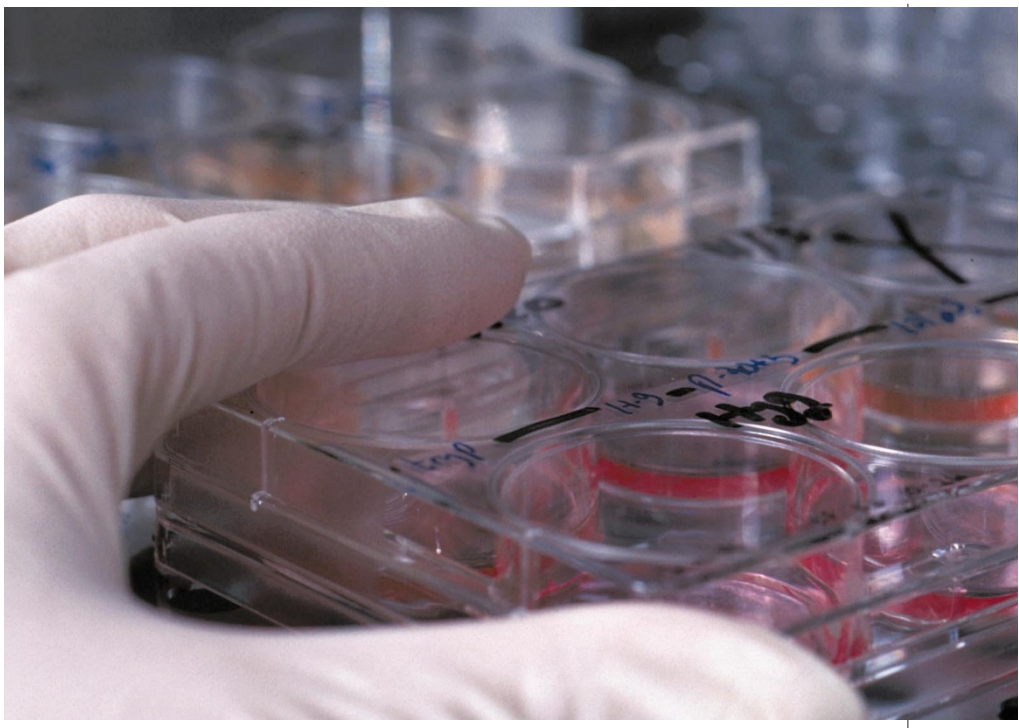
# Can they rebuild us?

The idea of therapeutic cloning, which offers the potential of growing replacement tissues perfectly matched to their recipients, is falling from favour. But there are alternatives, as Peter Aldhous found out.



Repair kit: can embryonic stem-cell cultures fulfil their promise of delivering replacement tissues?

UNIV. WISCONSIN

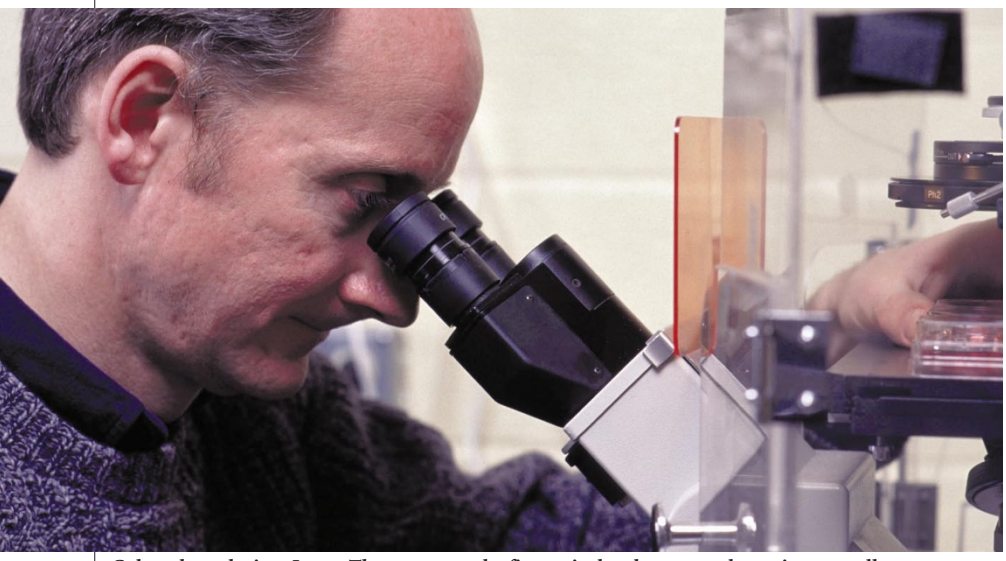
Take two of the biological breakthroughs of the late 1990s and combine them to produce a medical miracle — that is the thinking behind therapeutic cloning. The achievements are the cloning technology that in February 1997 gave us Dolly the sheep[1], and the successful creation the following year of cultures of human embryonic stem (ES) cells[2]. The promised miracle is the generation of 'personalized' replacement tissues to combat the ravages of ageing and disease. Genetically matched to the patient, these tissues would avoid the rejection problems that have always plagued transplant medicine.

