mathbf{u}) = -\nabla\rho + \rho g \mu \nabla^2 \mathbf{u} + \rho g$$

Where,
ρ = density, u = velocity vector,
t = time, P = pressure,
μ = viscosity, g = gravity vector.

$\boldsymbol{u} = u, v \text{ and } w$
$\boldsymbol{g} = g_x, g_y \text{ and } g_z.$

The velocity vector *u* is defined by u, v, and w
Where u, v, w represent the velocity components in the x, y and z directions respectively.

Similarly, the gravity vector is defined by g_x, g_y and g_z
Where g_x, g_y, and g_z represent the acceleration due to gravity in the x, y, and z direction.

Assuming incompressibility of the fluids Navier-Stokes equations becomes:

$$\nabla . \mathrm{u} = 0$$
$$\frac{\partial \boldsymbol{u}}{\partial t} + \mathbf{u}.\nabla \mathbf{u} = -\frac{\nabla \rho}{\rho} + \mathrm{v}\nabla^2 \mathrm{u} + \mathrm{g}$$

Re-writing the above equation into Einstein notation:

$$\frac{\partial \mathrm{u_i}}{\partial \mathrm{X_i}} = 0$$
$$\frac{\partial \mathrm{u_i}}{\partial \mathrm{t}} + \mathrm{u}_j \frac{\partial \mathrm{u_i}}{\partial X_j} = -\frac{1}{\rho}\frac{\partial \rho}{\partial \mathrm{X_i}} + \frac{\partial}{\partial \mathrm{X_i}}\left[\mathrm{V}\left(\frac{\partial \mathrm{u_i}}{\partial X_j} + \frac{\partial \mathrm{u}_j}{\partial \mathrm{X_i}}\right)\right] + \mathrm{g_i}$$

The above equations need to be modified toaccount for the effect of turbulent eddies within the bioreactor.The Reynolds Averaged Navier–Stokes (RANS) turbulence equations is use to represent turbulence:

$$U = \bar{u} + u'$$
Where,
U represents the instantaneous velocity vector,
$\bar{u}$ represents the average or main flow,
u' represents the turbulent velocity.

The above turbulent velocity definition can then be substituted into the Navier-Stokes equation and the equation changes to

$$\bar{\mathrm{u}}_j \frac{\partial \bar{\mathrm{u}}_i}{\partial X_i} = -\frac{1}{\rho}\frac{\partial \bar{\rho}}{\partial X_i} + \frac{\partial}{\partial X_i}\left[\mathrm{v}\left(\frac{\partial \mathrm{u_i}}{\partial X} + \frac{\partial \bar{\mathrm{u}}}{\partial X_i}\right)\right] + g_i - \frac{\partial}{\partial X_i}(\bar{\mathrm{u}}'_i\bar{\mathrm{u}}'_j)$$

Where,
$\bar{\mathrm{u}}$ represents bulk flow in the bioreactor and $-\frac{\partial}{\partial X_i}(\bar{\mathrm{u}}'_i\bar{\mathrm{u}}'_j)$represents the transport ofmomentum caused by turbulent flow.

Two-equation realisable k-epsilon model is based on the derivation of the RANS equations which is one of the most common ways of modeling these complex systems. This model introduces energy dissipation rate (ε) and kinetic energy (k) that helps in understanding the hydrodynamics of the system.

$$\mathrm{k} = \frac{1}{2}\mathrm{u}'_i\mathrm{u}'_j$$
$$\in = \mathrm{v}\frac{\partial \bar{\mathrm{u}_i}}{\partial \mathrm{u}_j}$$

The realizable k-epsilon model implements two additional transport equations, one for the kinetic energy and other for the energy dissipation rate

$$\frac{\partial \mathrm{k}}{\partial \mathrm{t}} + \frac{\partial(\mathrm{Ku_i})}{\partial \mathrm{u_i}} = \frac{\partial}{\partial X_\mathrm{i}}\left[\left(\mathrm{v} + \frac{\mathrm{v_t}}{\sigma_\mathrm{t}}\right)\frac{\partial \mathrm{k}}{\partial \mathrm{X_j}}\right] + \mathrm{v_t}S^2 - \in$$

$$\frac{\partial \in}{\partial t} + \frac{(\partial \in \mathrm{u_i})}{\partial \mathrm{X_i}} = \frac{\partial}{\partial \mathrm{X_i}}\left[\left(\mathrm{v} + \frac{\mathrm{v_t}}{\sigma_\varepsilon}\right)\frac{\partial \in}{\partial \mathrm{X_j}}\right] + \mathrm{C_1}S_\in - \mathrm{C_2}\frac{\in^2}{\mathrm{k} + \sqrt{\mathrm{v}\in}}$$

