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Pluripotent human stem cells

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Pluripotent Human Stem Cells

A Novel Tool in Drug Discovery

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Abstract

The need for new and improved pharmacotherapies in medicine, high late-stage compound attrition in drug discovery, and upcoming patent expirations is driving interest by the pharmaceutical industry in pluripotent stem cells for *in vitro* modeling and early-stage testing of toxicity and target engagement. In particular, human embryonic and induced pluripotent stem cells represent potentially cost-effective and accessible sources of organ-specific cells that foretell *in vivo* human tissue response to new chemical entities. Here we consider the potential of these cells as novel tools for drug development, including toxicity screening and metabolic profiling. We hold that despite various challenges to translating proof-of-concept screening platforms to industrial use, the promise of research is considerable, and close to being realized.

Increasingly stringent regulatory requirements render the process of drug development from initial discovery of new chemical entities (NCEs) through to US FDA approval for clinical use an incredibly labor-intensive, risky, and expensive endeavor. Estimates put the cost of discovering and developing a drug at over $US1 billion.[1,2] Despite increased funding for early-phase research and development (R&D), the number of drugs going to market is in decline, with late-stage compound attrition being a major problem.[3] Less than 10% of NCEs progress through the development process to become a marketed drug,[4] with most candidates failing during phase II clinical trials, suggesting poor translation from animal efficacy studies to human patients.[5] Preparing for clinical trials represents a massive financial outlay, which makes deferred rejection due to unanticipated toxicity or poor efficacy intractable.

Revised approaches to drug development incorporate numerous strategies to accelerate the process, reduce costs, and, importantly, flag risks early in the discovery pipeline. Traditionally, this is done through careful selection of candidates (limiting development to high-probability targets such as

G-protein coupled receptors [GPCRs]), by using disease-relevant cell-based model systems for proof-of-concept (POC) studies, adjunct to appropriate animal models for *in vivo* efficacy and toxicity studies.[4] Early-stage, cell-based screening represents an especially cost-effective way to filter out problematic compounds, with particular interest in models that closely resemble human organs and cells targeted by drug compounds.

Mammalian cell-based assays have become an important tool in the drug discovery process. When combined with enzymatic assays, they can provide valuable POC data by demonstrating target engagement and desired phenotypic responses. Importantly, cultured cells must retain or regain the phenotype of the normal *in vivo* cell or tissue in response to compounds. For example, the use of culture systems to study drug metabolism or insulin secretion currently require primary hepatocytes or pancreatic β cells, respectively, which mimic endogenous cellular processes following exposure to drug candidates.

While primary cells are usually considered the 'gold standard', there are many drawbacks to their use for compound screening or assay models for POC studies. Some cells such as adipocytes or keratinocytes are readily accessible from humans, while others such as hepatocytes or neurons are less easily attainable.[6,7] In most cases, primary cells are terminally differentiated and non-proliferative. This limits the expansion or scale-up of the cells *in vitro*, such that the cells must be harvested in large quantities, likely from many donors, which is often impracticable or unfeasible. Other problems arise from accessing tissue on demand, such as ensuring the quality of the preparation, as well as heterogeneity of cell behavior between donors. Finally, primary cells are invariably difficult to culture, and the cells can rapidly lose their native phenotype due to culture adaptation.[8] However, despite these shortcomings, primary cells are preferred to transformed or immortalized cells, which often lack critical characteristics of the tissue from which they were derived.

Given the limitations of current models, the pharmaceutical industry would undoubtedly benefit from new sources of cells for efficient and germane early-phase compound screening. Although novel, stem cells are predicted to fill the present gap as a readily available supply of relevant and different cell types, suitable for high-throughput application. Already, adult stem cells are being isolated and cultured for various pharmaceutical applications.[9] However, adult stem cells have not been identified in every tissue type, and in many cases are difficult to isolate and culture, often only accessible from post-mortem tissue. Alternatively, pluripotent human stem cells including embryonic stem (ES) cells and more recently discovered induced pluripotent stem (iPS) cells are less restricted, with theoretically unlimited potential to expand and form somatic cell types required by industry.[10,11] This article summarizes the potential of human ES and iPS cells as novel tools in drug discovery (see figure 1). Special consideration is given to toxicity testing as a major cause of late-phase attrition and it is proposed that, despite potential challenges, both cell types represent excellent alternatives to current *in vitro* screening paradigms, as relatively cost-effective and accessible sources of organ-specific cells that predict *in vivo* human tissue response to compound testing.