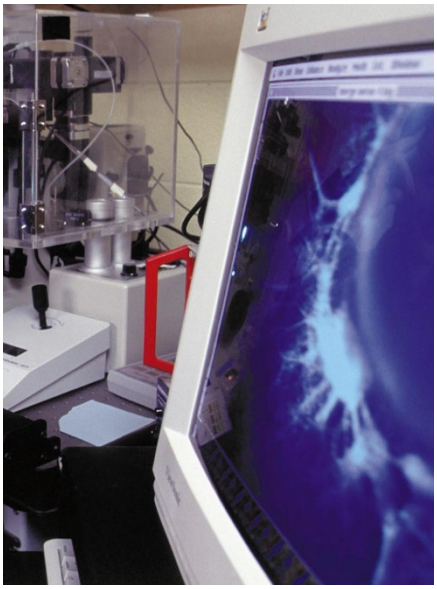
ES cells come from blastocysts — tiny embryos, just a few days old, that consist of a hollow ball of cells. ES cells can develop into any type of cell, and so could be cultured to grow replacement tissues, such as cardiac muscle to graft onto a weakened heart. Therapeutic cloning aims to create ES cells that are genetically matched to the patient by using the technique that created Dolly. A healthy cell from a patient would be fused with a donor egg cell stripped of its chromosomes. This would produce an embryo which, given the right conditions, should develop into a blastocyst from which ES cells could be harvested.

## High hopes

Enthusiasm for therapeutic cloning was initially high. In a December 1999 article in *Nature*[3], two leading cloning researchers declared their belief that such procedures would bring "the greatest eventual benefit" from the technology. And over the past few years, therapeutic cloning has featured prominently in the popular press accounts.

So to the casual observer, it may come as a surprise that many experts do not now expect therapeutic cloning to have a large clinical impact. Aside from problems with the supply of human egg cells, and ethical objections to any therapy that requires the destruction of human embryos, many researchers have come to doubt whether therapeutic cloning will ever be efficient enough to be commercially viable. "It would be astronomically expensive," says James Thomson of the University of Wisconsin in Madison, who led the team that first isolated





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Cultural revolution: James Thomson was the first to isolate human embryonic stem cells.

ES cells from human blastocysts.

But the field of regenerative medicine is not in the doldrums — far from it. Some stem-cell biologists argue that it might be possible to treat patients by manipulating the 'adult' stem cells that reside in many of our tissues. Others are busy collaborating with immunologists to develop strategies that will allow tissues grown from 'foreign' stem cells — including ES cells — to evade the body's immune system. And yet others believe that, in the long run, it may be possible to achieve the same goals as therapeutic cloning without a cloning step. They want to 'reprogramme' cells, reversing the developmental processes that made them adopt a particular specialized function, and turning them into 'ES-like' cells that can develop into any tissue.

## Double troubles

Therapeutic cloning is almost certainly possible. Researchers at Monash University and the company Stem Cell Sciences, both based near Melbourne in Australia, last year proved the principle by obtaining mouse ES cells from embryos that had been cloned from adult mouse cells[4]. But mammalian cloning is inefficient, even in the hands of the most skilled scientists. Of the 277 cells from Dolly's 'mother' that were fused with donor egg cells, less than 30 developed to the blastocyst stage[1]. At the time, experts believed the efficiency would improve. But despite feverish efforts by groups worldwide, progress has been disappointing. "We don't at the moment have any real handle on how to greatly increase the efficiency," admits Alan Colman of PPL Therapeutics near Edinburgh, the company involved in the Dolly experiments.

