

ISSUES IN MEDICINE

A new class of stem cells in South Africa: iPS cells

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Created from adult rather than embryonic cells, induced pluripotent stem (iPS) cells represent a breakthrough in stem cell science, and their pioneers have been recognised with the 2012 Nobel Prize in Medicine. These cells offer new hope in the treatment of pathogenetic diseases, but there is still a way to go on the road to effective therapeutic applications.

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The use of bone marrow, umbilical cord and other adult stem cells for the treatment of various conditions (mostly haematological) is familiar to SAMJ readers, as are the scientific and ethical debates surrounding the potential use of human embryonic stem (hES) cells for tissue repair.^{1,2} In the specialist medical and scientific literature, however, it is a new class of stem cells, induced pluripotent stem (iPS) cells, that are causing the greatest excitement of late, and with reason.

Human iPS cells are somatic (diploid) cells, obtained by relatively non-invasive procedures (e.g. skin biopsy), that have been genetically 'induced' to revert back to an embryonic stem-cell-like state. The term 'pluripotent' refers to a cell's ability to differentiate into any cell type in the body. Both iPS and hES cells are pluripotent. To turn a somatic cell into an iPS cell, simply stated, a very small skin biopsy is taken from a patient, a skin fibroblast cell culture is established *in vitro*, and the fibroblast is reprogrammed into an iPS cell. These immortal iPS cells can be grown *en masse in vitro* and differentiated into the particular cell type required for therapeutic or non-therapeutic purposes (Fig. 1).

The key advantage of iPS cells over other stem cells is that they are patient-specific (and therefore immuno-compatible) and can be grown in infinite amounts. Moreover, they are not dogged by the ethical and religious controversies associated with hES cells, yet still have the same properties as hES cells. They also offer the possibility of conducting 'clinical-trials-in-the-dish', providing a platform for drug screening, disease modelling and gene/cell therapy in pre-clinical studies.^{3,4}

How are iPS cells made?

When cell differentiation occurs, the cell follows a process of changes in gene activity whereby embryonic-specific genes are inactivated and differentiation-specific genes are activated. The end result of this differentiation 'programme' is a specialised cell of one type or another (e.g. cardiac muscle cells or neurons). To 'reprogramme' a fully differentiated adult cell into an iPS cell is surprisingly straightforward – all that is needed is reactivation of the embryonic regulatory genes and inactivation of the differentiation-specific genes.

This was achieved in ground-breaking studies in 2006/7 by Takahashi and Yamanaka, who showed that the ectopic expression of four key embryonic regulatory genes (Oct3/4, Sox2, Klf4 and c-Myc) reactivates endogenous pluripotency genes in mouse and human fibroblasts.^{5,6} These achievements won Yamanaka the 2012 Nobel Prize in Medicine.

iPS cells for therapeutic use

Many reports and reviews on the production of iPS cells and their possible therapeutic potential have rapidly followed. iPS cells have been

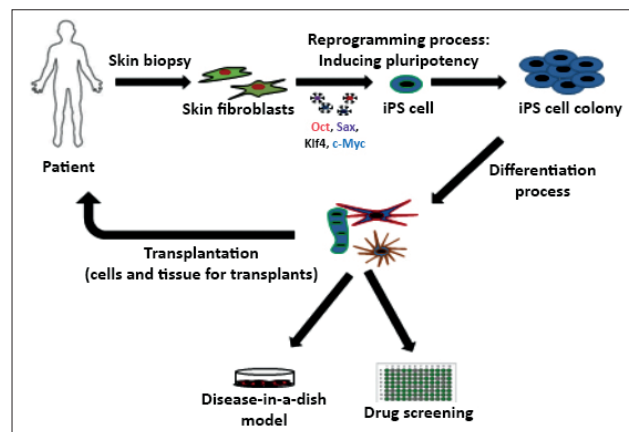


Fig. 1. Medical and research potential of iPS technology. The process of reprogramming a somatic cell into an iPS cell and back to a differentiated cell requires relatively few manipulations. Reprogramming requires the uptake of reprogramming factors, such as Oct3/4, Sox2, Klf4 and c-Myc, by the somatic cell. This leads to a number of changes, including changes in physical structure, gene expression and proliferative ability. Genes functional in the somatic cell get switched off and the endogenous stem cell-associated genes are switched on. All of these changes form part of the induction of pluripotency, which leads to the generation of embryonic stem-cell-like iPS cells and formation of iPS cell colonies. The differentiation process requires the introduction of factors required to force the iPS cells along a lineage of a specific target cell type. The target cells can be used to create disease models in a cell culture dish, for disease-specific drug screening, genetic manipulations to repair disease-causing mutations, or to bulk up cells for transplantation into the cell donor.

