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5 things to know before jumping on the iPS bandwagon

Induced pluripotent stem cells look just like embryonic stem cells, but are easier to create and free of the heavy ethics baggage. **David Cyranoski** separates fact from fiction in a burgeoning field.

Excited by their potential for biomedical research and therapy and lured by the ease with which they can be created, many researchers are looking into induced pluripotent stem (iPS) cells. Created from adult cells by a simple genetic trick, iPS cells seem to have regained an embryonic 'stemness' that might allow them to become any type of cell in the body. The concept is so appealing that some scientists and policy-makers even argue that related approaches such as therapeutic cloning and embryonic stem-cell research, which require the destruction of embryos, should be halted. But for biologists, iPS cells still present a black box. As resources pour in and patients' expectations rise, some scientists wonder whether the cells are being overhyped. Here, *Nature* looks at the status of the five most pertinent issues on people's minds.

"Expertise in human embryonic stem-cell culture is absolutely critical."
— Konrad Hochedlinger

1 Anyone can do it

When Shinya Yamanaka and his postdoctoral student Kazutoshi Takahashi from Kyoto University in Japan discovered that four genes could reprogram adult mouse cells, they kept it secret for nearly six months. They stopped having weekly laboratory meetings, and Takahashi fibbed to colleagues about the status of his work. All because the process is so simple. "If someone found out, they could have caught up in a flash," he says.

Familiar genes — *Oct3/4*, *Sox2*, *c-Myc* and *Klf4*, in Yamanaka's original recipe — do the trick[1]. The genes are cloned into viral vectors, and simply adding the vectors to a culture of skin cells under the right conditions results in reprogrammed cells.

But as simple as this procedure might seem, iPS cells are not easy to make. Kathrin Plath at the University of California, Los Angeles, estimates that each of the reprogramming genes (she used six) has only a 15% chance of making it into a given cell. Even if they all make it, the cell has only a 5% chance of being fully reprogrammed. The low efficiency presents a riddle for scientists, but with millions of cells available in a biopsy sample, it is not a roadblock. The trickiest part, says Konrad Hochedlinger from the Harvard Stem Cell Institute in Cambridge, Massachusetts, is finding the few cells that have been reprogrammed and culturing them. But the new iPS cells are picky: they require just the right culture conditions — much the same as those needed for embryonic stem cells — to stop them differentiating into more specialized cell types. "Expertise in human embryonic stem-cell culture is absolutely critical," says Hochedlinger.

Nevertheless, this type of expertise is becoming more common. Within a year, Yamanaka's results had been repeated and improved in mice by his own and two other groups. And since Yamanaka[2] and James Thomson[3] from the University of Wisconsin in Madison first produced human iPS cells independently of each other in November 2007, several other groups have now achieved that feat. The stakes are high; the technique is easy to learn; and researchers are flocking to the field, says Thomson. "The whole world is doing it now."

Status: Fact (mostly)

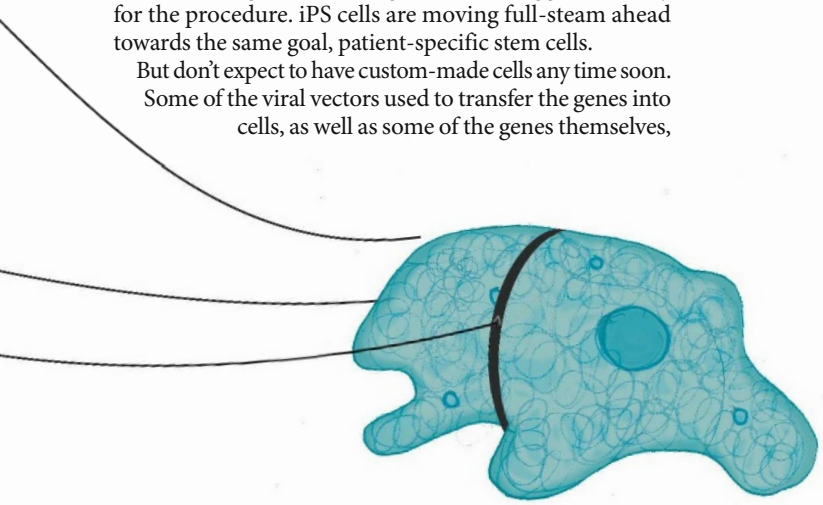
2 Everyone can have their own custom-tailored cells

'Therapeutic cloning' — cloning human embryos to generate stem cells that can be used to replace tissue that has been lost or damaged without the fear of rejection by the host's body — has floundered since it was first proposed

ILLUSTRATIONS BY C. ALLEN-FLETCHER

in the late 1990s, mainly because of the unexpectedly difficult challenge of acquiring the human eggs necessary for the procedure. iPS cells are moving full-steam ahead towards the same goal, patient-specific stem cells.