1. Pluripotent Human Stem Cells

Pluripotent stem cells have the potential to differentiate into any of the three germ layers: endoderm (e.g. pancreas, gastrointestinal tract, lungs), mesoderm (e.g. muscle, blood, bone) or ectoderm (e.g. epidermis, nervous system). They include natural ES cells and engineered iPS cells, which are by-and-large similar since both express typical stem cell genes and proteins,[12] share similar chromatin methylation patterns,[13] and form embryoid bodies *in vitro* and teratomas *in vivo*.[14,15]

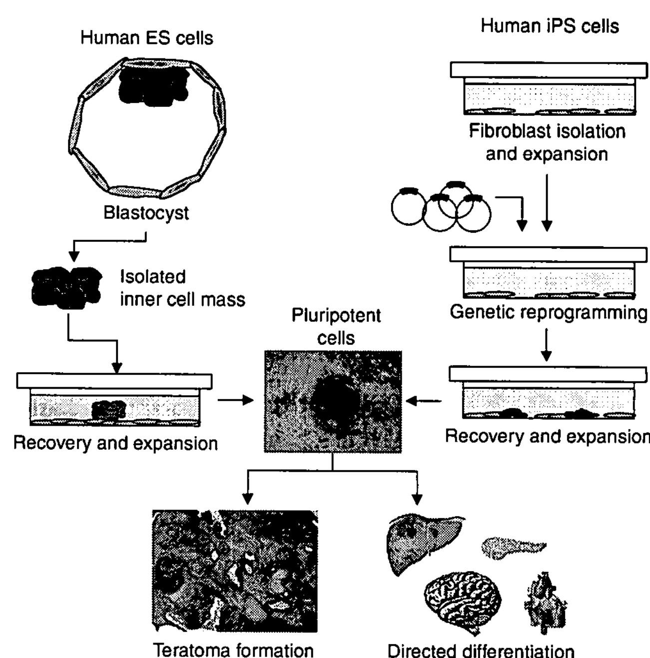

Fig 1. Schematic of human embryonic stem (ES) and induced pluripotent stem (iPS) cell production for *in vitro* modeling and drug discovery. Once derived, stem cells have unlimited potential to expand and differentiate to all somatic cell types, typically characterized by *in vivo* teratoma formation (comprising endoderm, mesoderm, and endoderm derivatives).

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Since their initial isolation[16] and ensuing prolongation as stable and expandable cell lines,[17] human ES cells have generated much hope as the first widely accessible pluripotent stem cells. Hundreds of lines have been produced worldwide from supernumerary blastocysts by established methods of isolation, culture, and preservation. Theoretically capable of differentiating into more than 220 somatic cell types,[10] the past decade has also seen the development of innumerable protocols to induce human ES cells into clinically relevant lineages including pancreatic β cells,[18] cardiomyocytes,[19] and neurons.[20] By applying optimal and standardized approaches to differentiation, efficient and cost-effective methods of stem cell expansion, derivative cell enrichment, and quality controlled (e.g. good manufacturing practice [GMP]-compliant) stem cell production, the use of ES cells for drug discovery will likely progress from being theoretical, and at best POC, to mainstream tools of the pharmaceutical industry. The ability to model disease states[21] by genetically modifying stem cells and their derivatives will undoubtedly broaden their application.

