Unlocking the full potential of induced pluripotent stem cells for drug discovery

Human stem cells were once viewed primarily as regenerative materials for tissue repair through cell therapies. However, advances in technologies and protocols mean that induced pluripotent stem cells (iPSCs) are now playing an increasingly important role in disease modelling and human cell-based screening assays. This roundtable explores what this means for drug discovery.

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The pharmaceutical industry's current issue with attrition in the R&D pipeline is complex and multifaceted. However, few would disagree that one of the biggest factors contributing to the poor rate of success in drug discovery is the lack of reliable and translationally useful disease models.

No matter how carefully studies are designed, animal models and immortalised cell lines cannot reflect the full complexity of human biology and disease mechanisms. While the use of human primary cells or human tissue samples is an attractive alternative for drug screening, these materials can be difficult to obtain, and require additional ethical considerations.

A more physiologically-relevant approach to the preclinical evaluation of drug efficacy and toxicity is the use of stem cells. Stem cells have the unique capacity for indefinite self-renewal and the capability to differentiate into multiple cell types. Pluripotent stem cells (PSCs) can differentiate into most cell types of the body, while tissue-specific stem cells, such as hematopoietic stem cells (HSCs), can differentiate into all mature cell types for a given tissue.

Human iPSCs possess a number of important properties that make them an excellent source of cells for disease models and compound screening assays. Not only do they have the potential to generate the full range of human cell types, they can be derived from any individual, even a patient. Consequently, patient samples can be used to create iPSC lines that harbour disease-causing mutations, which can subsequently be genetically modified to correct the mutation and study the disease in more detail, or to correct the cells for the development of novel cellular therapies. iPSCs generated from healthy donors can also be genetically modified to introduce disease-causing mutations to validate specific targets in the pathogenesis of a disease.

Furthermore, iPSCs can be expanded to large quantities and cryopreserved, and can be derived from easily accessible cell types, such as peripheral blood mononuclear cells or skin fibroblasts.

This ability to create customised, physiologically relevant models with relative ease opens up a wealth of new opportunities in drug discovery, particularly for disease areas where access to reliable cell models has historically been a stumbling block. These include neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, as well as certain cardiac abnormalities, which can be extremely difficult to detect using traditional models[1]. Therefore having complementary assays based on stem cell-derived cell lines would be an incredibly useful and cost-effective tool for drug discovery efforts.

"The availability of primary human brain or heart tissue for drug discovery purposes is limited," says Dr Gregor von Levetzow, Global Product Manager at Miltenyi Biotec. "Being able to generate iPSC-derived neurons and cardiomyocytes allows for large-scale drug screening, providing a more precise prediction of drug efficacy in humans than could be achieved with animal experiments alone."

From tissue repair to disease models and drug screening

The use of iPSCs has grown significantly over the past several years with large numbers of pharmaceutical companies adopting stem cells for toxicity testing and drug screening. However, this wasn't always the case, and this progress has been made possible in large part due to rapid advances in associated technologies[2].

Human stem cells were traditionally viewed as regenerative materials and largely limited to tissue repair applications. A lack of standardised culture and differentiation protocols prevented their use in translational settings, while time-consuming gene editing techniques of the past slowed down the development of stem cell-based disease models.

Over the last decade, stem cell maintenance and differentiation protocols have become much less complex. Culture techniques have now become substantially easier to use, leading to increased scale-up options for high-throughput drug discovery settings. Additionally, faster, more efficient and more reproducible gene editing technologies such as CRISPR-Cas9 have redefined what is possible when it comes to designing cell models of disease. Specific disease-associated mutations can now be more easily studied *in vitro*, enabling analysis of gene function and the underlying role in disease progression. These advances, alongside the enormous contributions to iPSC research made by Shinya Yamanaka and James Thomson's laboratories, have rapidly changed the stem cell field[3,4].

Today, reprogramming donor-provided somatic cells has become commonplace. The ease with which these cells can be derived from donors, both with and without disease-causing mutations, offers a very attractive way to study the genetic impact of many diseases. Human iPSCs can now be used to produce an almost unlimited number of cells critical for preclinical safety tests. These cells can be generated from cohorts of both healthy and patient donors with diverse genetic backgrounds, to provide a platform for toxicity screening. This makes them more representative of populations, and therefore a more translatable model.

