Feature

Regenerative medicine spoilt for choice

Embryonic stem cells, induced pluripotent cells, transdifferentiated cells – there is now a wide range of options for medical researchers trying to regenerate disease-affected tissues and organs. But which approach will win the day, and can science policy keep up with the bioethical implications of rapid progress? **Michael Gross** investigates.

Only ten years ago, 'stem cells' was a buzz word for all kinds of anticipated miracle cures, following the first production of human embryonic stem (ES) cell lines from blastocysts (early stage embryos) in 1998. As embryonic stem cells are pluripotent, they can generate the specialised cells for any kind of organ or tissue that one may want to create or repair, given the right enticement. While some countries, such as Germany, had qualms about the use of blastocysts discarded after IVF, others, including the UK, took the pragmatic view that these would have been destroyed anyway and took the lead in a rapidly growing and promising field.

Then, in 2006, Shinya Yamanaka showed that a cocktail of only four factors suffices to turn back the clock of embryonic development and turn a differentiated somatic cell into a pluripotent one, which came to be known as an induced pluripotent stem (iPS) cell. Earlier on, the cloning of Dolly the sheep had demonstrated that the clock can be turned back in a cellular environment, but the finding that researchers can achieve this *in vitro* with a relatively simple protocol came as a surprise. The bad news was that one of the factors was a known oncogene. While this factor could be eliminated eventually, it drew attention to the more fundamental problem that pluripotency includes the potential for the cells to become malignant.

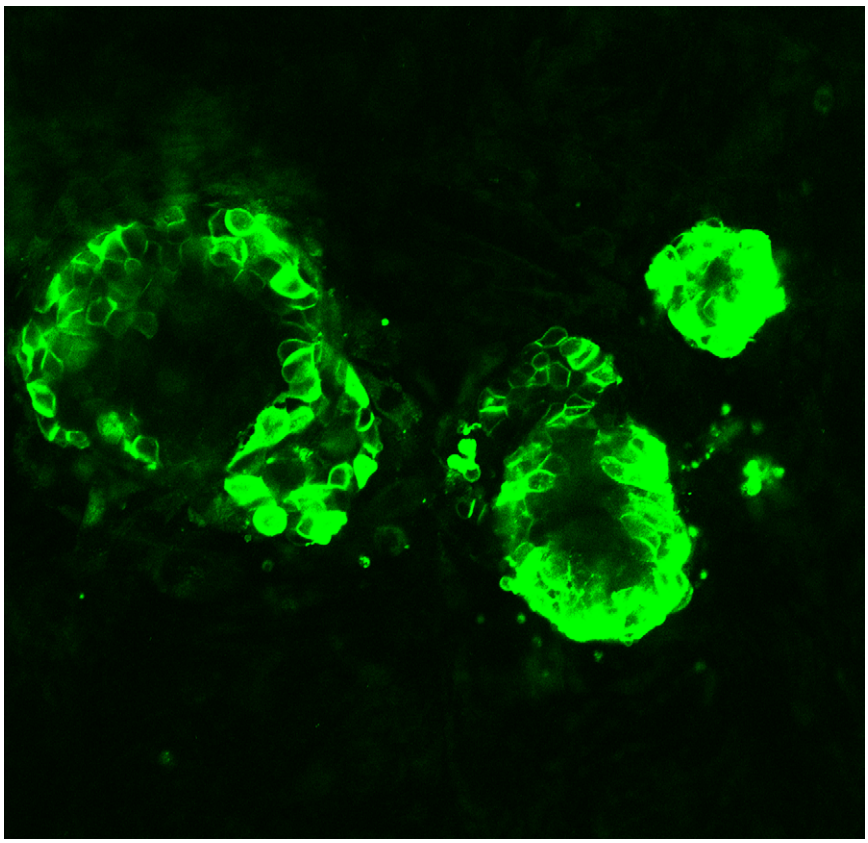
Recently, a third option came onto the scene, when direct conversion of one cell type into another was demonstrated first for mouse and then for human cells. Although the rules are still being established, this so called transdifferentiation appears to be possible even between completely unrelated cell types, without having to go via pluripotent cells.