The recent discovery of somatic cell reprogramming by viral delivery of four genes (*OCT4*, *SOX2*, *MYC*, and *KLF4*) to derive pluripotent cells[22] provides an attractive alternative to ES cell-based modeling, not least because of fewer ethical dilemmas for stem-cell production and the relative ease of sourcing cells, including disease-specific cells. Since initial breakthrough studies with mouse embryonic and human fibroblasts,[12,23] major goals for the field include the elimination of viral vectors and potentially mutagenic molecules from reprogramming, with concomitant improvement of induction efficiency.[22,24-29] Similar to human ES cells,[30,31] as optimal methods of iPS cell derivation are identified, increased attention is being given to understanding line-to-line variability and the full extent of ES and iPS cell likeness. While human ES and iPS cells share similar gene expression profiles, there appears to be a small number of genes consistently expressed differentially in iPS cells, indicating subtle epigenetic differences related to the reprogramming process.[32] Finally, in addition to the basic biology of iPS cells, there is increasing focus on the production and differentiation of disease-specific cell lines for *in vitro* modeling and longer-term autologous cell therapy.[21,33,34]

2. Drug Discovery and *In Vitro* Modeling

Pharmaceutical companies require well regimented screening systems to identify biologically active compounds with minimal toxicity. Typically, compounds are selected based on interaction and engagement of the chosen target (producing either the desired inhibition or activation).[35] The 'hits' are then filtered through a series of *in vitro* and *in vivo* assays to demonstrate efficacy and safety profiles before being declared as 'lead' and then 'candidate' compounds. Therefore, there is a need for cell-based model systems that accurately emulate the *in vivo* response, particularly for early-stage compound selection. While freshly isolated primary cells are often superior in performance compared with immortalized or transformed cell lines, they can be difficult to isolate and have limited proliferative potential and unstable phenotypes, affecting batch variability.[36] Nonetheless, since hepatocytes have long been implicated in the development of metabolic diseases, transformed hepatocytes such as HepG2 cells or freshly isolated non-proliferative hepatocytes have been popular for *in vitro* NCE screening (e.g. drug candidates for diabetes mellitus and obesity).[37] This is despite a tendency to dedifferentiate when culture adapted, which limits their use to a few days after isolation and causes batch variability.[38] Moreover, it remains to be shown how closely *in vitro* hepatocyte response matches the *in vivo* response with respect to, for example, cytochrome P450 (CYP) enzyme induction and drug interaction.[39] Similarly, other primary cell types such as human pancreatic β cells would be useful for drug discovery if they could be effectively supplied, isolated and stably maintained *in vitro*. Unfortunately, current methods are at best derisory, with cells tending to quickly lose functionality; thus, researchers often opt for cadaveric organ tissue isolations that are in limited supply and often comprise undefined mixed cell populations that are less relevant to tissue-specific POC studies.[36] In comparison, stem cells clearly retain replicative potential, and have demonstrated differentiation capabilities. Accordingly, the use of pluripotent cells and their derivatives are attractive alternatives to more conventional primary cells.

Despite the recognized potential of stem cells, the pharmaceutical industry has been slow to adopt them as tools for screening. This is likely due in part to the dearth of research relating to stem cell modeling, with few published examples of these cells as practical models in R&D and drug discovery. In addition, traditional methods for working with stem cells (in particular methods for human ES cell culture and differentiation) are onerous and complicated. However, as simpler, scaleable, standardized, and more efficient protocols are devised, a few leading pharmaceutical and biotechnology companies such as GE Healthcare (in partnership with Geron Corporation), Pfizer, and Novartis have begun to 'test the waters' of human ES cells for translational application.

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Recently, there have been several examples of high-throughput screening for small molecules influencing ES cell differentiation, mostly with respect to regenerative medicine applications. Pancreatic development and improved β-cell induction were recently addressed using murine ES cells expressing *Discosoma* spp. red fluorescent protein (dsRED) under the control of the *SOX17* promoter (an endoderm-expressed gene). The cells were used to screen around 4000 molecules resulting in 27 primary 'hits' subsequently narrowed down to two compounds that reproducibly induced endoderm formation in their ES cell model.[40] A similar approach has been adopted to optimize murine ES cell neurogenesis protocols.[41] Using the dual luciferase reporter clones to monitor Tα1 α-tubulin expression, the authors screened 975 compounds. Thirty-two hits were further analyzed to identify phenazopyridone, which was further shown to promote neuronal differentiation in human ES cells. A recent POC study using human ES cells has shown that the cells can be seeded into 384-well plates in single cell suspension and used for high-throughput screening using immunocytochemistry and high-content imaging.[42] In this case, the authors monitored pluripotency and lineage markers, which emerged from the human ES cell culture following compound treatment. Though preliminary and often optimized in murine pluripotent cells, these studies indicate that the use of pluripotent cells is feasible, and can produce hits. As improved culture conditions arise, we can look forward to further screens for regenerative medicine or using enriched human ES cell-derived populations for functional assays. Combined with an increasing confidence and interest in the potential of iPS cells, we predict a rapid expansion of pluripotent stem cell-based modeling for research and applied drug development. This includes modeling with cells provided by healthy donors, congenitally ill donors, or transgenic cells following *in vitro* targeted disease-related gene disruption.