What's more, whole genome sequencing can be performed on donor lines to identify specific mutations that are relevant to a disease. In this way, it is possible to combine clinical presentation with genomic information to create a model that essentially mimics a disease in a dish. These models allow researchers to assess how specific demographics and cell types react to novel drugs prior to any clinical tests, preventing drug failures much earlier in the discovery and development process, and thereby helping to reduce costs and improve success rates.

With the industry striving for more translational disease models, a particular focus is being put on 3D cell models. These models, grown in more physiologically-relevant conditions, have the potential to generate more pertinent preclinical insights that can be used to predict efficacy and toxicity.

"There's mounting data to show that liver cells derived from stem cells grown in 3D are a much better model by which to assess metabolic liability," says Dr Richard Eglen, Vice President and General Manager at Corning Life Sciences. "This ability to perform preclinical optimisation of compounds before they go into the patient is making a big impact. You can take precise genetic mutations from individual patient populations, and really mimic the action of your compounds in phenotypically-derived cells and get precise measurement of their liability towards metabolism and toxicity."

As recognition of the robustness and translational relevance of 3D stem cell-derived models grows, their use is set to have a particularly important impact on drug discovery research for applications where human cells and tissues act and behave differently to animal cells and tissues. The observance of more predictive behaviour with iPSCs – when

used in combination with a new generation of kinetic, live-cell assays, for example – is driving the adoption of these alternative assays in an industry increasingly looking to reduce its reliance on animal studies.

"Assays run on cell analysis platforms that employ built-in injection technologies and study biology in real-time are increasingly valued for their ability to model more complex cellular behaviour," says Dr David Ferrick, Senior Director of the Cell Analysis Division at Agilent Technologies. "It's hoped that the greater translational value and scales of economy these systems bring will facilitate fewer drugs failing in the development stage, especially after they have shown promising results in animals."

Tackling the challenge of slow maturation of human iPSCs

While stem cells have enormous potential in drug discovery applications and beyond, several challenges remain. One of the most pressing issues is the fact that in many cases, differentiated cells exhibit expression patterns and functional behaviour that are more closely matched with cells of a foetal age than those of an adult. This can be problematic for researchers working with cell types that are difficult to mature *in vitro*, such as cardiomyocytes and neural cells, as well as for those modelling late-onset diseases. Many engaged in stem cell research are searching for solutions to this issue.

One way to accelerate the human developmental clock in culture dishes is by manipulating the culture environment, and innovations in culture techniques and technologies are making this easier. "The challenge of slow maturation can be addressed using 3D cell culture scaffolds and matrices that mimic the adult extracellular environment, or through co-culture techniques that employ supportive cells and electrical or physical stimulation," explains Dr Yichen Shi, CEO at Axol Bioscience. Another solution involves direct programming of iPSCs by driving stem cell differentiation through transient expression of cell type specific transcription factors. "Introducing transcription factors into the cells by viruses, lipid vehicles or directly inserted into specific locations of the genome using CRISPR-Cas9 can also be effective," Shi adds.

In the latter case, when editing the genomes of iPSCs to generate isogenic pairs or to insert sequences that could act as detectors or reporters of specific biological end-points, the generation of single-cell derived clones is typically required. However, in the past, this has been problematic. "Historically, it's been a real challenge to isolate single iPSCs," says Dr David Piper, Director of Research and Development, Cell Biology and Synthetic Biology at Thermo Fisher Scientific. "There have been some great strides made in the development of media and supplements to support single-cell culture applications. Similarly, some of the latest Cas9 nucleases have exceptional purity and activity to support high efficiency editing in iPSCs."

Reducing variation in growth and differentiation potential

To support pharmaceutical pipelines, large numbers of high-quality, homogenous cell cultures are required. Currently, stem cells are typically manufactured in small scale dishes or T-flasks, and scaling-up stem cell production while ensuring quality and reproducibility can be challenging. To mitigate this issue, robust culture conditions and validated protocols that are amenable to scale are needed to ensure consistency between conditions and between experiments.

