Thriving for the Renewal of Life: Present Needs in Cell Therapy Translational Research

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Abstract

The stem cell field has grown very rapidly over the past decade and continues to be one of the most exciting areas of biomedical research. It is now known that stem cells are potential for improvement of pathological condition in many diseased organs, which is not possible in case of pharmaceutical drugs. Adult stem cells are most familiar for autologous and allogenic applications in different clinical indications. With the ability to produce an unlimited number of many kinds of human cells, the pluripotent stem cells have entered in the forefront of the regenerative medicine. However, several challenges must be overcome before clinical applications become a reality. More specifically, the challenges for the coming years are to extend multidisciplinary and multi-sector collaboration aimed at large-scale production of high-quality stem cell products, development of robust methods for characterization of cells, and assessment of therapeutic value. In this report, I have discussed about certain biological issues that might involve in determining the therapeutic potential and obtaining regulatory approval for the stem cell-based products. Other major aspect of this report has been manufacturing of cells and challenges for large-scale production.

Keywords

Bioreactor • Cellular characterization • Differentiation • MSCs • Pluripotent stem cells • Potency • Scale-up/scale-out • Suspension culture

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Abbreviations

ALF	Acute liver failure
CHD	Chronic heart disease
CLI	Chronic liver injury
CPCs	Cardiovascular progenitor cells
ESCs	Embryonic stem cells
HSPCs	Hematopoietic stem and progenitor cells
iPSCs	Induced pluripotent stem cells
LVEF	Left ventricular ejection fraction
MI	Myocardial infarction
MSCs	Mesenchymal stem cells
RPE	Retinal pigment epithelium
SCI	Spinal cord injury

20.1 Introduction

Regenerative medicine encompasses repair or replacement of damaged body parts to restore normal function [1]. With the advent of embryonic stem cells (ESCs) followed by induced pluripotent stem cells (iPSCs), there has been a paradigm shift in healthcare technology in the past 15 years, though cell-based therapy is not a new concept as first successful hematopoietic stem cells (HSCs) transplantation took place in 1968 [2]. Two different cellular approaches are generally followed in regenerative medicine for the replacement of cells; these are cell therapy and tissue engineering. While cell therapy involves direct administration of autologous/allogenic stem or differentiated cells in the diseased organ, in tissue engineering, cells are grown in the form of tissue on a biocompatible scaffold prior to implantation in the target site.

Despite step change in knowledge of stem cell biology, translation of basic/preclinical results into clinic has become more complicated. This is due to possibility of adverse consequences of the therapy, as in most cases stem cells are recovered from one tissue and introduce in another, hoping that either they will differentiate into target cells or secrete tropic factors for repair of the damaged organs. The fundamental questions that emerge from these two possibilities are:

(a) What kind of cells is supposed to be transplanted?
(b) Why is the transplant engrafted in the recipient's tissue, and if so, how long will it stay?
(c) How the transplant copes with the ectopic microenvironment?
(d) Can the transplant cause any adverse pathological changes in the recipient?

Other questions are pertaining to quality/potency aspect of expanded and differentiated cells; these are

(e) genetic instability during cell expansion;
(f) manufacturing processes underpinning cellular, molecular, and functional characteristics of the product; and
(g) purity and potency of the target cells.

As our understanding in some of these areas is still vague, the main challenges in cellular characterization and their interactions with ectopic site have taken lead over actual manufacturing processes to address biological questions about the cells and their clinical effects. This article will cover biological issues related to cells, tissue regeneration, and manufacturing processes.

20.2 Biological Issues Pertaining to Cell Therapy: Adult Versus Pluripotent Stem Cells

20.2.1 Adult Stem Cells

In clinical applications, bone marrow (BM)-derived hematopoietic stem and progenitor cells (HSPCs) are used as established standard of care for hematological disorders. In hematopoietic system, the regulators of HSPC's differentiation into specific lineages and characteristics of stem cell niche are known for many years. However, it is a concern to the viewpoint of regulation about these cells or BM-MNCs or MSCs when administered for the regeneration of non-hematopoietic organs. The obvious question is how cells response to non-supportive environment or whether the fusion of heterokaryons, if formed between donor and recipient cells, leads to oncogenesis. In case of MSCs, it is considered that cells do not differentiate into irrelevant tissue. Paradoxically, MSCs outside of their native environment may respond differently from their counterparts in the human body.