3. Drug Toxicity Assessment

Currently, one of the main contributors to late-stage attrition of compounds in the pharmaceutical industry is toxicity. In many cases, the appearance of unanticipated contraindications will not be apparent until clinical trials, following considerable cost and time in the development pipeline. Late discovery arises from the use of inappropriate *in vitro* and/or *in vivo* testing regimes that are poor predictors of clinical response. In principle, early *in vitro* toxicity testing using potentially limitless human ES or iPS cell derivatives represents a practical and cost-effective alternative to traditional approaches. Given the pharmaceutical industry's penchant for primary cardiomyocytes in toxicity screening, stem cell-derived cardiomyocytes are appealing.

3.1 Cardiotoxicity

Cardiomyocyte models are used in toxicity testing for several reasons. In addition to the high burden of cardiac infarct as a leading cause of death, adverse cardiac affects of drug candidates are a leading cause of late-stage attrition. The recent withdrawal of rofecoxib (Vioxx®) from the market due to cardiotoxicity highlights the immense cost in both legal fees and lost revenues caused by late-stage candidate failure.[43] Unfortunately, cardiotoxicity culminating in death is usually detected after large numbers of patients receive a drug during or after clinical trials and reinforces the importance of early detection.[44]

The complexity of cardiomyocyte function with a multiplicity of channel proteins presents several off-target mechanisms for compounds to interact with. The end result can be ventricular tachycardia, also described as torsades de pointes ('twisting of points'; TdP) based on the altered shape of the QRS complex of an ECG recording.[45] Importantly, TdP can quickly accelerate into ventricular defibrillation followed by cardiac failure and death.[46] Since it is rare, it is unlikely to be detected during traditional phase III trials. The risk of TdP arising is all the more alarming for drugs targeting non-life-threatening maladies. Current predictive tests include complex, variable, and low-throughput *in vivo* animal studies of elongated QT intervals in an ECG trace.[47]

Other serious although non-lethal problems relating to cardiotoxicity should be identifiable early in the drug discovery process. Again, methods of testing range from relatively simple high-throughput *in vitro* primary cell screens to more complex and expensive *ex vivo* (e.g. organ culture-based) and *in vivo* studies.[47] In humans, one of the main channel proteins associated with ionopathy is the hERG protein (human Ether-a-Go-Go).[48] This potassium ion channel is associated with elongated QT interval. A common initial assay is to measure hERG binding by target compounds, or to use more complex patch clamping to measure hERG ion channel activity.[47] Though routinely used by pharmaceutical companies (and endorsed by regulatory agencies)[49] these methodologies are limited by being dependent on the isolated hERG channel that is often over-expressed in HEK293 cells, which are not reflective of beating cardiomyocytes and their full complement of ion channels and surface proteins. More complex *ex vivo* cardiotoxicity screens such as the Langendorff assay (which

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employs excised rodent hearts) and *in vivo* animal studies are physiologically more relevant, although they are low throughput, potentially non-predictive of human myocardium, and expensive.[47] Importantly, while no amount of *in vitro* testing can match *in vivo* human trials, the inadequacies of existing pre-clinical assays make human stem-cell cardiomyocyte derivatives attractive alternatives for pre-clinical testing.