Variability between cell lines or sample sources is a particular hurdle. "Some groups are addressing this issue using gene editing technologies to enable derivation of isogenic lines to control for genetic background differences," explains Helen Hardiman, Bioengineering Product Manager at STEMCELL Technologies. Another source of variability is within the culture itself, and this can become a larger issue when large numbers of cells are required. An effective solution to this issue is to use a single large-scale suspension culture vessel instead of traditional well plates or T-flasks. "For human pluripotent stem cells (hPSCs), commercially available culture medium and scale-up systems are enabling researchers to generate large numbers of cells in a single, large-scale suspension culture vessel, thereby reducing variability."

Another issue is a historical lack of support for certain cell lines. In many cases, published protocols for the differentiation of iPSCs into disease-relevant cell types are either not available or are so complicated and labour-intensive that the differentiation protocol cannot be easily translated into a robust protocol for manufacturing cells at a scale large enough to accommodate drug screening assays. "Until very recently, most of the focus has been on specific disease-relevant cell types, most notably, cardiac, hepatic, pancreatic and neuronal cells. This means that other cell types like lung, endothelial, or intestinal cells traditionally haven't been well defined," explains Dr Liz

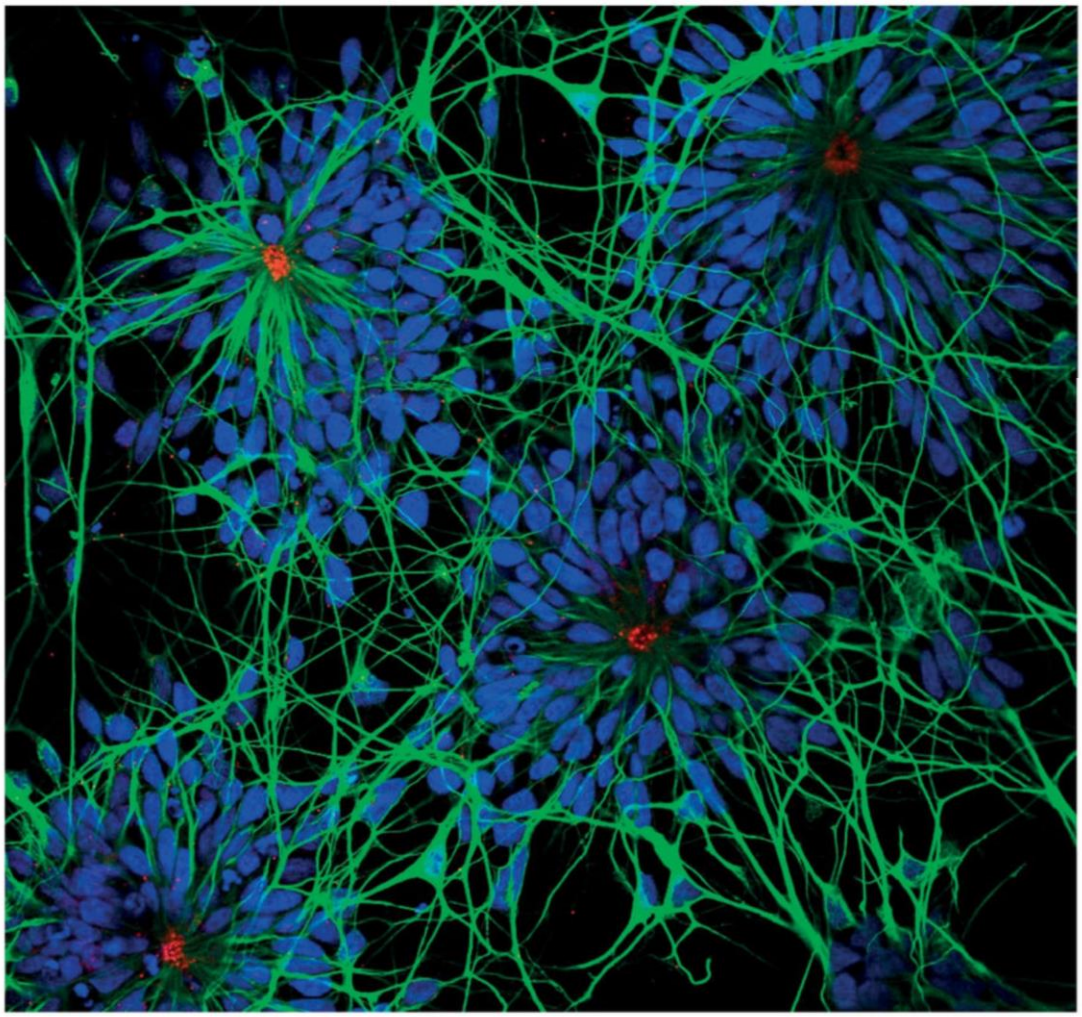

Figure 1
Human-induced pluripotent stem cells (iPSCs) possess a number of important properties that make them an excellent source of cells for disease models and compound screening assays. The image illustrates Axol's Human iPSC-derived Neural Stem Cells forming a rosette structure. Markers used: acetylated alpha tubulin (green), CEP135 (red), DAPI (blue)

Quinn, Associate Marketing Director for Stem Cell Technologies at Takara Bio. "Now, some vendors are commercialising complete systems for the differentiation of cells into various disease-relevant cell types, such as hepatocytes and beta cells. As a result, we should expect to see the publication of more differentiation protocols that will allow for scale-up on a level that will facilitate drug screening."

Commercially-available stem cell technologies

Many technology developers are heavily engaged in the stem cell field and working to overcome current challenges and realise the full potential of stem cells for drug discovery.