The magic of transdifferentiation

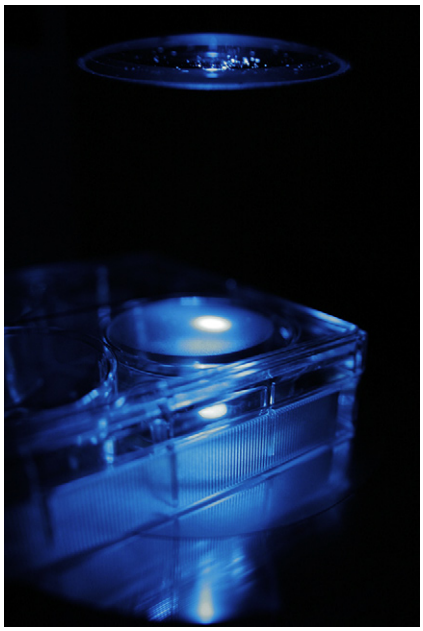
In February 2010, the group of Marius Wernig at Stanford reported the rapid and efficient conversion of mouse fibroblasts (a cell type found in connective tissue, which is also used for the production of iPS cells) into functional neurons *in vitro* (Nature (2010), *463*, 1035–1041). They achieved the conversion by inducing only three transcription factors specific for the targeted cell type. This was the first conversion between unrelated cell lineages — previous efforts had shown that one type of blood cell can be converted into another, and different types of neurons, as well as pancreatic cell types, can be interconverted.

Last November, the group of Mickie Bhatia at McMaster University at Hamilton, Ontario, Canada, reported a similar transdifferentiation success with human cells. This group had managed to convert human fibroblasts into blood progenitor cells expressing the marker CD45, shared by all types of leukocytes, without going through a pluripotent stage (Nature (2010), *468*, 521–526). These progenitors then differentiated into a range of blood cell lineages, including granulocytic, monocytic, megakaryocytic, and erythroid cells.

Earlier this year, the group of Sheng Ding at the Scripps Institute in La Jolla, California, reported the



Green light: Colonies of mouse ES cells growing on mitotically inactivated fibroblasts stained while alive with monoclonal antibodies to the surface marker SSEA-1, expressed by pluripotent cells. (Photo: Tim Davies.)



I spy: The light of a UV microscope diffracting through a 6-well culture plate containing embryonic stem cells. Image 'Paparazzi in the dark' by Christian Unger from *Smile of a Stem Cell* (www.estools.eu/estools/discovery/smile-of-a-stem-cell), copyright of University of Sheffield, collected and curated for the training and outreach programme of the ESTOOLS consortium.

conversion of mouse fibroblasts into functional heart cells or cardiomyocytes (Nat. Cell Biol. (2011), *13*, 215–222).

By avoiding any pluripotent states with their associated malignancy risk, the transdifferentiation approach appears to be safer than either ES or iPS cells, although this will have to be established in systematic studies. However, this direct route is also forsaking the opportunity to grow stable stem cell lines in culture, and thus to produce large amounts of cells for regeneration. The limited availability of suitable cells for conversion may well limit the usefulness of the transdifferentiation route, so it would be too early to write off pluripotent cells.

Pluripotent problems

Medical researchers now have three fundamentally different approaches to choose from, and it is far from clear which will offer the best options. Paul Fairchild, co-director of the Oxford Stem Cell Institute at the Oxford Martin School, comments: "Never has the landscape of regenerative medicine been so promising with advances in the use of adult stem cells and the availability of both embryonic and induced pluripotent stem cells. Nevertheless, the magnitude of the obstacles to be overcome should never be underestimated. The task ahead is, therefore, to begin sifting the chaff from the wheat to determine which approaches might lead to robust clinical applications with manageable levels of risk."

To begin the sifting process, five research groups have recently assessed the quality of iPS cell lines, as summarised in a recent comment in *Nature* by Martin Pera (Nature (2011), *471*, 46–47). The studies find that genetic and epigenetic abnormalities are much more frequent in iPS cell lines than in ES cells. Specific problems of iPS cells include gross chromosomal aberrations, which don't often appear in ES cells, mutation rates 10 times higher than in the fibroblasts from which the iPS cell lines were derived, and unexpected copy number variations (CNVs). At the epigenetic level, the researchers found imperfections in the reprogramming, leaving some characteristic marks of the cells of origin intact. There are indications that some of the faults arise by selection rather than accident, which would make them even harder to eliminate.

