

# Stem cells in regenerative medicine

Stem cell research has, justifiably, gained a high profile in the past decade. This has been fuelled not only by the fantastic therapeutic (and financial) potential but also by the emotive ethical and political implications. In this article, **Gavin Jell, Ian Bonzani and Molly Stevens** provide an overview for pharmacists

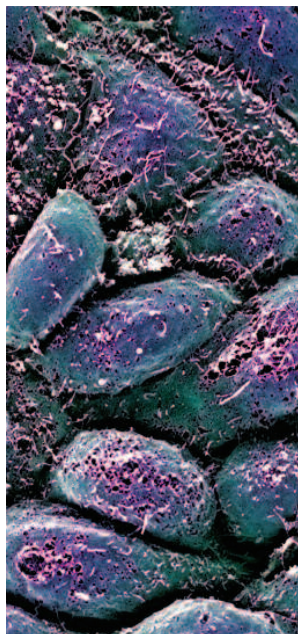
Stem cell research has already provided some outstanding contributions to our understanding of developmental biology and it offers much hope for the regeneration of diseased or injured tissues. Recent and rapid advances in the manipulation of both adult stem cells (ASCs) and embryonic stem cells (ESCs), combined with their potentially numerous applications, mean that stem cell research is a complex field, both scientifically and ethically.

## What is a stem cell?

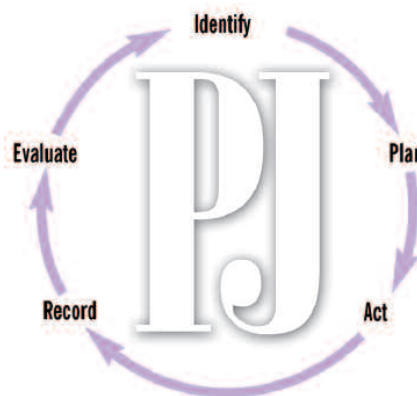
Despite 30 years of research this question remains contentious. However, most scientists agree that stem cells are capable, to some degree, of undergoing both self-renewal and differentiation into more functionally specialised, mature cells. When a stem cell divides daughter cells can, depending on the environmental stimuli, either remain stem cells or become more specialised, forming progenitor cells (eg, blood, bone and brain cells). The degree to which different stem cells can differentiate into other cell types is known as “plasticity” or the “differentiation potential”.

**Adult stem cells** ASCs are undifferentiated cells. More accurately they are called somatic stem cells because they can also come from fetuses, umbilical cords and infants. ASCs are believed to exist in small numbers in most tissues and organs (including the bone marrow, liver, gut, blood and brain). They are called on during tissue repair to replace damaged cells. Traditionally, the plasticity of ASCs is believed to be limited compared with that of ESCs — how much more limited is intensely disputed (see below) — but their use offers several practical advantages over ESCs, including ease of isolation and handling, and phenotypic stability. Bone marrow has been found to contain ASCs that are able to give rise to progeny of different tissue types. Two of the most researched subpopulations of bone marrow stem cells are mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSCs).

MSCs are non-haematopoietic, stromal cells that, under appropriate stimuli, can differentiate into bone, cartilage, fat, tendon and muscle cells. Originally isolated from bone marrow and stroma of the spleen and thymus, MSCs have more recently been isolated from other sites, including cartilage, periosteum, synovium, synovial fluid, blood vessels, muscle, and tendon. However, it is not yet clear to what extent MSCs are responsible for normal growth or maintenance *in vivo*. MSCs are an attractive option for cell-based therapy because of their potential for autolo-



Professor Miodrag Stojkovic/SPL



## Identify knowledge gaps

1. What are stem cells?
2. Who could benefit from stem cell research?
3. What is the next step in stem cell research?

Before reading on, think about how this article may help you to do your job better. The Royal Pharmaceutical Society's areas of competence for pharmacists are listed in “Plan and record”, (available at: [www.rpsgb.org/education](http://www.rpsgb.org/education)). This article relates to “keeping abreast of issues affecting pharmacy”.

## Definitions

**Differentiation** The process by which a cell, in response to stimuli, becomes more specialised.

**Transdifferentiation** The ability of a cell of one tissue, organ or system to differentiate into a cell type of another (eg, haematopoietic stem cells can become liver hepatocytes).