Early cardiomocyte induction protocols involved co-culture of human ES cells with a fibroblast feeder layer that expressed endoderm-like signals.[50] Protocols were progressively improved by eliminating feeders,[51] and serum-reduction for improved differentiation.[52] Finally, several groups have developed enrichment strategies by generating transgenic cell lines for cardiomyocyte selection through antibacterial resistance[19] or the use of small molecules to improve differentiation efficiency.[53]

Recent progress in differentiating human ES cells to functional cardiomyocytes has enabled sufficient levels of enrichment for electrophysiological studies using microelectrode array systems.[19,53] This has provided the impetus for several companies such as Reprocell (QTempo) and Roche (xCELLigence system) to further optimize and validate this type of assay for compound screening. Indeed, the company Cellular Dynamics International, who have a commercial interest in human ES cell- and iPS cell-derived cardiomyocytes for cardiotoxicity screening,[54] have partnered with Roche to assess well characterized kinase inhibitors using the iPS cell-cardiotoxicity model. POC studies are encouraging, with compounds known to interfere with cardiomyocyte ion channels showing measurable effects on human ES cell-derived cardiomyocyte function.[55]

While ES cells are becoming widely accepted as nascent sources of cardiomyocytes for early detection of toxicity problems, the potential of iPS cells is only now beginning to emerge. However, the advances of their embryonic counterparts will undoubtedly facilitate their acceptance and application. Recent reports provide POC of human iPS differentiation into cardiomyocytes[56] and potential for cardiotoxicity assays,[57] where cardiomyocytes show expected morphology and responded to β-stimulators, β-adrenoceptor antagonists (β-blockers), and ion channel blockers.

3.2 Embryotoxicity

The primary characteristics of ES cells are their ability to self-renew and pluripotency. These attributes make ES cells useful to model early development and embryotoxicity. The embryonic stem cell developmental toxicity test (EST), an *in vitro* screening assay used to investigate the embryotoxic potential of chemicals by determining their ability to inhibit survival and/or differentiation of ES cells, has become a popular tool for environmental toxicologists. For example, a recent study of nanoparticles demonstrated embryotoxicity using a mouse EST.[58] However, despite the apparent success of animal-based ESTs, like other non-human cellular models, they are not necessarily predictive of human biology. Importantly, human ESTs have recently been used to determine toxicity concentrations that produce 50% inhibition (IC_{50}) for fluorouracil and retinoic acid.[59] Moreover, the sensitivity of ES-derived cardiomyocytes to bile acids has been investigated as a model of obstetric cholestasis, an important cause of maternal delivery complications and fetal death.[60] This study showed that immature ES cell-derived cardiomyocytes of either human or mouse origin are more sensitive to the presence of taurocholate, one of the principal constituents of maternal bile acids, compared with mature cultured cardiomyocytes. The sensitivity presented as reduced and irregular contraction rate, depressed amplitude, and disorganization of contractile networks.

As human ES cell differentiation protocols become more refined, the use of embryotoxicity tests for drug screening will become more common by enabling extensive modeling of embryonic and fetal development using different precursor cell and tissue types. The extent to which iPS cells can similarly be used will depend on how 'embryonic-like' they really are. Time will tell!

4. Drug Metabolism Assessment

It is important to anticipate the metabolic profile of a given compound, since drugs are transformed into new chemical species with therapeutic and/or toxic effects. The simplest approach involves membrane fractions (microsomes) from human liver preparations. Microsomes are stable, can be stored for an extended time, and can be prepared in large quantities. They do, however, exhibit a limited expression of drug metabolizing enzymes and related CYP enzymes.[6] Cultured primary human hepatocytes provide the closest model of human liver, exhibiting typical hepatic functions and drug-metabolizing enzymes. Ideally, the latter should include stable expression of CYPs (including CYP1A2, CYP2A6, CYP2B6, CYP2Cs, CYP2D6, CYP2E1, CYP3A4, and CYP3A5), which are the primary mediators of phase I metabolism for the initial breakdown of compounds into metabolically active byproducts or other non-functional metabolites. Not surprisingly, traditional hepatocyte models are limited by being difficult to prepare, have limited scaleability, and experience batch-to-batch

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variability due, in part, to variable cytochrome expression.[61] In view of the limitations of current methods, human ES and iPS cell-derived hepatocytes would likely bolster the use of cellular and other *in vitro* models for metabolic risk assessment.