For therapeutic cloning to become affordable, the cloning step would have to be conducted efficiently by technical staff at individual hospitals. Human eggs are also in short supply, and in high demand for *in vitro* fertilization procedures. Peter Mountford, chief scientific officer of Stem Cell Sciences, believes these problems can be overcome, and argues that it is too early to give up on therapeutic cloning — but his has become a minority view.

Although the progress in improving the efficiency of cloning has stalled, research with human ES cells has continued — albeit in a restricted number of labs (see 'A tortured tale of supply and demand', overleaf). They seem to proliferate well in culture[5], and can develop in the laboratory into a wide range of different cell types[6]. At the Keystone Symposium on Pluripotent Stem Cells, held this February in Durango, Colorado, Melissa Carpenter of Geron, based in Menlo Park, California, reported on experiments in which she had allowed human ES cells to develop into 'neural progenitor' cells, which can develop into nerve cells. When she transplanted these into the brains of newborn rats, the cells seemed to continue their development and integrate into their new environment.

> Too much of this research is happening under the umbrella of biotech companies, which are understandably cagey.

Given these advances, stem-cell biologists are cautiously optimistic about the prospects of growing replacement tissues from ES cells. Geron is pushing ahead, and hopes to move into clinical trials within five years. But if the ES cells do not come from embryos cloned from the patient's own cells, the problem of rejection remains. In some cases, it may be possible to protect grafts grown from ES cells using relatively mild immunosuppressive drugs. The immune system has only restricted access to the brain, for example, so grafts of cells to replace the nerve cells lost in Parkinson's disease might survive without much assistance. But in most tissues, grafts grown from foreign ES cells would quickly be rejected.

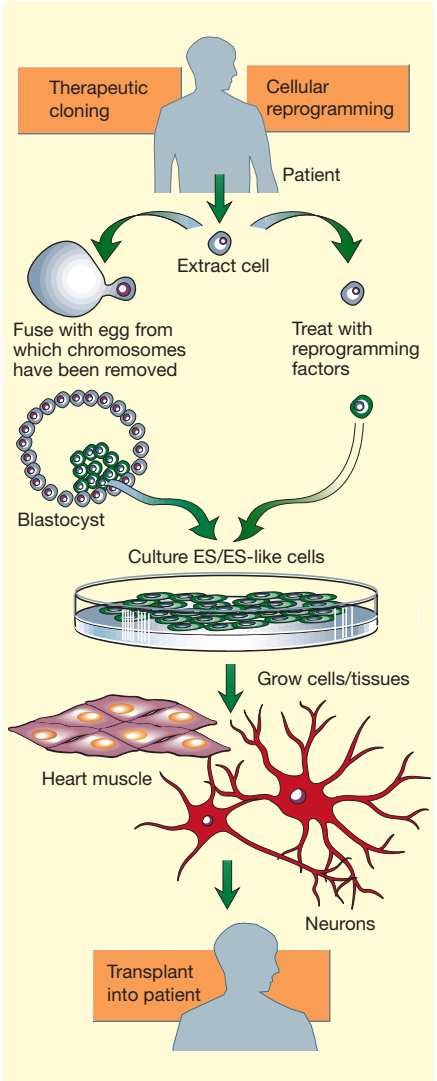
"The major issue in bringing this to reality is the immunological one," says John Gearhart, a stem-cell biologist at Johns Hopkins University in Baltimore. And because immunosuppression renders transplant patients susceptible to infectious diseases and cancer, there is a big incentive to develop 'tolerance' strategies that would allow tissues grown from ES cells to escape the attentions of the immune system.

There are many ways in which this might be achieved. One idea is to use antibodies to block or disable receptors carried by immune cells involved in rejecting foreign tissues. In mice, temporary treatment with such antibodies around the time of a transplant seems to prevent rejection[7] — although such strategies risk rendering patients tolerant to any bacteria or viruses they encounter during the treatment. Maggie Dallman, an immunologist at Imperial College London, also warns that tolerance regimes that work in rodents often do not transfer so well to larger animals, or people. "That's a general rule, and nobody quite understands why," she says.

