Stem cell technology – delivering the promise

The promise of stem cell technology as a tool for drug discovery, drug development and as a therapeutic modality is no longer in the future but part of contemporary healthcare. The speed which stem cells have been integrated into biomedical product development has surprised many, but stem cells in the form of bone marrow transplants have been around for several decades in medical practice.

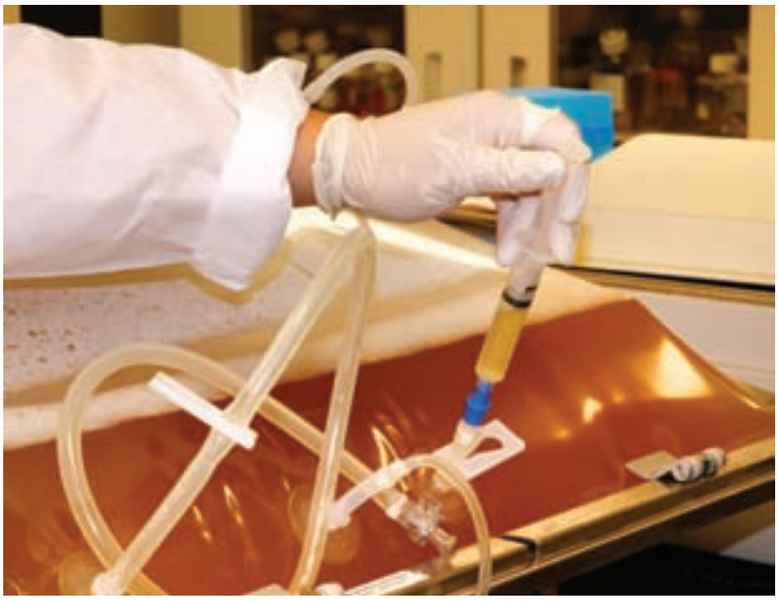
By Dr Aaron Heifetz

Most drug discovery programmes now use functional, cell-based assays for target hit identification and lead optimisation because of the desire to utilise the scientific understanding of signalling pathways. This has resulted in a capability to probe more complex targets and seek target modulation, rather than complete inhibition. Pharmaceutical companies have also employed stem cell technology for drug discovery and testing for more than 10 years. Stem cells may help us understand the complexity of human disease by studying cells as they become more differentiated, making nerves, skin, cartilage, bone and brain. Drug developers hope that characterising the signals and mechanisms of cell differentiation may yield information about how diseases arise and suggest new strategies for therapy. New medications are now tested for safety on specialised cells generated in large numbers from stem cell lines – reducing the need for animal testing – and cancer stem cell lines are used to screen potential anti-tumour drugs.

Thus, there are considerable efforts in adopting stem cell assays for drug discovery, since stem cells can differentiate into specific cell types that may not be available from human sources. Also, many available human cells have not been very good at predicting side-effects of newly discovered drugs as they enter the pipeline. In each example, however, it still must be proven that differentiated cells (derived from stem cells) are equivalent to the desired or target cells and can be validated as a drug screening tool. Critical areas of drug screening include cardiotoxicity, mutagenicity and immunogenicity.

Cell-based assays formats play a role in testing compounds for effects on proliferation and screening for inhibitors or modulators of cell growth. In addition, the effects of modulators of stem cell self-renewal will help define stem cells and their potential cell fates during differentiation. Studies to characterise both the natural and desired functioning of stem cells, progenitor cells and differentiated cells will be crucial. This focus on stem cell biology has yielded innovative technologies and cell-based tools for leading-edge research. This will hopefully translate into comprehensive drug discovery and development programmes which can bring new medicines to market faster and more cost-effectively. Furthermore, these advances should also translate into more robust manufacturing processes that supply novel therapeutics to clinical development programmes.

High throughput discovery and toxicology platforms used by pharmaceutical companies require large quantities of differentiated cells – cells that mimic the spectrum of human pathologies – and, the demand for those cell banks has opened new business opportunities for companies that provide biologic tools and contract manufacturing services. Cell



Cell manufacturing scale-up at Paragon Bioservices

banks are provided either in frozen stocks or 'ready-to-use' high throughput motifs. Contract manufacturing companies, such as Paragon Bioservices, are actively involved in addressing this need.