Where
$\sigma_\mathrm{k}$andσ_ε = turbulence number
$\mathrm{C_2}$ = constant value and

$\mathrm{C_1}$is determined by $\mathrm{C_1} = \max\left[0.43, \frac{S\frac{k}{\in}}{S\frac{k}{\in}+5}\right]$

Where, S is magnitude of the main flow strain rate sensor and is determined by

$$\mathrm{S} = \sqrt{2\left(\frac{\partial \mathrm{u_i}}{\partial \mathrm{X_i}}\right)^2 + \left(\frac{\partial \mathrm{u_i}}{\partial \mathrm{u_j}} + \frac{\partial \mathrm{u_j}}{\partial \mathrm{u_i}}\right)^2}$$

$\mathrm{v_t}$ is turbulent viscosity and is calculated by

$$\mathrm{v} = \frac{\rho \mathrm{C}_\mu \mathrm{K}^2}{\in}$$

Other scale up equations are

- **Reynolds number** $\mathrm{N_{RE}} = \frac{\boldsymbol{\rho}.\mathrm{N}.\mathrm{D_i}^2}{\boldsymbol{\mu}}$
  Where,
  ρ is density of the medium,
  Nis agitation speed,
  $\mathrm{D_i}$is diameter of the impeller, μ is medium viscosity.
- **Power number** is calculated by

$$\mathrm{N_p} = \frac{\mathrm{K_1}}{\mathrm{N_{RE}}} + \mathrm{K_2}\left(\frac{10^3 + 1.2.\mathrm{N_{RE}^{0.66}}}{10^3 + 3.2.\mathrm{N_{RE}^{0.66}}}\right)^{\mathrm{K_4}}$$

$$\mathrm{K_1} = 14 + \left(\frac{\mathrm{W}}{\mathrm{D_t}}\right).\left[670.\left(\frac{\mathrm{D_i}}{\mathrm{D_t}} - 0.6\right)^6 + 185\right]$$

$$\mathrm{K_2} = 10^{\mathrm{K_3}}$$

$$\mathrm{K_3} = 1.3 - 4.\left(\frac{\mathrm{W}}{\mathrm{D}_t} - 0.5\right)^2 - 11.4.\left(\frac{\mathrm{D_i}}{\mathrm{D_t}}\right)$$

$$\mathrm{K_4} = 1.1 + 4.\left(\frac{\mathrm{W}}{\mathrm{D_t}}\right) - 2.5.\left(\frac{\mathrm{D_i}}{\mathrm{D_t}} - 0.5\right)^2 - 7.\left(\frac{\mathrm{W}}{\mathrm{D_t}}\right)^4$$

Where, W isimpeller width and $\mathrm{D_t}$ = diameter of the vessel.

Average energy dissipation rate

$$\in = \frac{\rho}{\mathrm{V}.\rho}$$

Where, V is working volume of the vessel and P is the power input.

The power input is calculated by
$$\mathrm{P} = \mathrm{N_p}.\mathrm{N}^3.\mathrm{D_i^5}.\rho$$

Maximum shear stress ($\tau_{\max}$) is given by

$$\tau_{\max} = 5.33\dot{c}\rho\dot{c}(\in \dot{c}v)^{0.5}$$

Where, ν is the kinematic viscosity.

Tip speed calculated by

$$\text{Tip Speed} = \mathrm{N}.\frac{1\text{min}}{60\text{s}}.\pi.\mathrm{D_i}$$

(Shafa*et al* 2019)

DISCUSSION AND CONCLUSION

The path to industrializing cell therapies requires implementing appropriate characterization methods, standardizing materials and cell handling procedures in the manufacture of cell-based therapies. Cell-based therapies require large quantities of pluripotent stem cells. 3D matrix-free suspension cultures of hPSCs aggregates can be significantly expanded in a 100mL stirred-tank bioreactor. Expanding and improving the monitoring and control systems

is expected to increase culture stability and allow higher cell yields.