## Enter the engineers

Rather than manipulating the immune system to accept foreign grafts, some stem-cell biologists think it may be possible to genetically engineer ES cells so they become invisible to the immune system. "ES cells may need very little modification to make them universal donors," speculates Alan Trounson of Monash University, a reproductive biologist who is now branching out into stem-cell research. Various strategies could be used. Dallman and her colleagues, for example, are investigating a protein called Notch, which helps regulate immune responses[8]. They suspect that stem cells could evade the immune system if they were engineered to produce a protein to which Notch binds.

Gearhart, meanwhile, is interested in the possibility of customizing ES cells by genetic engineering to make them match the intended graft recipient. The rejection of transplanted tissues depends heavily on proteins produced by genes within a chunk of chromosome 6 known as the major histocompatibility complex (MHC). Replace the MHC of ES cells with the patient's MHC, and the immune system might be fooled into thinking the ES cells come from the patient. Replacing such a large gene sequence is technically difficult — but, argues Gearhart, not impossible.




**Growth industry: the concept of reprogramming cells, rather than cloning them, offers an alternative route to generating 'personalized' tissue grafts.**

Recent work with adult stem cells, however, has made some researchers question whether a tight focus on ES cells is necessary. Small numbers of stem cells exist in adult tissues, where they help to repair our bodies. Compared with ES cells, these adult versions are thought to have a more restricted capacity for development into different tissue types. But if they could be used as a source of replacement tissue, adult stem cells would avoid the destruction of a human embryo — a fundamental moral objection to approaches based on ES cells.

Adult stem cells could be harvested from healthy donors and used to grow replacement tissues for patients needing grafts. Once again immunosuppression or tolerance would probably be needed, but a novel way of using adult stem cells might provide a suitable tolerance strategy. Bone marrow contains haematopoietic stem cells (HSCs), which give rise to all of our blood cells, including those of the immune system. When HSCs are transplanted into the bone marrow of the recipient, the immune system can enter a 'chimaeric' state in which some of its cells are derived from the transplanted HSCs. These would, in theory, prevent the immune system from reacting against other cells transplanted from the same donor[9]. Last year, for instance, Judith Shizuru, Irving Weissman and their colleagues at Stanford University in California showed that mice given transplants of highly purified HSCs subsequently accepted heart grafts from mice genetically identical to those from which the HSCs came[10].

But if a patient's own stem cells could be used to grow replacement tissues, there would be no need to worry about rejection. With this aim in mind, researchers are again looking to bone marrow to provide a solution. Bone marrow contains stem cells that can give rise to a range of tissues including bone, cartilage and muscle. In April 1999, researchers with the company Osiris Therapeutics in Baltimore showed that cultures of these cells retain this potential[11]. And in this issue of *Nature*[12], Piero Anversa of the New York Medical College in Valhalla and his colleagues describe experiments in which they injected stem cells from mouse bone marrow directly into the cardiac muscles of mice with damaged hearts. They found that the stem cells developed into muscle cells and blood vessels, helping to repair areas of dead tissue. These experiments raise the possibility of repairing a patient's failing heart with cardiac muscle grown from his or her own bone marrow stem cells[13].

## Career change

Recent experiments in mice have also revealed that adult stem cells can develop in entirely unexpected ways. Neural stem cells from the brain, for example, have been transplanted into bone marrow, where they developed into blood cells[14]. Bone marrow stem cells have also been shown to migrate to the brain after being injected into the bloodstream, where they develop into cells that appear to be neurons[15,16]. These experiments have fuelled hopes of treating patients with their own adult stem cells.

But even the enthusiasts accept that there is a long haul ahead before therapies based on these discoveries are ready for the clinic. "We need to make this more robust," says Helen Blau, who works on adult stem cells at Stanford. Showing that small numbers of stem cells can migrate to another site in the body and develop into a cell type appropriate for that tissue is one thing; using them to repair damaged or diseased tissues is another.

Improving the situation will entail a search for cell-surface markers to identify the stem cells that can transform into a wide range of tissue types, and the development of methods to purify and selectively culture them. It may also require the discovery of the biochemical signals that attract stem cells to sites of tissue damage and direct their development to effect a repair.

Given these obstacles, some researchers believe it is also worth taking on the field's toughest challenge — finding a way to reprogramme any of the body's cells to create ES-like cells matched to the intended recipient without cloning an embryo. Interest stems in part from experiments reported in 1997, in which researchers led by Azim Surani of the Wellcome/CRC Institute of Cancer and Developmental Biology in Cambridge fused mouse white blood cells with embryonic germ cells[17] — cells from the developing reproductive system that share many characteristics of ES cells. The white blood cell nuclei appeared to return to an embryonic state.