derived from a wide range of cell types and differentiated into an equally broad range of cell types involved in neurological, haematological, metabolic, cardiac and vascular, pancreatic and hepatic conditions.⁷⁻¹⁰ Conditions in which treatment using iPS cells is being considered and actively pursued include genetic and acquired degenerative diseases, amelioration of the effects of ageing, and novel treatments for some of South Africa (SA)'s most serious infectious diseases. The true potential of iPS cells has yet to be established. Many concerns about their derivation must be addressed before they are ready for therapeutic use.

iPS cells and disease-in-the-dish models

Disease-in-the-dish studies have progressed much further. A broad spectrum of both monogenic diseases such as Duchenne's muscular

dystrophy and Huntington's disease, as well as complex adult-onset diseases (e.g. Parkinson's, Alzheimer's and schizophrenia, to name a few) have been modelled *in vitro*.^{9,10} How effective iPS cell-derived disease models are at reproducing the disease cellular phenotype, and at demonstrating the effects of drugs, is more easily recognised in early-onset diseases that have a strong genetic component and an identifiable disease-associated cellular phenotype. This is exemplified by a patient-derived iPS cell model for familial dysautonomia, which was used to show that the disease phenotype could be partially normalised by a plant hormone.¹¹

An example of the medical application of iPS cell technology was demonstrated by Itzhaki *et al.*,¹² who used an iPS-model for Long-QT syndrome to identify complex effects of cardiotoxic drugs and protective pharmacological agents for disease sufferers.

Our studies of spinocerebellar ataxia 7 (SCA7), a late-onset neurodegenerative genetic disease found almost exclusively in families of black origin in SA, exemplify our use of iPS cell technology in disease modelling.¹³ The development of effective therapies for a group of genetic conditions known as the polyglutamine (polyQ) diseases is particularly relevant to SA populations where, for example, the frequencies of spinocerebellar ataxia type 1 (SCA1) and type 7 (SCA7) are among the highest in the world.¹⁴

To date, our research has been primarily focused on the study of the SCA7 protein in transfected neuronal cell lines and transgenic mouse models.^{15,16} A unique clinical feature of this condition is loss of vision associated with retinal degeneration (in particular the macula), the aetiology of which is unknown. To explore the cause of retinal cell death in SCA7 patients, we have derived iPS cells from skin biopsies following standard protocols (UCT HREC ref. 460/2010 and 434/2011, both renewed in 2012 until 2013) and carried out in accordance with national stem cell regulations (promulgation in progress), cultured them *en masse*, and have successfully differentiated them into retinal pigment epithelial cells and neuronal cells.¹⁶ With these unique cells in hand, we are now able to investigate the cellular vulnerabilities conferred on the cells of the eyes of our South African SCA7 patients, and have a powerful tool to explore models of cell interactions and cell death. In addition, we are able to examine and observe patient-specific drug treatments that might eventually delay the onset of blindness arising as a result of cell death in South African SCA7 patients.

Risks, limitations and challenges

Despite the evident success of iPS cell technology to date, the field has been complicated by a number of challenges, not least that Takahashi and Yamanaka and many others used viral vectors (retrovirus, lentivirus) to transport the reprogramming genes into the somatic cells.^{5,6} The risk is that the viral integration itself could result in rogue cells with tumorigenic propensities. This is of particular concern as the technology rapidly moves towards clinical application.

Other methods of delivering reprogramming genes into cells use non-integrating agents of induction, such as the deleted persistent variant of the Sendai virus (SeVdp), viral-free non-integration approaches, proteins, mRNA and miRNA.¹⁷⁻²² Most non-integrating methods, however, result in reduced reprogramming efficiencies.²³ In our experience, induction with the Sendai virus has been most successful following our previous utilisation of retroviruses. Following these challenges, researchers have included rigorous quality-control steps to test the purity and integrity of iPS cells before differentiation.

Conclusions

Creating cells takes time and a great deal of care, and is not suitable for acute conditions. However, the rise of large-scale bio-repositories

may yet deal with this limitation. Technical research challenges and ethical controversies have delayed the progress of iPS cell technology and narrowed the scope of iPS cell banks to research projects, rather than treatment. This has resulted in recruiting iPS cells from patients and their family members rather than from larger population samples. However, in an unprecedented move, Yamanaka plans to create a bank of stem cells from fetal cord blood for therapeutic use in Japan, shifting the potential of iPS cell technology to new heights.²⁴

It is vital that the SA medical and scientific fraternity become familiar with iPS cell technology – a reality that has demonstrated evidence of proof-of-principle.¹⁰ Researchers and clinical practitioners should be encouraged to communicate with accredited stem cell or tissue culture laboratories, and should inform patients about potential iPS cell prospects. Bearing in mind the variation of disease phenotypes and clinical manifestations, it is important to build up an SA bank of patient-derived iPS cells from different patients diagnosed with the same disease for a more accurate representation of underlying pathology. There is no doubt that iPS cell technology and patient-derived disease-in-a-dish models have the potential: (i) to exponentially increase our understanding of underlying disease mechanisms and cellular phenotype responses to drug treatments; and (ii) to introduce novel treatments for some of SA's most serious diseases.

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