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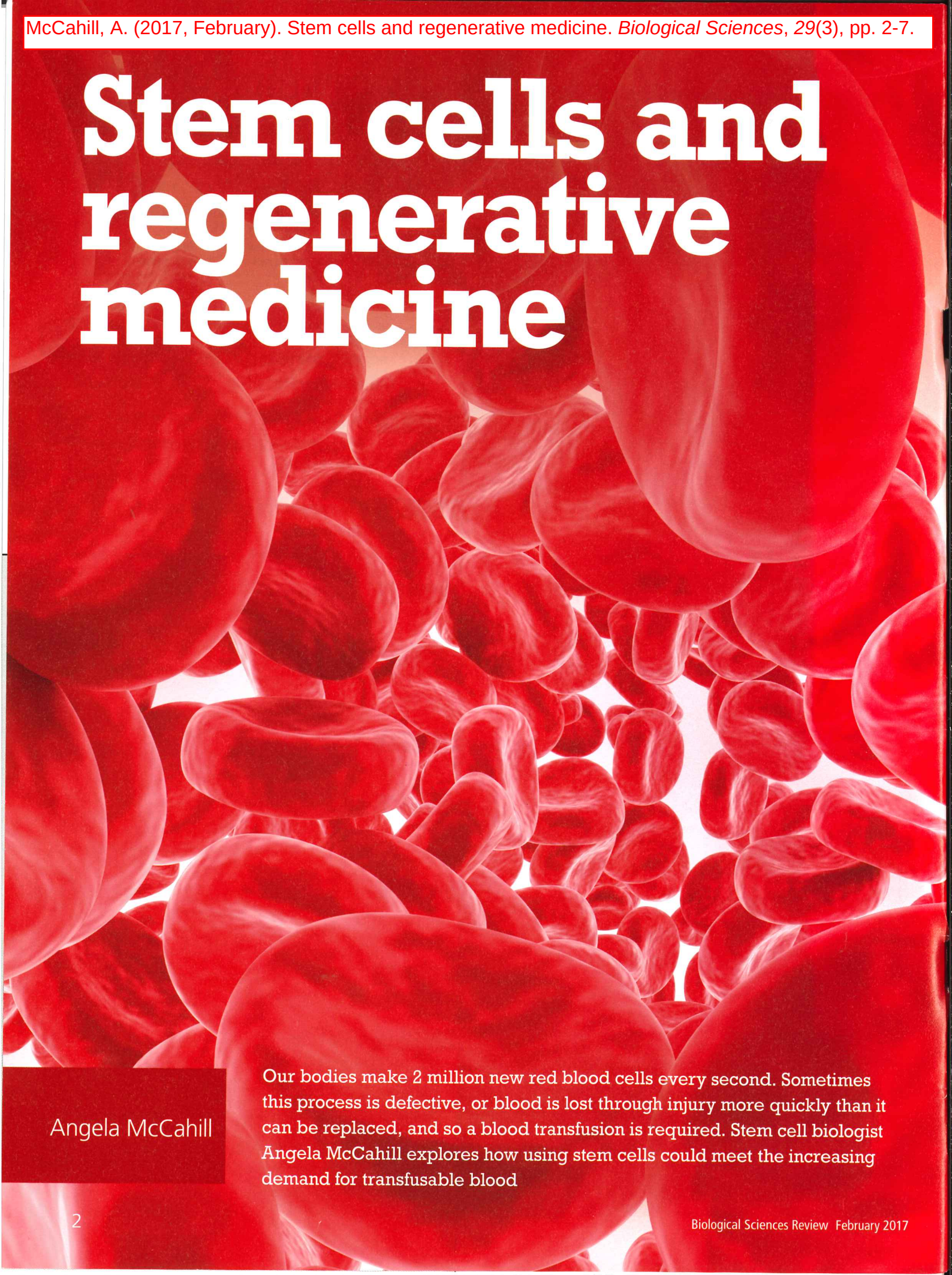
Stem cells and regenerative medicine



Angela McCahill

Our bodies make 2 million new red blood cells every second. Sometimes this process is defective, or blood is lost through injury more quickly than it can be replaced, and so a blood transfusion is required. Stem cell biologist Angela McCahill explores how using stem cells could meet the increasing demand for transfusable blood

Key words

Stem cells
Pluripotent
hESC
iPS
Blood

Stem cells are very important during both development and maintenance of adult animals. They are unspecialised cells which have a potentially infinite capacity to self-renew and can differentiate into specialised cells when required (see Biological Sciences Review, Vol. 29, No. 2, pp. 35–37). Blood stem cells (**haematopoietic** stem cells) reside in the bone marrow and self-renew to maintain a small but stable stem cell population. These cells can differentiate — mature — to give rise to the red blood cells (RBCs) that carry oxygen around the body, platelets for clotting and the white blood cells that form the immune system (see Figure 1).