Researchers at several of the leading com-

## A tortured tale of supply and demand

Given the breadth of their potential, one might expect that human embryonic stem (ES) cells would be the focus of attention for hundreds of research groups. But so far, only a dozen or so teams have entered the field. The issue was initially one of a shortage of cells. But now the main problems are political — with fears that the new US administration will ban federal funding for ES-cell research looming large.

So far, the main distributor of human ES cells has been the WiCell Research Institute, a non-profit spin-off from the University of Wisconsin in Madison, where in 1998 the cells were first cultured in James Thomson's lab[2]. His work was funded by the company Geron of Menlo Park, California, which has certain exclusive commercial rights to develop the ES cells for therapeutic applications.

In February last year, the University of Wisconsin announced that WiCell would soon start making Thomson's ES cell lines available to other research groups. Some researchers were initially concerned that WiCell wanted wide-ranging rights to rescind permission to work on the cells and to demand that they be destroyed. Those rights have since been restricted, and will only be enforced under specific circumstances — for instance if researchers use the cells for additional projects without written permission.

Given the time that has elapsed between the cells' creation and WiCell's formation, say stem-cell researchers, Geron got an important head start. Although researchers who use WiCell's ES cell lines can patent discoveries made using the cells, they may find that much of this territory has already been staked out. "We have submitted 36 patent filings on these cells," says David Greenwood, Geron's senior vice-president for corporate development.

Other supplies are now available. A group headed by Alan Trounson at Monash University, near Melbourne in Australia, is responding to requests to obtain cells from its human ES cell lines[6] — although high demand is putting the lab under pressure. "We are short on staff," says Trounson. Other ES cell lines are soon expected to become available from the Rambam Medical Center in Haifa, Israel.

But increased availability does not mean open access. Current legislation in France and Germany, for instance, prohibits embryo research, including work on ES cells — although the French government is proposing to lift its ban. In the United States, meanwhile, uncertainty as to whether federal funds will be freed to support ES-cell research is hampering progress.

Last summer, the previous US administration concocted a compromise that would allow researchers to use federal funds to work on ES cells, provided the ethically contentious step of isolating the cells from a human embryo had been achieved using other funding sources. But President George W. Bush's administration may now block the use of federal funds for ES cell research. And in the current state of limbo, few scientists have responded to a call from the National Institutes of Health (NIH) for ES-cell research proposals. By the 15 March deadline for documents to show that proposed research will comply with NIH guidelines, just three submissions had been received.

Even if federal funding is released, there may still be problems. The NIH has published criteria — including standards for informed consent from 'parents' of the embryos from which the cells were harvested — with which suppliers of ES cells to federally funded researchers must comply. WiCell's current cell lines do not meet these standards, and the NIH is still reviewing compliance documents submitted from Monash.

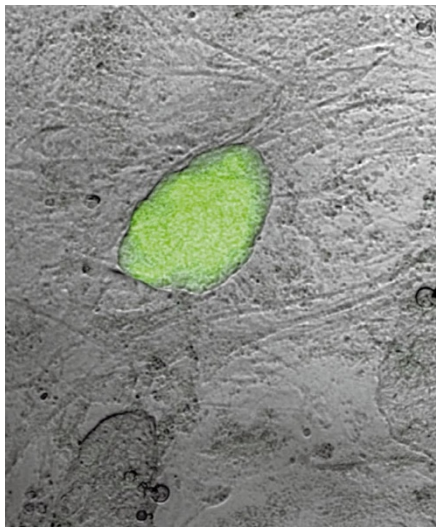
**Joanna Downer**

panies interested in regenerative medicine are now rumoured to be stripping the nuclei from ES cells and embryonic germ cells, and fusing them with various types of cells in an attempt to wind back the developmental clocks of the latter. In the process, they hope to learn how to rewind cell development without using ES cells.

One such company, PPL Therapeutics, claimed in January to have reprogrammed skin cells to form ES-like cells, some of which developed into heart muscle cells. But PPL has annoyed other researchers by refusing to produce data to back up the claim. The company also will not confirm whether cell fusion, or some other technique, was involved. "I don't think this is helpful," says Rudolf Jaenisch of the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology. "If you make an announcement, you have to say how you did it."