But don't expect to have custom-made cells any time soon. Some of the viral vectors used to transfer the genes into cells, as well as some of the genes themselves,



may cause cancer. Scientists expect that the heated race to find alternative systems, such as proteins or drugs that simulate the crucial genes, or safer ways to deliver the genes, will produce results soon — perhaps in the next two years.

Nevertheless, says Hans Keirstead of the University of California, Irvine, the "greatest challenge still exists: the generation of high-purity, clinically relevant cell populations". It takes a couple of months to establish a cell line, several more to expand it, more still to differentiate the iPS colonies into the cell types required, a few more to expand those, and then a good half-year of testing to ensure that the cells do not form tumours. The cells also have to be processed in facilities that adhere to 'good manufacturing practice', which adds greatly to the cost.

To use custom-made cells "would take a ridiculous amount of money", says Yonehiro Kanemura, a neurosurgeon at Osaka National Hospital in Japan. It would be several times more, he estimates, than the current, rare, patient-specific skin grafts, which cost as much as US$100,000. The roughly two-year process is also too slow to treat disorders such as spinal-cord injury, which require prompt treatment if the damage is to be minimized.

Kanemura's solution is 'ready-made' iPS cells. This April, working with Yamanaka and Hideyuki Okano of Keio University in Tokyo, he will start establishing a national library of therapy-ready cell lines from donated placental and cord-blood tissue. At first he plans to use viral vectors, switching to virus-free lines as these become available. Over the next five years, he aims to generate 200 iPS cell lines and 200 neuronal cell lines derived from those cells.

These cell lines will not be patient-specific, but Kyoto University's Norio Nakatsuji estimates that 50 well-chosen lines could provide close immunological matches for 90% of the Japanese population. People who need the treatment urgently could use the best immunological match, whereas those with chronic disorders might decide to fork out the money for a line specific to them, says Kanemura. Rich people might want to bank their own iPS cells for a rainy day — a desire that some companies will no doubt try to capitalize on.

Status: Fiction (unless you're rich)

"Grafting even a very small number of undifferentiated stem cells carries a risk of tumorigenesis."
— Arnold Kriegstein

3 The cures are on their way

iPS cells will probably provide models for disease first, cures later. Researchers could soon culture a 'disease in a dish' of, say, motor neurons from a patient with amyotrophic lateral sclerosis, heart muscle cells from a person with heart disease, or retinal cells from a patient with macular degeneration. Those lines could be screened and observed as they develop, and biotech companies could test preventative or therapeutic drugs on them.

The University of California, Los Angeles, and the Harvard Stem Cell Institute, among others, are discussing plans to start iPS cell banks for this purpose. Hochedlinger says that the stem-cell institute is considering "the major diseases — neurodegenerative, metabolic, cardiovascular, diabetes".

Reaching the clinic will depend, like modelling, on how faithfully iPS cells differentiate into the affected cell type as well as on the development of safe and effective ways to deliver them into the body. The foundations are already being laid.

Rudolf Jaenisch, from the Massachusetts Institute of Technology in Cambridge, for example, used blood progenitor cells created from mouse iPS cells to treat a mouse with a humanized version of sickle-cell anaemia[4]. He says that blood disorders, in which clinicians have considerable experience in transplanting cells, might see early application of iPS cells. Kyoto University's Jun Takahashi, who studied neuronal precursor cells derived from embryonic stem cells in monkey models of Parkinson's disease[5], is now pursuing clinical treatment with neuronal precursor cells derived from both embryonic stem cells and iPS cells. He hopes that the cells will be in clinical trials within five years.

Whether cultures of differentiated cells for therapeutic use still retain undifferentiated embryonic stem cells or iPS cells is a point of grave concern. "Grafting even a very small number of undifferentiated stem cells, perhaps as few as one pluripotent cell, carries a risk of tumorigenesis," says Arnold Kriegstein of the University of California, San Francisco. Everyone will be watching closely a clinical trial planned for the middle of this year — the first trial of embryonic-stem-cell-based treatment — in which the pharmaceutical firm Geron of Menlo Park, California, will be implanting oligodendrocytes derived from embryonic stem cells in patients with spinal-cord injuries. "Application of iPS cells largely depends on how that trial goes," says Okano.

Unpublished work by Okano, based on iPS-cell treatment of spinal-cord injury in mice, could accelerate application. He claims that he has a method to weed out the potentially dangerous cells before they are transplanted into the mice.

Status: Too soon to tell

4 Embryonic stem cells are the same as iPS cells

"There are no major differences, yet," says Plath, based on a rigorous characterization of morphology, chromosome profile and gene expression of human iPS cells[6].

But everyone is hedging their bets as dozens of scientists start to examine the key question: whether iPS cells will differentiate as stably and diversely as embryonic stem cells. For the time being, iPS pioneers are looking at subtler hints, such as protein markers that characterize the two types of cells. But

NATURE|Vol 452|27 March 2008

Hochedlinger says that "markers don't mean anything". Some tumour cell lines express protein markers of pluripotency but don't make anything other than tumour cells, for example.