Agilent's Seahorse XF technology enables reliable prediction, monitoring and tracking of cell fate transitions to accurately identify pluripotency and differentiation transitions. Agilent Seahorse XF Analyzers simultaneously measure mitochondrial and glycolysis energy pathways in live cells using label-free, solid-state sensor cartridges in microplate format. Together with the Seahorse XF Assays and Kits range, which includes the XF Cell Mito Stress Test Kit and XF Glycolytic Rate Assay Kit, this platform can be used to take metabolic measurements by which reprogramming efficiency, differentiation and lineage commitment can be determined.

Axol Bioscience has a broad range of products for stem cell research, including a large number of iPSC-derived cells that comprise neural, cardiac, vascular, immune and renal cells, among others. Axol also offers an extensive collection of human iPSC-derived disease models, including those for Alzheimer's disease, Huntington's disease, frontotemporal dementia, epilepsy and a wide range of cancers. Isogenic human iPSCs, developed by introducing disease-associated mutations into cell lines using CRISPR-Cas9 gene editing, are also available (**Figure 1**).

Corning Life Sciences provides advanced cell culture technology to ensure stem cell viability, reproducibility and regulatory compliance. The company specialises in innovative solutions for the generation of *in vivo*-like 3D models, such as Ultra-Low Attachment vessels, Corning Matrigel® matrix and Transwell® permeable supports. Corning recently added a 1536-well Spheroid Microplate to its line of Spheroid plates. To help streamline work with 3D cell cultures in an automated environment, the 1536-well spheroid microplate features a novel and proprietary design that allows scientists to generate, culture, assay and analyse 3D multicellular spheroids in one microplate. The spheroid microplate eliminates the need for a transfer step in visualisation or biochemical assays, reducing the risk of cell damage or loss. The 1536-well format takes spheroid plates out of the research lab and into high-throughput screening, enabling researchers to drive a larger volume of compounds through drug screening in less time, using a more physiologically relevant 3D cell culture (**Figure 2**).

Figure 2
Advances in 3D stem cell models are helping to break barriers in drug discovery. The 1536-well spheroid microplate from Corning allows scientists to generate, culture, assay and analyse 3D multicellular spheroids in one microplate

Miltenyi Biotec offers a range of instruments and reagents to drive a standardised approach to generating iPSCs. The company's CliniMACS Prodigy Platform provides a fully-automated, closed-system approach to generating differentiated cells. All protocol steps during cell culture and differentiation, including passaging, timed cytokine exposure

Figure 3
Advances in automated devices for stem cell differentiation improve standardisation, cost reduction and high-throughput capabilities. The CliniMACS Prodigy Platform from Miltenyi Biotec is a fully-automated, closed-system approach to generating differentiated cells