Biomaterials provide support for cell attachment, protect cells during shipment and cryopreservation, purification, isolation and growth. Biomaterials also regulate cell functions through specialized nanostructures and chemical modifications, in order to meet the requirements of cell production in a quality-controlled manner and to fulfil clinical demands. Natural biomaterials undergo some problems such as potential influence of xenogenic components and batch differences, which influences the safety and stability of the cell products. These drawbacks of natural biomaterials impact the FDA approval process which slows down the clinical process. Biomaterials provide mechanical and biological signals to cells that impact differentiation, proliferation and migration. A better understanding ofinteraction between biomaterial and cells during cell production will help in the adoption of biomaterials in therapeutic cell culture processes. Determining which biomaterial closely mimic the specific cell niche and is more appropriate for a certain cell type still remain as a challenge. To ensure the purity of cell products by removing residual biomaterial components is an important step while using, biomaterials in therapeutic cell production.

Cell therapies are rapidly developing and novel therapies such as CAR T cells have progressed towards clinical applications. Research on stem cell therapies continues to accelerate and attract attention. Progress in cell trans-differentiation and dedifferentiation, use of umbilical cord blood will help to overcome ethical issues related to cell sources and genome modification will allow versatile cell engineering. The pioneering works in the clinical trials and long-standing expertise of transfusion medicine in cell-based therapies, iPSC-derived hematopoietic cells represents a promising cell type for the initiation of further iPSC-based clinical trials. Especially, the recent advances in GMP-compliant generation and differentiation of human iPSCs in combination with scaling up approaches of hematopoietic differentiation along with using iPSC-derived blood cells clinical translation can be achived. Various preclinical studies demonstrate the safety and efficacy of various iPSC-derived blood cells, more work is still required to generate transgene-free human stem cells for clinical application. Transplantation of macrophages into different tissues could be the most important for clinical translation.

A hiPSC stirred suspension culture system has been developed with a focus on free scalability in the range of 50 ml and 3 l, process yield, maintenance of cell quality and ease of use by incorporating a well-established single-use stirr tank bioreactor, as it is flexible and fits into existing standard adherent culture workflow, and is scalable from a 125-ml spinner to a 3-l bioreactor. The hiPSCs cultured over 14 days with our workflow maintain pluripotency and will be in undifferentiated state. A quantity sufficient for disease modelling, cell transplantations, high-throughput drug screening and tissue patches can be produced using scaling up bioreactor. This newly developed approach can be used to explore the possibility of directing differentiation of hiPSCs in stirred suspension culture vessels towards various lineages, as well as to use this culture system to the large-scale production of progenitor or precursor cells. This helps to use hiPSCs and their derivates for cell therapy and disease modeling. Human pluripotent stem cells both (hiPSCs and hESCs) share a key properties of unlimited self-renewal and pluripotency to differentiate into any cell types in the body. hiPSCs has been considered as an effective replacement of hESCs and is a promising candidate cell source for cell-based therapy and regenerative medicine. Human iPSCs also have their own advantages: a) they avoid the ethical issues surrounding the use of human-embryo-derived stem cells. b) they can be autologouslyderived from patient tissue thus eliminating immune rejection upon transplantation. A novel and effective CFD modeling approaches is adapted for the differentiation of iPSCs to scale up and controll bioprocess utilizing a GMP iPSC cell line.