**Dedifferentiation** The regression of a (normally specialised) cell to a less specialised cell.

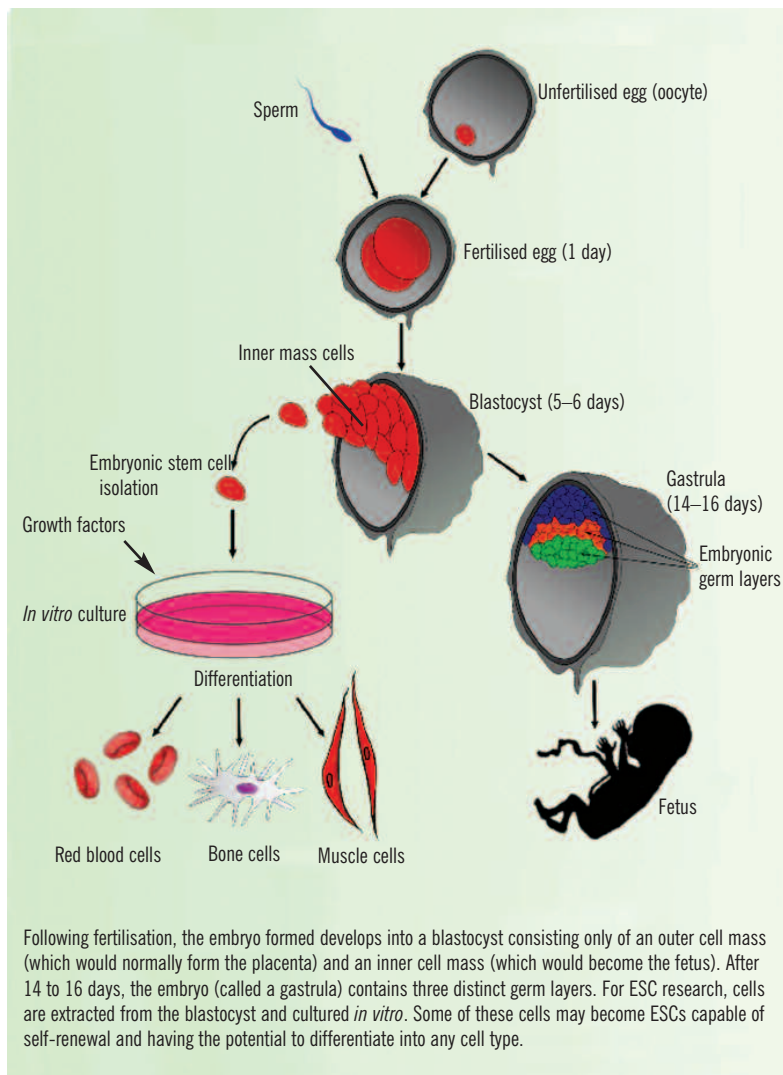
**Molly Stevens, PhD, MRPharmS**, is a reader in the department of materials and the Institute for Biomedical Engineering at Imperial College London. Within her research group, **Gavin Jell, BSc, PhD**, is a senior postdoctoral associate and **Ian Bonzani BSc**, is a Marshall Scholar. Correspondence to: [m.stevens@imperial.ac.uk](mailto:m.stevens@imperial.ac.uk)

gous cell-based therapies and their relatively low immunogenicity.

HSCs are stromal cells that can differentiate into all types of blood cell, including platelets. Successful HSC transplants have been performed for a number of years, using autologous bone marrow and allogenic umbilical cord blood to treat patients with non-malignant and malignant haematopoietic disorders, such as leukaemia. However, such procedures are hampered by the rarity of these stem cells.

**Embryonic stem cells** Although ASCs have been used successfully for over 30 years, ESCs have only recently (1998) been isolated from humans. ESCs are cultured from the undifferentiated inner mass cells of a blastocyst (an early stage embryo that consists of 50 to 150 cells, see Figure, p696). Once established, an embryonic stem cell line is immortal (ie, capable of continuous proliferation without differentiation).

Compared with ASCs, ESCs are more pluripotent (see Definitions, p697) and able to self-renew, which makes them a versatile therapeutic option. ESCs, however, cannot be used directly in cell therapies because they form tumours. The therapeutic potential of ESCs, therefore, relies on their directed differentiation into a particular specialised cell