Initially BM-MNCs, later MSCs from BM, adipose tissue (AT), and Wharton's jelly (WJ) have become popular in clinical trials, particularly for cardiovascular and renal diseases, liver cirrhosis/fibrosis, and immunomodulation [3]. This was primarily due to homing ability of the cells [4] and secretion of immunomodulatory cytokines and tropic factors by MSCs [5]. The issue here is how transplanted MSCs respond to new microenvironment (niches) when it is proinflammatory in nature. Cells home to this new microenvironment either undergo apoptotic death or disappear from the sites of homing to protect themselves or favorably respond to the new microenvironment. As in most clinical and some preclinical studies, MSCs or donor-derived cells have not detected in the organs even after the regeneration; it has been considered that tropic factors and/or exosomes secreted by the cells are responsible for tissue regeneration [6, 7]. Like other organs, in acute liver failure (ALF), hepatic environment is highly toxic, and there is massive immune-mediated apoptosis or necrosis of native or transplanted hepatocytes. Poor viability and functions, due to lack of supportive environment and increased immune-mediated cell death, are the major physiological barriers in successful clinical applications of any cell-based therapy. Despite proinflammatory nature of

the damaged tissue, many reports emphatically demonstrated multi-lineage differentiation potential of MSCs in vivo. These converge into three important issues: stem cell potency, safe limits of the proinflammatory cytokines that do not cause harm to the transplant, and their tissue engraftability. How much do we understand regarding these? There are enormous scopes for extended study on these areas. For example, it is necessary to comprehend how damaged tissue microenvironment causes harm to MSCs when proinflammatory cytokines are required to induce immunosuppression by them [8]. Further homing is compromised to the donor cells due to decrease of SDF-1α/CXCL12 axis or other potential trafficking signals. Early passage MSCs are shown to express high level of CXCL12, whereas SDF-1α is expressed by the injured tissue. It has been shown that trafficking of MSCs, via upregulation of SDF-1α, to the region of ischemia leads to the improvement of cardiac functions [9]. The chemotactic signals drive HSCs/MSCs to home to the injured organs, whether it is irradiated bone marrow or injured liver or heart. The expression of SDF-1α by damaged tissue happens to be high at the beginning and, later with time, reduces near to normal level after regeneration. The above study and other perhaps indicate the importance of the time of delivery and localization of MSCs at the damaged site.

The clinical trials and meta-analyses showed that transplantation of BM-derived cells is safe, and there is 3.96% increase of left ventricular ejection fraction (LVEF) and also improvement in post-infarct remodeling; however, the efficacy of this therapy for myocardial infarction (MI) and chronic heart disease (CHD) continues to remain debatable [10–12]. This was due to modest improvement, uncertain benefits for the long term, and even absent of benefits in several studies. Outcomes of the therapy not only depend on the potency, number of cells, and route of delivery; the optimum time of cell delivery appears to have an important role. It is known that the effectiveness of therapy is governed by the ability for early attenuation of left ventricular remodeling post-MI, which is caused due to lowering expansion of infarct and ventricular dilatation. Since most of the pathophysiological changes are initiated in early (<3 day) phase of remodeling [13], improvement of long term prognosis can be expected if cells are administered within this period. Modest outcomes of BMC therapy in case of MI were thought to be due to the intervention at the late stage of remodeling.

I would like to discuss another important clinical indication, that is, liver cirrhosis. Chronic liver injury (CLI) is marked by persistence inflammation, in which monocytes/macrophages play a central role. Macrophages are considered master regulators in the progression as well as the resolution of liver fibrosis. In fibrosis regression, the infiltrating macrophages adopt fibrolytic phenotypes to secrete MMPs for the degradation of excess ECM components [14]. Hence, the most pertinent question emerges: Whether suppression of chronic hepatic inflammation is an obligatory for the treatment of fibrosis? In immune suppressive environment, does enough number of fibrolytic macrophages present? In the past, many clinical trials were conducted for the treatment of cirrhotic liver by using MSCs [15–18]. As MSCs have immunosuppressive properties, also are profibrogenic due to secretion of fibrogenic molecules [19], and directly differentiate into myofibroblasts in

experimental mouse model of liver fibrosis as well as in patients [20, 21], it is necessary to revisit the functional analysis of these cells for the treatment of CLI. Additionally, it is essential to evaluate the paracrine role of MSCs in terms of the protection or induction of apoptosis in activated hepatic stellate cells (HpSCs), to observe whether the secretory factors promote or inhibit myofibroblastic differentiation [22]. Unless the answers of these questions are available, treatment of cirrhosis patients with MSCs will not prove much beneficial.