On the other hand, research with ES cells still faces hurdles at the legal and bioethical front. The European Court of Justice currently deliberates a case that might end up with a complete ban on all patents based on the use of human ES cells. A recent statement from Yves Bot, the European Advocate General, although not legally binding, appeared to point in this direction. While acknowledging that embryonic stem cells are not equivalent to embryos in the eyes of the law, Bot indicated that the fact that embryonic cell lines are originally derived from fertilized human eggs means patents cannot be granted for techniques that involve the use of embryonic stem cells (www.eurostemcell.org).

Stem cell researcher Oliver Brüstle from the University of Bonn, whose patent on producing brain cells from ES cells triggered the court case when it was challenged by Greenpeace in 2004, commented: "The Advocate General has taken a more restrictive view than the European Commission or any of the European Member States that have taken a position on this issue. No-one in Great Britain or Sweden would think of questioning a patent of this kind."

Putting the case in the global context, Brüstle said: "One wonders why the EU spends millions of Euros supporting the development of therapies based on embryonic stem cells, if practical progress towards the clinic is to be blocked by patenting restrictions. While stem cell technologies are already beginning to reach patients in the US and Asia, we are still discussing policy issues and wasting Europe's valuable competitive edge."

The decision of the court is expected in around May.

Translational research

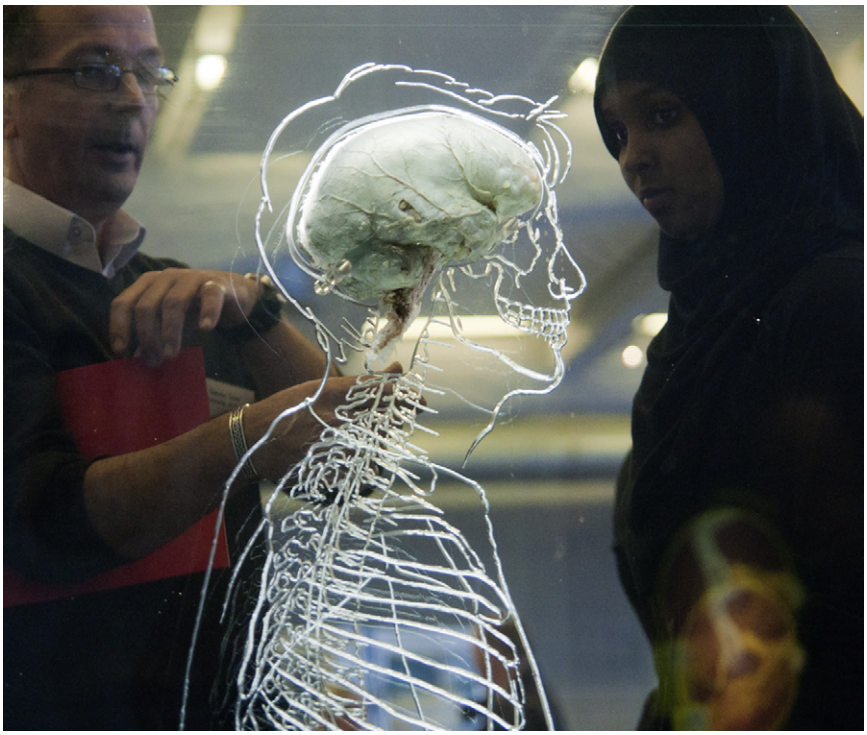
Meanwhile, researchers at the medical applications front are already working towards regenerating certain defective cell types from whatever precursor or stem cell is available. The group of John Kessler at the Northwestern University recently attracted media attention with a report of brain cells grown from human ES cells (Stem Cells (2011) DOI: 10.1002/stem.626). The cell type created in Kessler's experiments is one that is affected by the early stages of Alzheimer's disease, so the hope is to be able to treat the disease by regenerating the population of these cells.