Figure 4
Feeder-free culture systems prevent the exposure of PSCs to animal pathogens. The Thermo Fisher Scientific Gibco™ StemFlex™ medium supports the robust expansion of feeder-free pluripotent stem cells (PSCs) and is optimised to support novel applications, including single-cell passaging and genome editing

and expansion, are performed on one device using a single-use tubing set composed of bags, fluidic paths, centrifugation chamber and temperature control element. Integrated IPC/QC sampling pouches provide the option to take a sample without compromising the sterility of the system. By reducing hands-on steps performed by an operator, the system can increase reproducibility during differentiation and expansion, allowing for more robust subsequent drug screening experiments that are suitable for clinical scale-up (**Figure 3**).

STEMCELL Technologies offers robust culture systems for optimum reproducibility. This includes the TeSR™ range of human embryonic stem and induced pluripotent stem cell culture media, a family of feeder-free media that is produced using prescreened materials to ensure the highest standard of batch-to-batch consistency. In addition to mTeSR™1, the most widely published feeder-free hPSC culture medium, product offerings include the ArciTect™ CRISPR-Cas9 system for genome editing, the CloneR™ supplement for robust generation of clonal hPSC cell lines without the need for single-cell adaptation and mTeSR™3D, an optimised suspension culture medium to enable seamless scale-up. Differentiation of hPSCs towards specific lineages is also supported with the STEMdiff™ product line of specialised kits for generating diverse cell/tissue types from all three germ lineages (ectodermal, endodermal and mesodermal), including neural, hematopoietic, mesenchymal, pancreatic progenitor and cardiac cell types, as well as cerebral and intestinal organoids.

Takara Bio's Cellartis DEF-CS 500 Culture System allows iPSCs to grow in a 2D-monolayer that is unlike the traditional colony culture approach. The 2D-monolayer allows cell generation to be readily scaled, giving scientists more control over how many cells are formed in a flask, plate or dish. Cellartis DEF-CS 500 media is designed to ensure cells maintain pluripotency and remain karyotypically normal, for optimal results. Additionally, Takara Bio has recently launched a kit containing all the necessary additives to allow researchers to generate hepatocytes from their own iPSCs. The kit is based on a differentiation protocol that mimics embryonic development to offer a universal and robust approach to hepatocyte cell growth and has been tested against more than 20 iPSC lines. These differentiation kits form part of Takara Bio's complete solution for hepatocytes which also includes fully-differentiated frozen cells and outsourced services for the generation of iPSC-derived hepatocytes from customer provided iPSC donor lines.

Thermo Fisher Scientific offers a broad range of products for stem cell research. For cell culture, its Gibco™ range of media, supplements and cell culture reagents are optimised for reproducibility and performance. The Gibco™ StemFlex™ medium and Gibco™ RevitaCell™ supplement support the robust expansion of feeder-free pluripotent stem cells and are optimised for more challenging applications such as single-cell passaging, gene editing and reprogramming. In addition, for iPSC gene editing, Invitrogen TrueCut Cas9 Protein v2 is a next-generation CRISPR Cas9 protein that has been engineered to maximise editing efficiency. The product is transfection-ready and eliminates time-consuming cloning steps (**Figure 4**).

Delivering the promise of personalised medicine

Undoubtedly, disease models based on stem cells will play a key role in realising the goals of precision medicine. Stem cell research is now being combined with single-cell analysis, allowing researchers to study the effects of drugs on individual cells as they go through the differentiation process. This is allowing multiple disease models to be generated from a single donor, giving scientists the opportunity to explore the effects of novel treatments on multiple organs or cell types, while maintaining the genetic information contained within them. By creating disease models from individuals

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with different ethnic backgrounds, drug susceptibilities or resistances to infections, it is hoped that this will better enable drug discovery researchers to develop more personalised treatments and even create new cellular therapies that can be used to cure disease.

Advances in stem cell technology have transformed drug discovery over the past decade. Thanks to the latest cell culture systems, iPSC-derived cell lines and more standardised protocols, researchers are already accelerating the delivery of much-needed treatments to patients using more translationally-relevant models. With the next generation of innovations set to overcome current challenges in stem cell research, a new frontier in drug discovery awaits. DDW