**Figure: Embryonic stem cell differentiation**

### Panel 1: Major advances in stem cell research

- 1907 European scientists realise that all blood cells come from one "stem cell".
- 1963 Bone marrow injected into irradiated mice is found to be able to self-renew and differentiate.
- 1968 The first bone marrow transplant (adult stem cells) is successfully used to treat severe combined immunodeficiency disorder.
- 1972 Mouse teratoma (a tumour that contains tissues derived from all three embryonic layers) cell line is established.
- 1981 A pluripotent mouse cell line is established.
- 1996 The first mammal (Dolly the sheep) is cloned from ASCs using somatic cell nuclear transfer.
- 1998 A pluripotent human cell line is established.
- 2003 Oocytes from mouse embryonic stem cells are derived, demonstrating that ESCs can be totipotent.
- 2004 The world's first stem cell bank opens in the UK to store, characterise and supply ethically approved, quality controlled cell lines for research and, ultimately, treatment.
- 2004 A human embryonic stem cell line from an adult (somatic) cell isolated from a living person is generated. This might allow ESC therapies or regenerative medicine with patients' own DNA, preventing immune rejection.
- 2005 A new technique to extract ESCs without damaging the embryo is developed.
- 2005 A new human embryonic stem cell line, free from animal components, is created. (Existing human embryonic stem cell lines used animal cells and serum for culture, which could present human health risks.)
- 2005 World human embryonic stem cell bank is established in South Korea. This may help scientists avoid government restrictions on cloning.

(eg, insulin-producing  $\beta$ -cells for diabetes), before administration.

To prevent spontaneous differentiation of ESCs *in vitro*, precise culture conditions must be maintained with specific growth factors, such as leukaemia inhibitory factor. The signalling pathways underlying self-renewal and pluripotency of ESCs in culture have only recently begun to be unravelled and the ability to guide ESCs towards a specific cell type will require a further leap of understanding.

**Trans- and dedifferentiation** Future ASC developments may rely on the recently discovered phenomenon known as transdifferentiation. The multipotency of ASCs is traditionally believed to be restricted to cell lineages found within their tissue of origin. However, a number of experiments have since shown that stem cells from one tissue have the ability to give rise to cell types of completely different tissue. Examples include HSCs from bone marrow that can transdifferentiate into neural, myogenic and hepatic cells. Transdifferentiation is believed to occur in several diseased tissue states and metaplasias, such as gastric carcinoma and liver fibrosis. Although several studies offer extensive data to support ASC transdifferentiation, some researchers have attributed this plasticity to cell-fusion rather than true differentiation.

The holy grail of stem cell research is to dedifferentiate ASCs so that they have the same differentiation capacity or plasticity as ESCs. This would negate both ethical objections to ESC research and the need for techniques to prevent immunological rejection. Recently, human ESCs have been created by fusing individual ASCs with ESCs. The cells developed stem cell characteristics and demonstrated the ability to dedifferentiate given the right environmental signals. Future advances in understanding these signalling factors may enable ASC dedifferentiation into embryonic-like stem cells without fusion to an ESC.

### Cloning and immune rejection

Therapeutic cloning refers to the transfer of the nucleus of a somatic (adult) cell into an enucleated donor oocyte, using a technique called somatic cell nuclear transfer (SCNT). In theory, the oocyte's cytoplasm reprogrammes the transferred nucleus by turning off all the somatic cell genes and activating the embryonic ones. ESCs are then isolated from the inner cell mass of the cloned embryo and the embryo is destroyed. The cloned ESC would then carry the nuclear genome of the patient (the cell donor) and, therefore, after directed cell differentiation, the cells or tissues could be transplanted without immune rejection. Therapeutic cloning should not be confused with "reproductive cloning", where the SCNT-cloned embryo is reimplanted into a surrogate mother to produce cloned offspring, such as Dolly the sheep (see Panel 1).

The generation of human ESCs from a somatic cell isolated from a living person has

recently been reported.<sup>1</sup> In these studies, numerous embryos were used to generate cloned embryos, which led to the derivation of viable embryonic stem cell lines. Each cell line was confirmed to be genetically identical and, therefore, immunologically compatible with the cell donor.

An alternative approach to prevent immune rejection is to engineer stem cells to be less immunogenic; indeed, the ambitious proposal of universal donor cells may be a future possibility. A more achievable short-term goal to minimise rejection, however, would be to build up stem cell banks with a vast array of cell lines representing, as close as possible, the complete range of immune profiles. The world's first stem cell bank was opened in the UK in 2004.

### Applications of stem cells

Stem cells have the potential to revolutionise current medical practice by a variety of methods, including cell replacement therapies, tissue engineering and the activation of resident *in vivo* stem cells. An impact may also be made in the pharmaceutical industry where stem cells would enable the development of disease models, thereby assisting in more effective screening of potential drugs. For example, cells, tissue and organs with degenerative disease could be developed to allow more accurate and better controlled screening methods for potential drug therapies. For this consideration, a number of cloned embryonic stem cell lines with inherited disease have already been created.