Another part of the central nervous system that is very popular with experimental medicine is the retina. In the quest for ways to reverse the loss of sight, cell-based regeneration is in direct competition with bio-electronic approaches based on artificial sensors. At a recent meeting, for instance, Mandeep Singh from the Nuffield Department of Ophthalmology at the University of Oxford reported preliminary animal studies suggesting that retinal regeneration can be treated with precursor cells, which, after injection into the collapsed retina started to develop synapses and make contact with bipolar cells. The treatment was also successful in restoring some retina function, even though, intriguingly, there was a placebo effect in the control experiments, due to the body's own cells re-colonising

the space created when inactive control material is injected into the collapsed retina.

Short of creating replacement tissues, stem cells can be useful in allowing researchers to create a disease model *in vitro*. This is one of the approaches pursued in the field of Parkinson's disease. Recently, researchers at Stanford University have cultivated brain cells derived from skin cells (via iPS cells) of a patient with Parkinson's disease and have been able to observe the disease symptoms in the cell culture, specifically the accumulation of α-synuclein, an increase in oxidative stress, and an increased sensitivity to a range of stress factors (Cell Stem Cell (2011), *3*, 267–280). Having an 'out of body' model of the diseased tissue of a living patient should help drug development in general and may also enable the researchers to develop personalised treatment options in the future.

One organ that decays dramatically with aging is the thymus. The loss of function starts in early adulthood and leads to changes in the composition of the immune system in later life, as evident from an increased susceptibility to infection and a weakened response to vaccinations. Clare Blackburn from the MRC Centre for Regenerative Medicine, University of Edinburgh, is using ES cells and fetal thymic stem cells in an attempt to bypass the bottleneck of tissue supply for thymus transplants. "We hope one day to be able to boost immune system function by regenerating or replacing the thymus, but to do this we need to understand the fundamental mechanisms which regulate the epithelial cells in the thymus over the lifespan and how these are impacted by aging," Blackburn explains. "Our research is focused on regulation of thymic cell identity by key transcription factors, and also by the cell's external environment (Nature (2010), *466*, 978–982). Work of this type is the essential foundation for controlling tissue stem cells — both *in vivo* and *in vitro* — with the degree of precision currently possible only for pluripotent stem cells."



Brain box: Certain populations of brain cells affected by degenerative diseases are among the prime targets of regenerative medicine. The photo shows a real human brain displayed as part of the About Us exhibition at At-Bristol, launched in March. Left, Steve Gaze from the University of Bristol's Centre for Comparative and Clinical Anatomy, who worked closely with At-Bristol's exhibition team on the development of the Real Brain exhibit. (Photo: At-Bristol.)

Prickly questions

In fast-moving research fields like genomics or stem cells, politics can easily be left behind. As former MP Evan Harris told a stem cell symposium at Oxford in March, this problem gets even worse when the research involves morally complex issues such as dealing with human embryos. "Politicians don't like these issues," Harris said, "so we don't get timely legislation." Still, the UK has been relatively lucky due to the fact that the pioneering achievement of the first IVF birth in 1978 shocked politicians into creating the 1990 Human Fertilisation and Embryo Act, which was then cautiously updated in 2008. Harris called the 2008 bill "a missed opportunity" as it tiptoed around the critical issues of current research and anticipated therapies.

One important outcome of the 1990 Act was the introduction of the Human Fertilisation and Embryo Authority (HFEA), which not only supervised IVF clinics but also acted as a bioethics council in prickly questions surrounding stem cells, therapeutic cloning, and pre-implantation diagnostics. As the current, Conservative-led government is planning to abolish the HFEA and transfer some of its functions to the Human Tissues Authority (HTA), it is not yet clear what kind of guidance in bioethics questions there will be in the future.

If and when human cell lines, be they ES or iPS cells, enter medical practice, the ethical and intellectual property issues surrounding such cell lines will certainly require some work, as a recent report from the Hinxton Group, an international thinktank dealing with stem cell policy and bioethics (www.hinxtongroup.org), points out. At the moment, there are stem cell banks in various places, but there is no central resource where researchers could find information about existing cell lines and the associated intellectual property rights. The Hinxton Group report recommends establishing a central hub for stem cell information and patents, along with improvements to international coordination of research and the sharing of materials and data.

At this moment, the future of regenerative medicine appears to be wide open, but it is less than clear whether society will be able to handle what this future may hold.

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