Early studies of teratomas and spontaneously differentiating cells showed that human ES cells are capable of becoming cells expressing hepatocyte markers.[62-64] More refined differentiation protocols have been based on hepatocye development *in situ* and murine ES differentiation. However, despite hepatocyte conditioned medium and acidic fibroblast growth factor (FGF1) improving the efficacy of differentiation, only ~6% of cells were albumin expressing.[65] Protocols have more recently improved, with the use of activin A and Wnt3A increasing induction to 90% albumin-positive hepatocyte-like cells.[66] Importantly, these cells expressed a large complement of other hepatocyte-specific markers including alpha-fetoprotein (AFP), hepatocyte nuclear factor 4-α (HNF4A), tyrosine aminotransferase (TAT), tryptophan oxygenase (TO), apolipoprotein E (APOE), and several CYPs. Also, POC for metabolism modeling was demonstrated by comparing CYP1A2 activity of human ES cell-derived hepatocytes to isolated native human hepatocytes. ES cell-derived hepatocytes performed admirably, achieving 4–24% activity (depending on the clone tested) of the primary cells.[66] This reinforced previous work, which showed that enriched human ES cell-derived hepatocytes expressed a panoply of cytochrome genes and CYP1A2 activity (around 25% activity compared with primary human hepatocytes).[67] When transplanted into severe combined immunodeficient (SCID) mice, the authors demonstrated engraftment and release of human liver-specific proteins into the serum even after 75 days. Collectively, these studies show the potential of pluripotent stem cell-derived hepatocytes for metabolic profiling.

5. Disease Modeling

Clearly, there are many obstacles to the clinical use of pluripotent stem cells. These include demonstrable alternatives to viral vector-based methods of iPS cell derivation, and platforms for large-scale stem cell culture and differentiation. A more near-term application is disease modeling.

Disease modeling with ES cells is possible by targeted gene disruption and cell-line isolation from congenitally defective blastocysts identified by preimplantation genetic diagnosis (PGD). While gene targeting by, for example, RNA interference has been reported,[68-70] poor transfection and cloning efficiencies limit efficacy.[71,72] Recent efforts to improve homologous recombination show promise,[21,73] although there remains an ongoing concern for the clonal selection of karyotypic abnormalities.[74] Genetic manipulation of ES cell derivatives may be a better alternative to undifferentiated ES cells, with two recent reports indicating lentiviral-based transduction of self-renewing human neural-lineage restricted progenitor cells.[69,75,76] PGD is used to detect genetic defects before implantation of *in vitro* fertilization (IVF) embryos. Once identified, ES cell lines can be derived from defective embryos for disease modeling. Examples of PGD-derived cell lines include Huntington disease[77,78] and fragile X syndrome[79]

The accessibility of disease-specific iPS cell lines makes them leading candidates for disease modeling and drug discovery.[80] While their ability to recapitulate cellular mechanisms of disease states remains to be determined, numerous lines have been derived. Examples include trisomy 21, Huntington disease, amyotrophic lateral sclerosis, Gaucher disease, Duchenne muscular dystrophy, and type 1 diabetes, among others,[33,34,81,82] with POC for drug screening applications provided using spinal muscular atrophy (SMA)[83] and familial dysautonomia[84] cell lines.

SMA cells are characterized by loss of function of the *SMN1* gene (encoding survival of motor neuron 1, telomeric) and are capable of robust expansion and neuronal differentiation, but exhibit a marked motor neuron degeneration, which mirrors the *in vivo* phenotype of SMA patients. To determine if the 'diseased' iPS cells respond to compounds, the authors studied the response to valproic acid, a compound that increases SMN1 protein levels.[85] In both SMA fibroblasts and SMA-derived iPS cells, valproic acid resulted in increased nuclear SMN1 aggregates.[83] Based on this result, it is possible to envisage a high-content imaging approach to screen for compounds that could restore motor neuron function in patients with SMA.