20.2.2 Pluripotent Stem Cells

At present, in clinical application, autologous or allogeneic adult stem/progenitor cells are used for repair or regeneration of diseased organs. However, in case of ESCs/iPSCs, due to their ability to form teratoma, derivatives of these cells are used. The derivatives of pluripotent stem cells are heterogeneous population that varies in their differentiation stage, lineage identity, and other biological characteristics. The question is how intensely should this heterogeneity be analyzed? Does these analyses should be similar in case of adult and pluripotent stem cells and their derivatives? Since adult stem cells possess finite life span and primarily committed to a define lineage, the cellular heterogeneity or presence of "biological unknowns" is not a serious issue. For cell therapies based on pluripotent stem cells, it is essential to identify and separate both residual undifferentiated cells capable of forming teratoma and contaminating cells that are capable of forming ectopic tissues. The significance of understanding these contaminating cells can be realized by FDA's temporal decision to stall Geron Corporation's phase I clinical trial of GRNOPC1 against spinal cord injury (SCI). GRNOPC1 contains hESC-derived oligodendrocyte progenitors that showed to have remyelinating and nerve growth-stimulating properties leading to restoration of locomotion activity and kinematic scores in rat model of acute SCI. The decision of FDA was due to the formation of larger cysts in 50% test animals, though cysts were benign. Cysts formation was presumably due to the presence of contaminating (intermediate) cells. Later, Geron addressed this issue by developing new molecular markers and release criteria that demonstrated lower number of cysts.

Therefore, the major questions for the clinical development of hESC-/iPSC-derived products are:

(a) Does differentiation process allow to achieve the clinical endpoint?
(b) How subpopulation of cells are identified with biological characteristics?
(c) Do standard assays are sensitive enough to determine cellular heterogeneity after sorting?
(d) How to monitor interaction between cells and their niches?
(e) Do system to deliver cells and control of homing are in place?
(f) Are available methods sensitive enough to monitor clinical efficiency (potency) of the cells?
(g) Does functional integration of transplant with damaged organs can be determined?
(h) Can a sensitive method for understanding the immune response be adopted?

(i) How a sensitive noninvasive assay method can be implemented to track the transplant?
(j) Can a large animal model and cell line derived from the same animals to validate the proof of the concept be used?

Few of the above issues are further clarified for the benefit of the readers.

Besides Geron Corporation's attempt for the treatment of SCI, retinal pigment epithelial cells are produced from hESCs and iPSCs and used in clinical trials for the treatment of macular degeneration [23, 24]. The current differentiation protocols of pluripotent stem cells to any lineage of importance do not produce fully mature cells, and thus the ability of these cells to become functionally active after engraftment needs to be confirmed in a suitable disease model. The efficiency of differentiation of hESCs/iPSCs is low, and the end product always gives a mixture of cells with different phenotypes; some of their biological identity may be not known. Therefore, it is crucial that only established or standardized protocols are adopted for differentiation. Reexamination of the protocols using growth/differentiation factors versus small molecules is obligatory. It is important to keep in mind that the ultimate objective is to standardize a protocol that is highly efficient to generate desired cells that are functional and stable [25, 26]. In this connection, functional and molecular diagnosis (e.g., epigenomic, proteomic, transcriptional profiles, etc.) for each differentiation stage should be carefully carried out. The 3D culture system often imitates in vivo environment, leading to the formation of native cellular/tissue architecture as found in the case of hepatocyte-like cells from ESCs [27]. This is supposed to be the best culture system by which pluripotent stem cells are expanded and differentiated; thus a properly optimized culture condition is warranted. In cell therapies, the vast majority of donor cells are reported to die soon after transplantation into humans; the knowledge regarding the interaction of transplant with the microenvironment of diseased tissue/organ is premature. It is most essential of investigating tissue milieu for the expression of cytokines/growth factors. It is needless to mention that serum analyses will add up in understanding of ectopic production, if any.