Many companies have developed unique stem cell technologies and are now relying on service companies to produce and distribute these tool platforms to a wide range of users. Vivo Biosciences, Inc has developed a novel human biomatrix system for cultivating human stem cells (eg derived from adipose, CNS and MSC) for long-term growth and differentiation studies in both 2D and 3D bioscaffold culture. This approach is in development for use in real-time cell-based assays for the xCELLigence System, co-developed by Roche-ACEA.

In 2006, scientists made more news in stem cell research when they identified conditions that would allow some specialised adult cells to essentially be reprogrammed genetically to assume a cell-like state, by being forced to express genes for maintaining the defining properties of embryonic stem cells. This type of stem cell is called induced pluripotent stem cells (iPSCs). Unlike embryonic stem cells, iPSCs are made in the lab (while adult stem cells naturally occur in the human body). Since they are obtained from the patient's own cells (such as from skin) the ethical issues that plagued human embryonic stem cells are avoided.

Patient-specific iPSCs can offer a supply of genetically identical cells that can be differentiated into all somatic cell types for potential use in regenerative therapies or drug screening and testing. As the techniques for generation of iPSC lines are constantly improving and include small molecule effectors, new uses for human iPSCs are emerging. These include *in vitro* disease modelling, high throughput drug discovery and population specific screening. This technology promises to revolutionise the field of medicine and offers new hope for understanding and treatment of numerous diseases. It is widely believed that iPS cells have the greatest potential for drug discovery and patient therapies.

Testing the toxicity of pharmaceutical candidates in lab animals to support the safety for human clinical trials is notoriously unreliable. Often compounds that appear safe in rodents prove to be toxic in humans. In order to predict toxicity in cell models, many investigators are using embryonic and somatic progenitor cells to monitor the behaviour of stem cells exposed to new compounds via disruption of cell-to-cell interactions and interference with expected development or differentiation. Using embryonic stem cells or iPSCs to create human heart cells could be a viable and scientifically exciting alternative to animal testing – saving precious time and money spent on the wrong drug candidates.

Pro-arrhythmia (development of cardiac arrhythmias as a pharmacological side-effect) has become the single most common cause of the withdrawal or restrictions of previously marketed drugs. The development of new medications, free from these side-effects, is hampered by the lack of an *in vitro* assay for human cardiac tissue. According to Caspi, et al (Stem Cells Dev 2009 18(1):161-72), human embryonic stem cell-derived cardiomyocytes (hESC-CMs) assessed with a combination of single cell electrophysiology and microelectrode array (MEA) mapping can serve as a novel model for electrophysiological drug screening.

Cellular Dynamics has the ability to generate iPSC lines from the blood samples of hypertensive patients. The iPSC lines can then be differentiated into ventricular heart cells for use in genetic studies. Differentiated iPSC lines may enable a new level of research into the genetics and mechanisms of certain diseases that previously has not been possible due to the unavailability of primary human cardiomyocytes for functional studies.

Making stem cells from the skin of adults rather than embryos also makes it much easier to create cell lines that are ethnically diverse, letting researchers better judge the safety and effectiveness of drugs on a wide range of people. As reported in 2010 by Waters (*Bloomberg Business Week*), Roche Holding AG scientists tracked the changes