Reference

1. Mehdi Shafa, Krishna M Panchalingam, Tylor Walsh, Thomas Richardson, Behnam AhmadianBaghbaderani, Computational fluid dynamics modelling: a novel and effective approach for developing scalable cell therapy manufacturing Processes. Wiley online publication, 2019. doi: 10.1002/bit.27159.
2. Haake, K., Ackermann, M., &Lachmann, N, Concise review:Towards the clinical translation of induced pluripotent stem cellsderivedblood cells – ready for take-off. Stem Cells Translational Medicine, 2018. https://doi.org/10.1002/sctm.18-0134.
3. Kwok, C. K., Ueda, Y., Kadari, A., Günther, K., Ergün, S., Heron, A., …Edenhofer, F. Scalable stirred suspension culture for thegeneration of billions of human induced pluripotent stem cells usingsingle-use bioreactors. Journal of Tissue Engineering and RegenerativeMedicine,2018.doi: 10.1002/term.2435.
4. Meng, G., Liu, S., Poon, A., &Rancourt, D. E. Optimizing humaninduced pluripotent stem cell expansion in stirred-suspension culture.Stem Cells andDevelopment, 2017. https://doi.org/10.1089/scd.2017.0090.
5. Ruoyu Chena, Ling Lia, Lu Fenga, YixueLuoa, MingenXub, Kam W. Leongc,,RuiYaoa, Biomaterial-assisted scalable cell production for cell therapy, Department of Biomedical Engineering, Columbia, USA, 2019. doi: 10.1016/j.biomaterials.2019.119627.
6. Gary M. Pigeau1, Elizabeth Csaszar1, and Aaron Dulgar-Tulloch1, Commercial Scale Manufacturing of Allogeneic Cell Therapy 2018. https://doi.org/10.3389/fmed.2018.00233.
7. Cells Neta Lavon, Michal Zimerman, and Joseph Itskovitz-Eldor, Scalable Expansion of Pluripotent Stem, BiochemEngBiotechnol 2018.doi: 10.1007/10_2017_26.
8. Abbot, S., Agbanyo, F., Ahlfors, J. E., Baghbaderani, B. A., Bartido, S., Bharti, K., Zoon, K, Report of the international conferenceon manufacturing and testing of pluripotent stem cells. Biologicals, 2018.DOI: 10.1016/j.biologicals.2018.08.004
9. Borys, B. S., Roberts, E. L., Le, A., &Kallos, M. S, Scale-up of embryonic stem cell aggregate stirred suspension bioreactor culture enabled by computational fluid dynamics modeling. *Biochemical Engineering Journal*, 2018. 133, 157–167.
10. Laco, F., Woo, T. L., Zhong, Q., Szmyd, R., Ting, S., Khan, F. J., Oh, S,Unraveling the inconsistencies of cardiac differentiation efficiency induced by the GSK3β

inhibitor CHIR99021 in human pluripotent stem cells. Stem Cell Reports, 2018.
doi: 10.1016/j.stemcr.2018.03.023.

11. Shafa, M., Yang, F., Fellner, T., Rao, M. S., &Baghbaderani, B. A, Human-induced pluripotent stem cells manufactured using a current good manufacturing practice-compliant process differentiate into clinically relevant cells from three germ layers. Frontiers in Medicine, 2018.doi: 10.3389/fmed.2018.00069.
12. Kropp, C., Kempf, H., Halloin, C., Robles-Diaz, D., Franke, A., Scheper, T., Olmer, R, Impact of feeding strategies on the scalable expansion of human pluripotent stem cells in single-use stirred tank bioreactors. Stem Cells Translational Medicine, 2016.doi: 10.5966/sctm.2015-0253
13. Harvey, A. J., Rathjen, J., & Gardner, D. K, Metaboloepigenetic regulation of pluripotent stem cells. Stem Cells International, 2016.
https://doi.org/10.1155/2016/1816525
14. Higuchi A, Kumar SS, Benelli G, Alarfaj AA, Munusamy MA, Umezawa A, *et al*. Stem cell therapies for reversing vision loss. Trends Biotechnol, 2017.
doi: 10.1016/j.tibtech.2017.06.016.
15. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. Cell Transplant, 2016.doi: 10.3727/096368915X689622.
16. Martin U. Therapeutic application of pluripotent stem cells: challenges and risks. Front Med, 2017.doi: 10.3389/fmed.2017.00229
17. Trounson A, DeWitt ND, Pluripotent stem cells progressing to the clinic. Nat Rev Mol Cell Biol, 2016.doi: 10.1038/nrm.2016.10.
18. KempfH, Andree B, Zweigerdt R, Large-scale production of human pluripotent stem cellderived cardiomyocytes. Adv Drug Deliv, 2018.doi: 10.1016/j.addr.2015.11.016.
19. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, JemalA. Global cancer statistics: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018.
20. Wei W, Luo J. Thoughts on Chemistry, Manufacturing and Control of Cell Therapy Products for Clinical Application. HUM GENE THER 2018.
21. Abdeen AA, Saha K. Manufacturing Cell Therapies Using Engineered Biomaterials. TRENDS BIOTECHNOL 2017.
22. Poorebrahim M, Sadeghi S, Fakhr E, Abazari MF, Poortahmasebi V, Kheirollahi A, *et al*. Production of CAR T-cells by GMP-grade lentiviral vectors: latest advances and future prospects. Crit Rev ClinLabSci 2019.

How to cite this article:

Muktha Bharadwaj S (2021) 'Approaches For Scaling Up Stem Cells Used In Therapeutic Process', *International Journal of Current Advanced Research,* 10(05), pp. 24502-24508. DOI: http://dx.doi.org/10.24327/ijcar.2021.24508.4862

****