Indeed, the commercial secrecy cloaking much of the work on cellular developmental reprogramming is causing widespread frustration. "Too much of this is happening under the umbrella of biotech companies, which are understandably cagey," says Richard Gardner of the University of Oxford, who last year chaired an expert panel that reported on the issues surrounding stem-cell research for Britain's Royal Society.

Other approaches thought to be under investigation behind closed company doors may be inspired by work on the African clawed toad, *Xenopus laevis*. Cloning amphibians is technically less challenging than cloning mammals — *Xenopus* have been routinely cloned since the 1960s. Biologists have recently started to identify the molecules in *Xenopus* egg cells that underpin the developmental reprogramming involved. And these findings provide hints about some of the changes needed to wind back development without a cloning step.



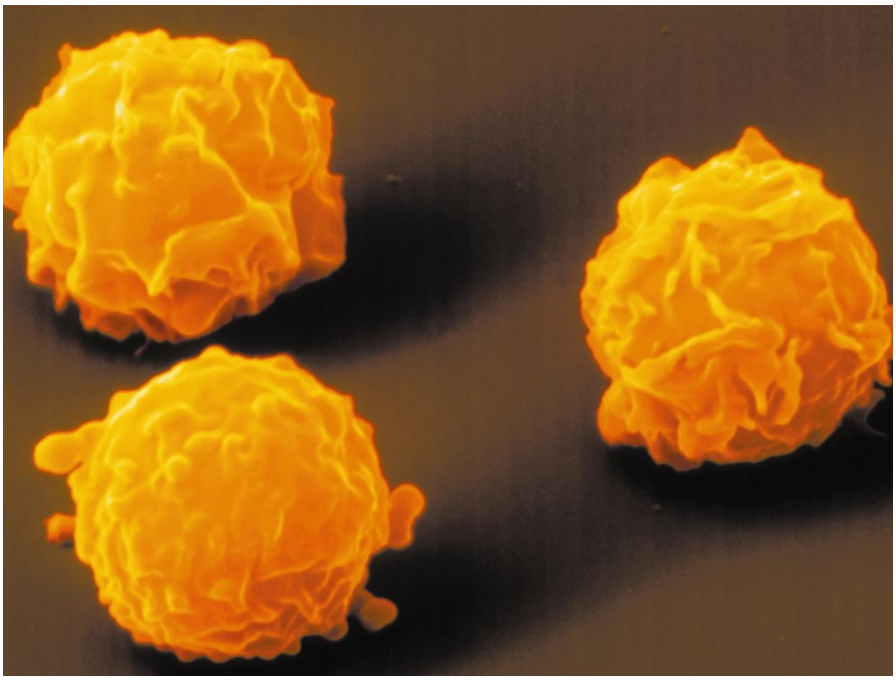
A. SURANI

**Fresh start: Azim Surani's cell fusions (right) suggest that developmental reprogramming is possible.**

The DNA in every cell is associated with proteins that regulate the expression of the cell's genes. As cells move towards their specialized adult functions, some of these proteins are removed, while many more are added. So, among other processes, reprogramming means undoing these changes. In the 1990s, while studying cloning in *Xenopus* at the National Institute of Child Health and Human Development in Bethesda, Maryland, Alan Wolffe implicated a protein called nucleoplasmin in this process. Nucleoplasmin helps strip DNA from the histone proteins around which it is wound in the chromosomes of mature cells[18] — thought to be a necessary stage in cellular reprogramming. And last year, he led a team that showed that an enzyme called ISWI removes another protein, called TATA-binding protein, that associates with the DNA of adult cells[19].

Such discoveries may merely be scratching the surface of the reprogramming mechanism. But other researchers intend to conduct systematic screens for cellular reprogramming factors. Surani, for instance, is now embarking on experiments with cells engineered to contain 'reporter' genes that should switch on if the cells are reprogrammed. Surani plans to insert a library of genes into the engineered cells, to find those that activate the reporter gene.

Enthusiasm for therapeutic cloning may have dimmed, but regenerative medicine is still a hotbed of activity, with molecular and cell biologists, immunologists, geneticists and developmental biologists all claiming a piece of the action. If the pace of discovery holds up, stem cells might eventually deliver a medical miracle. ■



SPL

**Rich resource: stem cells from bone marrow could be used to repair a variety of tissues.**

**Peter Aldhous is *Nature*'s chief News and Features editor.**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