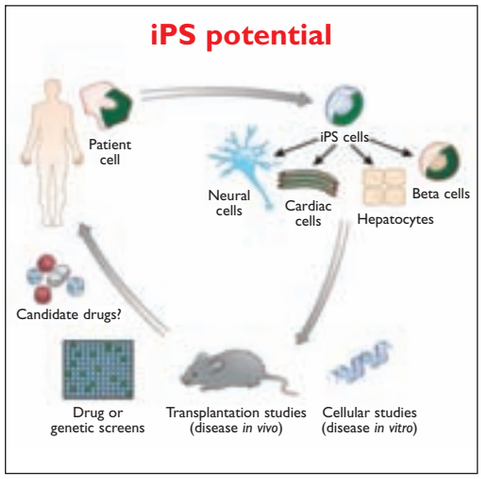
in the beating of heart muscle made from iPSCs exposed to an anti-cancer compound and duplicated the side-effect previously only seen in patients. The experiment showed that human tissue grown from stem cells can mimic the side-effects of medicine that are seen in people. Large pharmaceutical companies are using stem cells to test experimental drugs in an effort to dramatically reduce the cost and risk of the discovery process. More evidence that iPSCs can create heart cells for short-cut drug testing came when the Roche team used them to confirm cardiac toxicity from an antiviral medication that it had been developing.

Umbilical cord blood is a potential vast source of primitive hematopoietic stem and progenitor cells available to reconstitute the hematopoietic system and/or restore immunological function when used as a source for bone marrow transplantation. There are more than 40 private family cord stem cell banks in the US and an estimated 120 additional firms internationally offering these services. Cord Blood Banking is one way to preserve stem cells from diverse populations, which may offer research samples for new medical advancements and drug discovery.

Broxmeyer, et al (May 2011 in *Blood*) evaluated recovery of functional hematopoietic progenitor cells cryopreserved for 20 years. Highly efficient recovery (80-100%) of multipotential hematopoietic progenitors was apparent and CD34(+) cells isolated from cryopreserved Cord Blood had engrafting capability in immunodeficient mice reflecting recovery of long-term self-renewal. In this study, functionally responsive CD4(+) and CD8(+) T lymphocytes, generated iPSCs with differentiation representing all three germ cell lineages *in vitro* and *in vivo*.

Thus, an additional source of cells that may be used for stem cell applications or converted to iPSCs for personalised medicine lay in the wealth of individual's cells and tissue stored in both public and private cell banks. Since the drug discovery and diagnostic technologies that utilise human cells are rapidly evolving, these approaches may allow people to one day have their own cells available to be used in coupled diagnosis, drug screening and personalised treatment of their diseases. Companies, such as Next Healthcare Inc, focus on this leading edge of personalised healthcare by providing tissue and cell storage solutions to individuals through their physicians.

The outlook for regenerative medicine is bright. Companies are poised to replenish their technology pipelines with developing stem cell science from research universities and early stage biotech. The

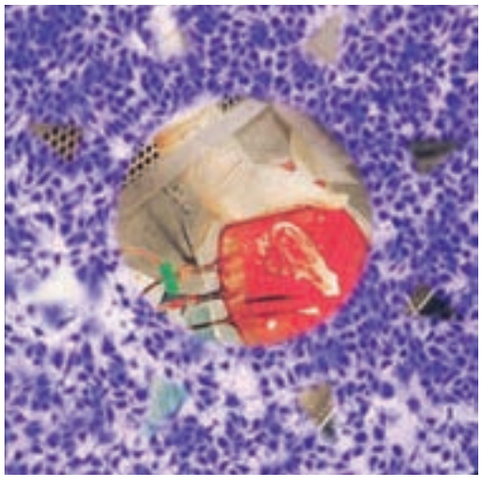


iPS cell uses

stem cell market was estimated to be $21.5 billion globally in 2010 and is forecast to reach $63.8 billion by 2015 – including both tools and therapeutics. The knowledge gained in drug development has been integrated with new programmes for cell therapy and combination drug and cell therapies. Many large pharmaceutical companies are developing internal and external regenerative medicine programmes, including Pfizer, Johnson & Johnson, Shire and GlaxoSmithKline. Some of this expansion is based on knowledge gained by employing stem cell technology in the drug discovery process; others, such as Shire, are acquiring commercial stage companies, like Advanced Cell Technologies.