As heterogeneity is inherent in the differentiation process, it is critical for the development of robust assay to identify different subpopulation of cells (heterogeneity), due to the fact that potency may change with the cellular phenotype. The word "potency" in case of cell-based product is not as straightforward like pharmaceutical drugs. The potency of a cell product is indeed linked with the mechanism by which it involves in tissue regeneration. Irrespective of the cell types, potency can be measured by the ability of cells' homing to the target site, ability to secrete trophic factors for diverse biological activities, propensity of differentiation into functional tissues, and ability to survive for the longer period of time. In this regard, I would like to introduce the terminology "negative potency," which refers to the undesirable cells that are harmful to the recipients. Those cells belonging to this category are undifferentiated stem-like (tumor inducing), acquire mutations during expansion, committed to other lineage and migration defective, undergo apoptosis in response to inflammatory cues, etc. For a successful clinical translation,

it is essential that the assay methods to determine potency are available, and a robust method for absolute removal of cells associated with negative potency is in place.

Transplanted cells often engraft in the target organ; however, functional integration with the damaged tissue is a major challenge. For some cell types, such as β cells and RPE, just engraftment of cells is sufficient to ameliorate diabetes and macular degeneration, respectively. In these cases and in many others, though transplant physically incorporate in the existing tissue, the coordinated function is not essential for reversal of the diseased state. In cases like regeneration of neurons, cardiac tissue, etc., functional integration is imperative. In neurons, functional integration of engrafted cells is ensured by (a) passive and active membrane properties, determined by whole cell voltage-gated inward and outward current, (b) synaptic integration, and (c) expression of functional neurotransmitter receptors. A study demonstrated that mouse ESC-derived neural precursors have the capacity to develop into functional neurons, which can integrate synaptically into the recipient's brain circuitry [28]. Similarly, functional integration is crucial in regeneration of infarcted heart muscle. Functional integration of cardiomyocyte-like cells in MI heart is expected to cause attenuation of left ventricular remodeling, increase in LVEF and mechanical function, and improvement of electrophysiological property. Obtaining functionally engraftable cells, posttransplantation is the crux of the success of regenerative medicine. Trilineage potential cardiovascular progenitor cells (CPCs) of embryonic mouse heart were found to express intracellular transcription factors Islet 1 (Isl1) and Nkx2.5. It has been revealed that in differentiating iPSCs, $Flt1^{+}Flt3^{+}$ cells have similar trilineage cardiovascular potential that is enriched of $Isl1^{+}/Nkx2.5^{+}$ CPCs. In mouse, iPSC-derived CPCs are differentiated into cardiovascular lineages that became authentic adult cardiomyocytes both in morphologically and electrophysiologically [29]. In this connection, a predictive cardiac tissue model, engineered heart tissue (EHT), was proposed to assess functional cell integration. The changes of molecular and electrophysiological properties of EHT, caused by the presence of test cells, are expected to predict their composition [30]. For example, mouse ESC-derived CPCs integrated in EHT will enhance the amplitude of tissue contraction and exhibit electrophysiological integration. However, mouse cardiac fibroblasts, if present, will interfere with the electrical signal propagation [30]. Overall, this system will be potentially useful in quality assessment for the pluripotent stem cell-derived CPCs.

Last but not the least is the development of a sensitive noninvasive method to monitor cell migration in vivo. It not only determines engraftability of the transplant, their presence in nontarget tissues/organs will also be evaluated as it may cause detrimental due to the formation of ectopic tissue. The current technologies for clinical imaging are magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), single photon emission tomography (SPECT), and multimodality methods; however, the ability to track the fate and function of transplanted cells using these methods has several limitations [31]. The sensitivity of above imaging techniques is related to the concentrations of contrasts (e.g., nanoparticles, SPIO, or chemical agents, ^{111}Indium-oxine, ^{18}F-fluorodeoxyglucose, ^{99m}-technetium, etc.) in the cells, which is determined by

cell uptake, their retention, lost due to leaching, or dilution with cell division. Thus these methods are not reliable for long-term applications. Indirect labeling with genetically modified reporter genes is good for short- and long-term applications in animals, but in the human subject, safety and immunogenicity issues will attract attention of the regulatory body. Among the many techniques under clinical investigations, direct labeling technique using gadolinium chelate contrast—MRI for myocardial regeneration by c-kit^{+} cardiac cells [32] and ^{18}F-FDG contrast—and PET imaging for pancreatic islets [33] are promising.