The use of human cells and tissue produced from stem cells may also reduce the need for animal testing and the accompanying issues of interspecies variation. It

## Definitions

**Multipotency** Ability to produce cells of a closely related family of cells (eg, only blood cells).

**Plasticity** The potential to differentiate into different cell types.

**Pluripotency** Ability to grow into any cell type except for totipotent stem cells. Pluripotent cells are unable to form a complete organism.

**Progenitor** Progenitor cells can produce only one cell type but can self-renew. This distinguishes them from non-stem cells.

**Totipotency** Ability to differentiate into any type of cell. Totipotent cells can create a complete organism (eg, zygote cells and the cells produced by the first few divisions of the fertilised egg cell are totipotent).

would also enhance the testing of hazardous chemicals for embryotoxicology and pharmacotoxicology.

**Cell therapy** Cell therapy is traditionally defined as the replacement of diseased or dysfunctional cells with healthy ones. There are two broad categories of cell therapy: the direct administration of ASCs obtained from a donor and the *in vitro* culture and differentiation of ASCs or ESCs before administration.

An example of direct administration is bone marrow transplantation. This is successfully used to treat leukaemia and other blood disorders. Fetal nerve cells have also been transplanted (experimentally) into the brains of people with Parkinson's disease for the past 10 years. The aim is for ASCs, when transplanted, to colonise the target site, where tissue specific factors will cause the stem cells to differentiate into the desired cell type.

For the purpose of replacing a host of cells, transdifferentiation of ASCs offers potential advantages of developing therapeutic cell numbers based on the *in vitro* expansion of a few isolated cells. Even better, embryonic stem cell lines have the potential to differentiate into all adult tissues and together with therapeutic cloning this could present an astounding (if currently futuristic) way of replacing all types of damaged adult cells with genetically identical healthy cells. To this end, human ESCs have been directed to differentiate into neurones as a potential treatment for neurodegenerative disorders.

**Tissue engineering** One of the biggest problems in transplantation medicine is the lack of suitable donor organs and tissues. Recent progress in tissue engineering and stem cell research offers the possibility of organising the cells into three-dimensional structures that can be used to repair damaged tissues. Tissue engineering scaffolds can be seeded with stem cells, grown in culture, and subsequently grafted into a damaged organ.

**In vivo stem cell activation** Numerous researchers study the subtle chemical language that activates and recruits stem cells during normal tissue repair. Mastering this language may enable, by creating the right cascade of signals, the activation of dysfunctional stem cells or the quicker recruitment of stem cells already present in the body (eg, HSCs which circulate in the blood could be recruited to wounds using the right chemical signals). This would eliminate the need for laboratory-grown cells. Insulin growth factor-1, has been shown to improve muscle healing by summoning stem cells to injured muscle tissue, and is regularly used for treating injuries to racehorses.

As important as understanding how to activate stem cells, however, is how to turn stem cells off after repair. Several researchers have shown evidence that a number of cancers may be caused by stem cells remaining in an activated, self-renewing state.

## Panel 2: Who might benefit from stem cell research

**Heart disease** Heart disease is the leading cause of death in developed countries. Clinical studies suggest that adult stem cells may benefit heart conditions, including myocardial infarction and advanced heart failure. Stem cell repopulation of damaged myocardial tissue by trafficking bone marrow stem cells into the circulation using cytokine drug treatments is promising.

**Neurodegenerative disorders** Parkinson's disease and Alzheimer's disease currently afflicts, respectively, 110,000 and over 500,000 people in the UK. Neural stem cells found to reside throughout the central nervous system have been shown to differentiate into neuronal cells when transplanted into aged rat brains, significantly improving cognition.

Mouse embryonic stem cells, directed to differentiate into neurones and transplanted into brains, have been shown to cause neurogenesis in mouse disease models.

**Diabetes** An estimated 150 million people have diabetes worldwide, with numbers predicted to double in the next 20 years. ASCs isolated from the pancreas, liver and bone marrow, and transplanted into the pancreas of a patient with diabetes have been shown to differentiate into islet-like clusters that produce insulin. In some mouse models glycaemic control can be achieved.

**Orthopaedic defects and joint disease** Twenty million people suffer from some form of arthritis in the UK. Autologous, *ex vivo* expanded bone marrow osteoprogenitor cells transplanted into patients with large bone defects show good initial osteointegration and promote full limb recovery within 12 months.

When mesenchymal stem cells were injected into osteoarthritic goats, further cartilage destruction was retarded and meniscus tissue regenerated.