And reports of the iPS cells' properties have been conflicting. Thomson, for instance, found that iPS cells not only expressed similar genes to embryonic stem cells, they also expressed them more consistently[7]. This means that their differentiation might be more predictable than that of embryonic stem cells. However, Robert Lanza of Advanced Cell Technology, a biotech firm in Los Angeles, California, says that iPS cells are much more variable. "Embryonic stem cells all do more or less the same tricks. But some iPS cells express just a few markers of pluripotency, some express all," he says. "The resulting cell types will presumably differ as well."

Even if iPS cell lines seem to differentiate into the cell of choice, some variation between lines is unavoidable. Each line will require rigorous testing, says Keirstead, who is involved in the Geron trial. "A different line may have a different tumorigenic potential, differentiation potential, migratory potential, and react with the host in a unique way. It represents a different product, so must be fully tested as such." For the same reason, Plath recommends doing any tests or drug screens with multiple lines.

Despite some scepticism about iPS cells, many key researchers embrace them as a preferred alternative to embryonic stem cells. "Only time will tell, but I know where I'm going," says Thomson, who was the first to establish human embryonic stem-cell lines in 1998. If things go as he predicts, it could be the end of an era. "If you can't tell the difference between iPS cells and embryonic stem cells, the embryonic stem cells will turn out to be a historical anomaly," he says.

Status: Fact (so far, anyway)

"If you can't tell the difference between iPS cells and embryonic stem cells, the embryonic stem cells will turn out to be a historical anomaly."
— James Thomson

5 iPS cells have no ethical issues

Days after Yamanaka and Thomson announced the creation of human iPS cells, President George Bush hailed the research as a sign of "scientific advancement within ethical boundaries" — a feat for which he gives himself partial credit.

A week later, though, Yamanaka told *Nature*: "We are presenting new ethical issues, maybe worse ones, because many people can do this — and without telling anybody." Yamanaka was concerned that someone might use iPS cells to derive gametes — human reproductive cells. Eggs and sperm could both be derived from iPS cells from a man, for example, and then be used in an *in vitro* fertilization procedure. The result would not be an identical clone because genes reassort during formation of the gamete. But it would be "strange and potentially dangerous", says Yamanaka.

Gametes from iPS cells could meet demands for infertility treatments. And producing eggs from male iPS cells would allow a gay couple to produce offspring between them. (Lesbian couples would be out of luck, as Y-chromosome genes are needed to produce sperm.)

Such fertility treatments would be plagued by safety issues, but judging from experiments with embryonic stem cells, they won't happen soon. Morphologically similar versions of both eggs and sperm have been derived from embryonic stem cells, but only one group has reported that embryonic-stem-cell-derived gametes — mouse sperm in this case — led to live births when combined with normal eggs, and the results have yet to be repeated[8].

iPS cell adventurers might also try to create a live, cloned human. Jaenisch managed to clone mice, by transferring iPS cells into a specially developed embryo made by fusing the cells of a two-cell embryo. By putting these embryos into surrogate mothers, Jaenisch produced several fetuses that were genetically identical to the iPS cell source. (There were no live births, but Jaenisch says that is only a matter of trying.)

Repeating the experiment in humans would, according to Jaenisch, "be possible in principle". He adds, however, that because "more than 100" embryos are probably needed to make it work, it would be unrealistic and a ridiculous thing to do. But as fertilized embryos are easier to get than the fresh eggs used in cloning, some maverick might give it a try. iPS cells generated from a person could also be inserted into a fertilized embryo to make a chimaeric baby.

These reproductive strategies would probably fail, at least with the current state of the technology. But given the rapid rate of innovation and the wide range of iPS cell capabilities, dangerous experiments will be more difficult to monitor. "Before, you had a specific community to focus in on — the practitioners of assisted reproduction. [With iPS cells] it will be difficult, especially in a place such as the United States, where there is so much dependence on self-regulation," says Paul De Sousa of the Scottish Centre for Regenerative Medicine in Edinburgh.

Yamanaka's concern about the ethics drove him to lobby the government for regulation. On 21 February, Japan's science ministry sent all universities and research agencies a notification specifically forbidding "the implantation of embryos made with iPS cells into human or animal wombs, the production of an individual in any other way from iPS cells, the introduction of iPS cells into an embryo or fetus, and the production of germ cells from iPS cells".

Status: Fiction (depends on what you want to do)

Yamanaka says that society, not scientists, must quickly deal with the challenges that iPS cells present. "I am proud of this technology, but I feel a great responsibility," he says. ■

David Cyranoski is *Nature*'s Asia-Pacific correspondent.

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See Editorial, page 388.