20.3 Manufacturing of Therapeutic-Grade Cell Products

Science for manufacturing of therapeutic-grade cells is still immature; there is a global demand for the development of scalable manufacturing processes for the same. Here, I will focus on challenges and bottlenecks of cell culture process development and the present trend of bioreactor development for manufacturing stem cell-derived products. Manufacturing of stem cell products demands consistent, high-quality, and scalable production processes. Again, stem cells become sensitive in culture due to different chemical and physical environments than that are prevailing in the native state. Due to these complexities in manufacturing requirement, there is a fair chance that products are adversely affected. To handle this, in 2013, US Food and Drug Administration (FDA) released the guidance [34] which recommends general preclinical program design for investigational cell therapy and gene therapy products.

In cases where cell replacement therapy is proved beneficial, primary donor cell transplantation may not be adequate to meet the clinical need. Expansion of therapeutically active cells will require compensating low number harvested from donors/patients. This is possible only when a scalable manufacturing process is developed before or along with the clinical trial. The aim of the manufacturing process is to increase cell number at reasonable cost without compromising with the therapeutic potency. Though manufacturing lot size is dependent on the clinical indication, cell dose, and number of subjects, it may be in the order of trillions of cells [35, 36]. The current manual culture technology is not sufficient to meet this requirement, and new manufacturing methods must be developed.

20.3.1 Manufacturing Challenges

There are several manufacturing challenges that explain why large-scale stem cell culture is complicated and unpredictable, and why industry foresees the risk for expanding the manufacturing facility. Some of these challenges are explained below:

1. *Cellular heterogeneity*, *potency*, *safety*, *and stability of the product*: These are specific released criteria of the final products. Getting homogeneous preparation

of stem cell-based products is rather difficult, on regulation viewpoint it is necessary to demonstrate that cellular heterogeneity does not cause any harm to the recipient. FDA released guidance for the industry to carry out safety and potency tests; however, no single current measurement tool is available that can absolutely define a cell and its clinical efficacy [34, 35]. There is also no reliable method in place to identify single unsafe cells in a large therapeutic population.

2. *Dose and cell type*: Presently numerous competing cell types are used in clinical trials for a single indication; these are either from autologous or allogeneic sources. The autologous therapy requires a facility that can handle multiple individual samples without cross-contamination, therefore unlikely to be amenable to the conventional batch manufacturing process familiar in pharmaceutical industries. Further, each cell type may require a specific manufacturing process that causes the major hurdle in the way of process optimization. The scale of manufacturing also depends on final dose of cells, route of administration, and cell type. Clinical trial findings have no clear indication on above issues.
3. *Scale of production*: For autologous primary hematopoietic stem cell transplantation (HSCT), harvesting and processing of cells are conducted in the hospital premises. Furthermore, the application for small niche like cornea and inner ear hair cells manufacturing is partly or fully conducted at small scale on the hospital premises. In large-scale clinical trials (single/multicentric) of allogeneic MSCs, ESC-/iPSC-derived cells as well as in case of standard therapy, manufacturing of cells in hospital premises is not advisable as it requires special expertise and facility. Involvement of manufacturing industry in these cases can provide a solution; however, it is difficult to design large-scale manufacturing facility at present as clinically efficacious cell dose is poorly defined.
4. *Lack of flexibility of manufacturing processes*: After the clinical trials, manufacturer's flexibility to modify the production process is forfeited.
5. *Diverse technological requirements*: The concept of generic cell culture system, like in traditional recombinant therapeutic proteins, is lost as stem cells and their derivatives require a plethora of culture environments for expansion and differentiation. For example, mechanical stimulation is preferred for the formation of functional osteogenic [37] and chondrogenic [38] lineages from stem cells, and culture of vascular endothelial cells prefers pulsatile flow [39]. Again, many cell products grow better in 3D frameworks and require supporting cell-mediated paracrine signaling.
6. *Process control strategy*: For recombinant protein production using CHO or BHK cell line, the control strategies followed to maximize the product yield are generally based on stabilization of cultures at a steady-state condition. In contrast, any stem cell-based production is a dynamic process as the committed progenitors or differentiated cell populations are evolved with time. Thus a dynamic control strategy is likely more effective to correct and regulate the minute fluctuations in culture conditions [40].