Familial dysautonomia is a disease characterized by sensory and autonomic neuron degeneration. The mechanism of disease progression is not clearly understood but is thought to be associated with a point mutation in *IKBKAP*, resulting in a tissue-specific splice defect.[86] iPS cell-based modeling has shown defects in *IKBKAP* splicing and neurogenesis that can be resolved by treatment with candidate compounds such as kinetin.[84]

Given the potential of pluripotent cell lines for research and translational application, their use should entail careful and standardized culture and cell banking, complimented by a baseline level of characterization to assure their capacity for self-renewal, pluripotentiality, and minimal sample-to-sample and batch-to-batch variation. Such measures will maximize their usefulness as a valuable resource for understanding the cause and pathology of disease and drug discovery.

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6. Scale-Up and Good Manufacturing Practice Compliance

A major requirement for drug discovery is large, homogeneous populations of stem cells and their derivatives for early-phase high-throughput compound screening. Ideally, the number of cell batches should be minimized and the size of individual batches maximized. While traditional culture methods are inadequate,[17] innovations in human ES and iPS cell culture and expansion offer practicable alternatives including feeder-free and 3-dimensional platforms for large-scale cell production.[87-92] While removal of feeder cells has posed less of a challenge (using, for example, basic FGF [FGF2] fibroblast-conditioned media and various growth substrates),[93-96] advancing to single-cell culture from clumped-based methods has been more difficult, though not insurmountable. Many groups have employed trypsin or other enzymatic approaches to stem-cell expansion[97-99] despite concerns for karyotypic stability of the cells.[100] More recently, several groups have shown that Rho kinase (ROCK) inhibitors facilitate long-term, single-cell culture of human ES cells.[101] This has paved the way for improved cell expansion using cell factories or stirred suspension cultures on microcarriers.[88] Whether or not a similar approach can be applied to iPS cell scale-up remains to be shown.

Improved methods of ES and iPS cell culture are complemented by improved quality of cell-line derivation. Until recently, all human ES cell lines were derived and maintained under laboratory-grade conditions, without standard quality control including validation of pathogen status, risks of genetic and phenotypic instability, traceability, and cross-contamination. Quality control strategies are essential for high-quality stem cell-line production necessary for stem cell research and translational applications such as drug development. Recent examples of best practice include the derivation of cell lines under GMP conditions for clinical compliance.[102] The option to use such lines for drug discovery is set to expand, with programs supported by the UK Medical Research Council and a project between the Waisman Clinical Biomanufacturing Facility and WiCell Research Institute to produce GMP human ES cell lines. Such lines will be attractive for both drug discovery and cell-based therapeutics.

7. Challenges

Despite the potential of pluripotent human stem cells, their acceptance by the regulatory authorities and wholesale adoption by the pharmaceutical industry for drug development is not without its challenges. As biologicals, they tend to be unstable in culture, causing variability from passage to passage, batch to batch, and line to line.[31] For example, cells that are derived and/or cultured using the same methods inevitably exhibit phenotype instability and differences in differentiability. This seems irreconcilable with the rigorous statistical uniformity tests applied to assay development for drug discovery. To be used successfully as screening tools, the cells must behave consistently from assay to assay in order to pass typical pharmaceutical industry assay validation criteria.

Other challenges are less specific to human ES and iPS cells but rather common to cell-based assayology in general. These include devising strategies for single-cell adaptation of stem cells for automated handling and high-throughput plating and screening, as well as managing the 'edge effect' of cultured cells, evaporation and gas exchange.[103]

8. Conclusions and Future Perspectives

Pluripotent human stem cells offer new and compelling ways to discover and develop a new generation of medicines. Benefits arise from a potentially limitless cell supply, the opportunity for large-scale cell banking for ease of access and quality control, relevance to *in vivo* human tissue response, and early application in the drug discovery pipeline. Moreover, efficient differentiation to functional cell types such as hepatocytes and cardiomyocytes will ensure a reliable supply of cells for metabolism and toxicity testing of drug candidates. Collectively, these features are expected to decrease the risk of late-stage attrition of NCEs, reduce R&D costs, and increase the likelihood and rate of *bona fide* drug discovery – primary objectives for the pharmaceutical industry. While there are significant challenges to advancing current POC platforms, these will be addressed by improved and standardized methods of growing and screening cells. Importantly, investment by the pharmaceutical industry into human pluripotent stem cell R&D will undoubtedly assist to overcome the remaining hurdles to full-scale industrial application and accelerate the drug development process.

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