*Figures taken from Health Statistics Quarterly 27:2005*

## Panel 3: The stem cell debate

### Arguments for

#### Embryonic stem cells

- Donor permitted cells harvested from *in vitro* fertilisation discarded embryos, which would be destroyed, should be viewed on the same moral grounds as tissue harvested from recently deceased tissue donors.
- IVF routinely discards all "unselected" embryos, showing that many embryos die while only one survives. This is a common procedure with ethical approval.
- The decision to discard embryos is independent of research motives.
- ESC tissue is only harvested after the decision to discard has been made no coercion tactics can exist.
- Government restrictions will lead to private enterprises forging ahead and leaving a safe regulatory umbrella.
- The private sector is unlikely to use funds for improved research safety and regulation.

#### Therapeutic cloning

- Embryos cannot be considered individuals until further development.
- Tissues can be developed that are genetically identical to the patient, thereby preventing immune rejection.
- The efficiency of ESC isolation and somatic cell nuclear transfer cloning techniques has greatly improved, but further research is necessary.

#### Reproductive cloning

- Genetic diseases can be eliminated.
- Transgenic animals for xenogenic therapies can be produced.
- Technology should not be limited.

### Arguments against

- Opponents of deriving ESCs from discarded IVF embryos argue that human beings must not be used as a means to satisfy other ends.
- Human life begins at conception.
- Removing cells from the embryo (which until recently also destroyed the embryo) is a decision that can only be made by the donor.
- Embryos have the potential to be stockpiled and turned into commodities for ESC research purposes.
- Women will be exploited and coerced into giving embryos.
- The government has a duty to uphold the morals and ethics of its citizens.
- The US government will only fund research conducted using an existing 26 embryonic stem cell lines. No federal funding goes to the derivation of new ESCs from IVF or otherwise.

- Cloned embryos are destroyed; this is killing a potential human being.
- Large numbers of embryos are needed to create a single cloned embryonic stem cell line (eg, Hwang *et al* used 185 eggs for 11 cell lines!).
- Advances in this field will inevitably lead to successful reproductive cloning.

- Large numbers of malformed babies would have to be born to produce one normal clone.
- Designer babies should not be created.
- There will be loss of individuality.

## Ethical issues

Recent ethical debates concerning stem cell research has mostly concentrated on the derivation and intended uses of new lines of ESCs. These debates can be separated ethically as well as scientifically into those concerning cloning and issues concerning ESC production from discarded *in vitro* fertilisation embryos. Panel 3 outlines some of the reasons that have been put forward for and against stem cell research.

There are major differences in the world's political views regarding stem cell research. Policies range from the more permissive (eg, in the UK, Spain, Switzerland and South Korea where derivation of new embryonic stem cell lines has been granted) to conservative (eg, in the US and Canada where a ban has been placed on the use of federal funds to finance the development and use of new embryonic stem cell lines) to restrictive (eg, in Germany). The effectiveness of governments

in balancing global public policy concerning ESCs will affect the overall speed and safety of future developments.

## Remaining research hurdles

Several hurdles remain before the enormous potential of stem cells can be realised. These include greater efficiency in ESC isolation and in establishing embryonic stem cell lines, overcoming immunogenic issues, and the characterisation of stem lines using sophisticated genetic and proteomic technologies. There is also a need for the greater standardisation of techniques and procedures to ensure accurate reproduction of studies. Further limiting the use of animal products in stem cell culturing methods will help to improve the immunogenic health risks associated with contaminated stem cells.

Research in dedifferentiation of ASCs is also vital. However, the most significant hurdle in stem cell research is the identification of the signals that determine the differentiation, and the signals that inhibit activity after injury repair. The progress of stem cell research has been evident by the shift of focus from broader questions concerning stem cell potency and regeneration potential to more specific biological questions regarding stem cell survival and capacity to populate host tissues, as well as their effectiveness after topical implantation and circulation.

## References

1. Hwang WS, Lee BC, Lee CK, Kang SK. Human embryonic stem cells and therapeutic cloning. *Journal of Veterinary Science* 2005;6:87–96.

## Resources

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## Action: practice points

Reading is only one way to undertake CPD and the Society will expect to see various approaches in a pharmacist's CPD portfolio.

1. To learn more about stem cell research and therapy, visit <http://stemcells.nih.gov/index.asp>
2. Research other possible future treatments for diabetes.
3. Consider the potential benefits that stem cells could present for treating Parkinson's disease and Alzheimer's disease. Discuss with another pharmacist, how stem cell therapy could affect pharmacy.

## Evaluate

For your work to be presented as CPD, you need to evaluate your reading and any other activities. Answer the following questions: What have you learnt? How has it added value to your practice? (Have you applied this learning or had any feedback?) What will you do now and how will this be achieved?