20.3.2 Cell Culture Technology: Clinical-Grade Products

20.3.2.1 Expansion of Stem Cells

Stem cell-based products may be classified into two general categories: patient-specific (autologous) and off-the-shelf (allogeneic). The bioprocess requirements in these two categories of products are essentially different. The former one deals with production/processing of cells on an individual basis, at the hospital premises, whereas large-scale manufacturing is carried out in case of off-the-shelf products. Owing to the demand of limited number of cells, patient-specific products are preferably manufactured in a disposable system. However, cost of production and process salability are two important issues for bulk culture of therapeutic-grade cells. In cell culture products, two different manufacturing approaches are followed to increase the capacity of production: (a) horizontal scale-out (replication of many small units) and (b) vertical scale-up (volumetric scale-up). At present horizontal scale-out method is followed to increase the capacity of stem cell-based products.

Three approaches are currently adopted for the expansion of patient-specific stem cells and their committed progenitors; these are "open" laboratory-scale culture systems (petri dishes, T-flasks, and multilayered flask). Due to the nature of open culture system, online monitoring, control, and evaluation of key parameters to determine product yield are not possible. Basic culture environment is controlled by placing flasks inside a CO_2 incubator. Due to low cost and easy in handling, many therapies have been developed using open culture flask technology. However, flask culture suffers from limited capacity thus cannot cope with the demand of phase III trial and onward. Even though stem cell clinical requirement is not fully understood, large-scale automated bioreactors (closed type) have been tested for expansion of therapeutic-grade cells. One such commercial-scale system, known as "wave bioreactor," was introduced by GE Healthcare and Goodwin Biotechnology. This is a cGMP compliance closed cell culture system containing disposable horizontal pillow bags (gas permeable) of different capacities, made of biocompatible polymer. Both free suspension and microcarrier-adhered stem cells can be cultivated in these bags. Such system was originally developed for expansion of plant and routine animal cells. Disposable gas permeable bags with media perfusion system have been largely utilized for clinical-scale expansion of HSPCs [41, 42].

Development of a large-scale unit culture system has been urged in which vertical scale-up is possible and process control becomes simple. One way of doing this is adopting stem cells in free suspension culture while retaining safety and therapeutic efficacy. The technique by which recombinant CHO and BHK cell lines were adopted to grow in suspension for the production of therapeutic proteins is known. On similar line, an attempt was made to adopt human ESCs and iPSCs for suspension culture using defined media. ESCs were expanded in culture plate up to 17 passages without compromising with the expressions of pluripotency markers and potential of differentiating into derivatives of three germ layers. Interestingly, the expanded cells maintained the stable karyotype [43]. The ability for expansion of these cells in scalable stirred tank bioreactors and subsequent determination of their therapeutic efficacy are yet to be evaluated.

In cases where stirred single cell suspension culture is inappropriate, other options may be adopted. It is known that 3D cell culture technique closely mimics natural tissues and organs than when grown two-dimensionally in a culture flask. In 3D culture, cells attach to one another and interact through gap junctions via exchange of ions and small molecules. The additional benefit in this culture system is that many growth factors are presented to cells while bound to extracellular matrix or stromal layer for enhanced biologically relevant signals as in the case of stem cells niche. Furthermore, shear-sensitive stem cells are expected to grow better and retain functional properties, if cultured in porous scaffolds. Cultispher-S, Cytodex 3, and other microcarriers were successfully used for the expansion of ESCs and MSCs [44–47] in suspension bioreactor.