Stem cells are key to replacing cells lost in degenerative diseases and for repairing cells in damaged tissue, similar to organ transplants of the past. Stem cells or their differentiated cell products offer a probable and manufacturable source of replacement cells to treat diseases including Parkinson's, stroke, heart disease and diabetes. Many companies are involved in regenerative medicine with more than 1,100 clinical studies under way involving stem cell therapy in the US, Europe and Japan. These cell therapy companies include Aastrom Biosciences, Cellerant, Geron, Cytori, Osiris Therapeutics, StemCells, BioE and ViaCyte. There are more than 200 companies developing stem cell products for the biopharmaceutical industry. Companies that supply the products and services that support research and development, include Life Technologies, Sigma-Aldrich, Cellular Dynamics International, IPierian and ViaCord.

Mesenchymal stem cells (MSCs, also known as bone marrow stromal cells or skeletal stem cells) are multipotent stem cells that can differentiate into chondrocytes (cartilage cells), osteoblasts



Generation of stem cells from bone marrow

(bone cells) or adipocytes (fat cells), making them ideal candidates for tissue engineering. MSCs can contribute to the regeneration of bone, cartilage, muscle and tendons. It has also been shown that, when transplanted systemically into animals, they are able to migrate to the sites of the injury. Scientists are currently examining the potential of generating healthy heart muscle cells in the laboratory and then transplanting those same cells into patients with chronic heart disease.

While progress in this area is exciting, more work remains in both process and clinical development. One of the critical factors limiting growth of the cell-based therapy industry is the lack of expertise in product development and specialised manufacturing that will be required to bring these products to market. Challenges include achieving high cell densities, control of cell differentiation and production of uniform cell populations, which maintain the desired phenotype and function. Small scale suspension culture systems for undifferentiated hESC and iPSCs have been developed and offer an approach for large-scale propagation of undifferentiated pluripotent cells for clinical and translational applications. This work requires the systematic study of relationships between cell characteristics, cell density, oxygen, and cell function. Lovett, et al (Tissue Engineering Part C: Methods. December 2010) reported changes in the expression profiles of hMSCs differentiated under varied oxygen tensions and showed different tissue-specific oxygen requirements for adipogenic (20% O_2) and chondrogenic (5% O_2) differentiation. In addition to process control strategies, expansion of attachment dependent cells, such as MSCs, offer additional challenges. Expanded cells must maintain a desired phenotype and have the functional potency as the cells from well characterised cell banks. Many companies are working on new biomaterial scaffolds, bioreactors and bioprocess systems to meet the needs of producing large numbers of well-characterised stem cells. These companies include EMD Millipore, BD Biosciences, ATMI and Xcellerex.

In many ways, the level of complexity for cell products is higher than for biologics. The need to supply a large number of highly characterised and documented cells exists on an international basis. Medical tourism is increasing; patients are seeking treatments for life threatening diseases that are currently not approved for sale in the US. In addition, there is a need for the many cells that are being administered under approved experimental clinical trials internationally.

This is a perfect niche for contract manufacturing companies to support development and clinical supply for regenerative medicine companies. Companies such as Lonza, Progenitor and Paragon Bioservices, Inc all offer contract services to drug and regenerative medicine companies. Use of outsourced suppliers for stem cell products fits with current pharmaceutical company strategy to focus internal resources on core capabilities and outsource pre-clinical and clinical stage manufacturing. Recognising all of the potential in the field of stem cell therapies and the possibility of finding solutions for public health imperatives, Paragon Bioservices, Inc and the University of Maryland's Center for Stem Cell Biology and Regenerative Medicine recently created a stem cell initiative to explore how they could advance the scientific research in this exciting field. This public-private partnership is for the development and manufacturing of stem cell therapies. The Stem Cell Technology Consortium is openly seeking wider participation from multiple universities, State and Federal agencies, and private companies. DDW

Dr Aaron H. Heifetz *supports Paragon's expanding business segments in cell therapy, stem cells and regenerative medicine. Aaron has 20 years of industrial management experience within biomedical/ biotechnology businesses with process and product development, manufacturing and commercialisation responsibilities. He was Vice-President, Business Development at Cognate Bioservices, a manufacturer of cell therapy products and was Vice-President, General Manager and Site Director of Cambrex Bio Science Baltimore, a contract developer and manufacturer of biologics and vaccines.*