20.3.2.2 Manufacturing Therapeutically Active Cells

Once stem cells are expanded to a desired number, they are induced for differentiation into target lineages following appropriate procedures. As mentioned before, stem cells are not allowed to differentiate into mature cells but into therapeutically active form. The therapeutic active cells are committed to a specific lineage, which when engrafted to diseased organ assume mature phenotype. Expansion of stem cells and their differentiation into therapeutically active form are essentially conducted in two different culture conditions in separate systems, either open or closed type. There are even more challenges in this stage of manufacturing, which can be appreciated from the example of Advanced Cell Technologies (ACT) cell therapy project. ACT manufactured retinal pigment epithelium (RPE) from the GMP-grade MA09 human ESCs [48] for the treatment of macular degeneration. In brief, the manufacturing process is as follows: stem cells are expanded on inactivated murine embryonic fibroblasts; dislodge stem cells are allowed to form embryoid bodies in different culture conditions till pigmented RPE colonies are visible. Manually isolated RPE are further expanded and cultured till appropriate phenotype is appeared. The key manufacturing challenges for this production process are (a) establishing a differentiation protocol that generates large number of therapeutically active RPE in minimum culture time; (b) developing an automated isolation and purification method of RPE; (c) reducing manufacturing steps, in turn optimizing cost of production and protecting cells from harmful effect of dislodging enzymes; and (d) developing a close, automated, and scalable clinical manufacturing process. It will be extremely valuable if expansion of stem cells and their differentiation are conducted in a single bioreactor in the presence of different culture environments.

20.3.3 Models for Commercial Production

Technological development for manufacturing stem cell products has been initiated but is premature at this stage; there is an immense scope for further process development and manufacturing. Two alternate ways by which stem cell products can be made available to the patients:

1. FDA-approved independent commercial organizations provide scale-out services for manufacturing stem cell products. In this cases, patient's cells are harvested and cold-shipped to the manufacturing site; products are returned back to the patients for infusion within a fixed time frame. This model of manufacturing of products is cost intensive.
2. Clinical development pathway deals with manual production of phase I material, which could be open or semi-closed system. This is followed by transfer of manufacturing process to a scalable, closed, and automated manufacturing system after the success of the initial clinical trial [49]. This model allows confident buildup, and thus easy transition of process know-how from early to late phases of clinical trial would be possible. It is also expected that overall investment cost will reduce and also cost-effective production is possible.

Since stem cell therapies moving toward late phase of clinical development the selection of suitable manufacturing technology becomes increasingly important. By this way, the potential pitfalls in process development and scale-up for manufacturing products are identified and timely attended. At present MSC-based therapies are majorly supported by the supply through manually operated open system. If the projected number of patients is increased to 1000, the requirement for clean room area and personnel will be prohibitively high making proportionally increase of costs of production. This suggests the necessity for the parallel development of expansion platform that is fully closed and automated, so that product supply line is maintained. Manufacturing of cells in large-scale suspension culture is given a license if encouraging results are derived in clinical trial using products obtained from the smaller system. This means, the scale-up of closed suspension culture process needs to be initiated just after the evaluation of phase I/II clinical trial results. The early implementation of suspension culture may facilitate safe transition from laboratory to clinical-scale production. The clinical-scale application is specific; $1–2 \times 10^8$ HSPCs cells would be sufficient for a myeloablated patient; while for the treatment of MI or adoptive immunotherapy, about 10^{10} functionally differentiated cells are administered [40]. Above doses provide a reasonable idea regarding the capacity of a bioreactor and other accessory equipment that would be necessary to support large-scale trial for 1000 patients and above.

20.3.4 Future Perspective

Following areas in which further improvement is warranted:

(a) Development of methods for expansion of cells in closed volume, automated culture system,
(b) Improvement of cell yield and efficiency of differentiation,
(c) Use of small molecules and synthetic matrix instead of growth factors/cytokines and biological matrices, respectively,

(d) Development of powerful methods for characterization of cells in culture and determining their biological potency in vivo,
(e) Understanding the biology of the damage tissue niche involving regeneration, and
(f) Development of safe and sensitive methods to monitor homing and migration of cells.

Conclusions

Stem cells have taken the central stage of the discovery of future medicine. The enormous potential of stem cell therapy to repair and/or regenerate disease organs has been recognized in various preclinical studies. As compared to these results, few clinical trial outputs are found to be either inconclusive or contradicting. There is a need to perform global, multicentric clinical trials based on common protocols for those indications in which already encouraging results have been obtained in the proof-of-concept studies. It is equally important to work on pluripotent stem cell differentiation program by which high purity therapeutically active cells are obtained by simple changeover of the culture environment from cell expansion to differentiation mode. As clinical development pathway is the best model for commercial production, it is necessary that this perception is introduced right at the beginning of the development of a stem cell product.

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