Medical use of stem cells

Stem cells are extremely useful in biomedical research. It is not possible to study developmental processes in humans by dissection and experimentation. Studying the differentiation of stem cells into specialised cells gives us insight into the normal development of human tissues. Stem cells also have potential to improve the development and testing of new drugs. Testing new drugs on human cells, such as heart or liver cells which have been grown from stem cells, will give a better idea of their effectiveness and toxicity than testing new drugs on animals. Stem cells also have vast potential in the field of regenerative medicine, where tissues derived from stem cells can be used to repair or replace damaged tissue or body parts.

The first stem cell therapy was bone marrow transplantation. This has been used to treat blood disorders, including the blood cancer leukaemia, since the 1960s. The blood stem cells in the donated bone marrow have the capacity to replace the entire haematopoietic system by producing all the different types of blood cells required during the lifetime of the new recipient (see Figure 2). One of the most promising stem cell treatments currently being developed is cartilage transplant, which aims to relieve the pain caused by cartilage loss in joints such as the knee and hip. Disorders of the eye are also the focus of much research, and a stem cell product which helps replace damaged cells in the cornea (the transparent layer at the front of the eye) has recently been developed to treat pain and vision loss.

Different types of stem cell

The three different types of stem cell are adult, embryonic and induced pluripotent stem cells. Their origins differ, as shown in Figure 3. Many adult stem cells are unipotent, meaning that they

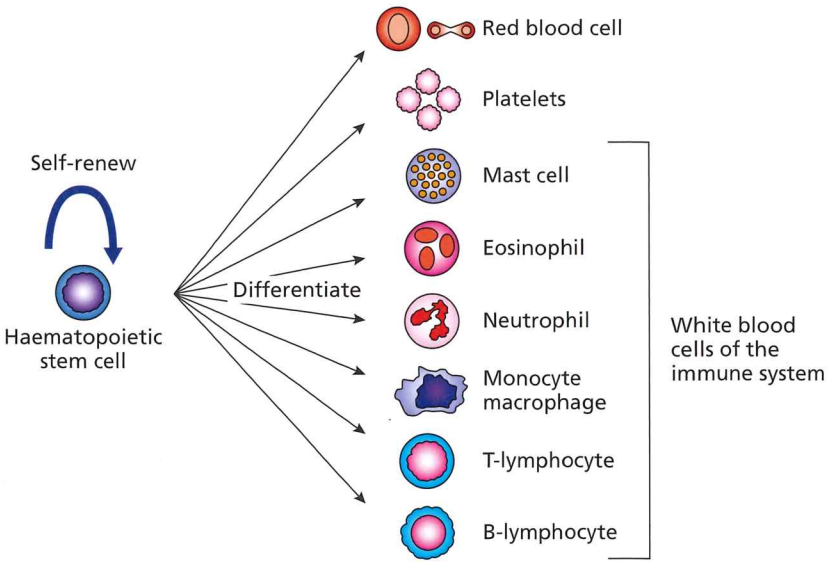


Figure 1 Haematopoiesis — the differentiation of blood cells

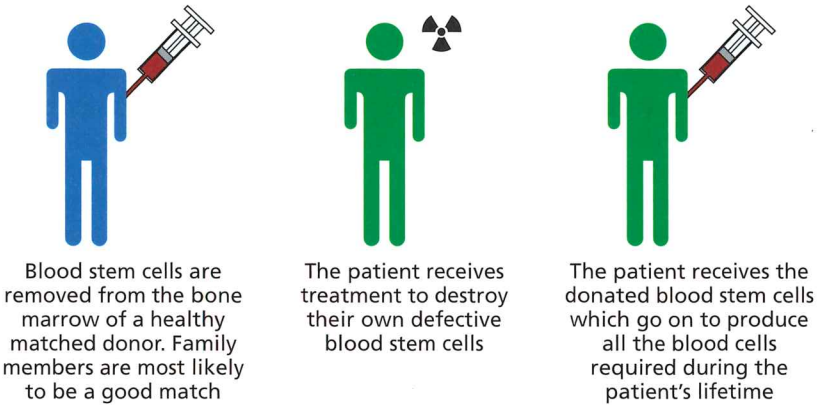


Figure 2 Bone marrow transplant — the original stem cell therapy

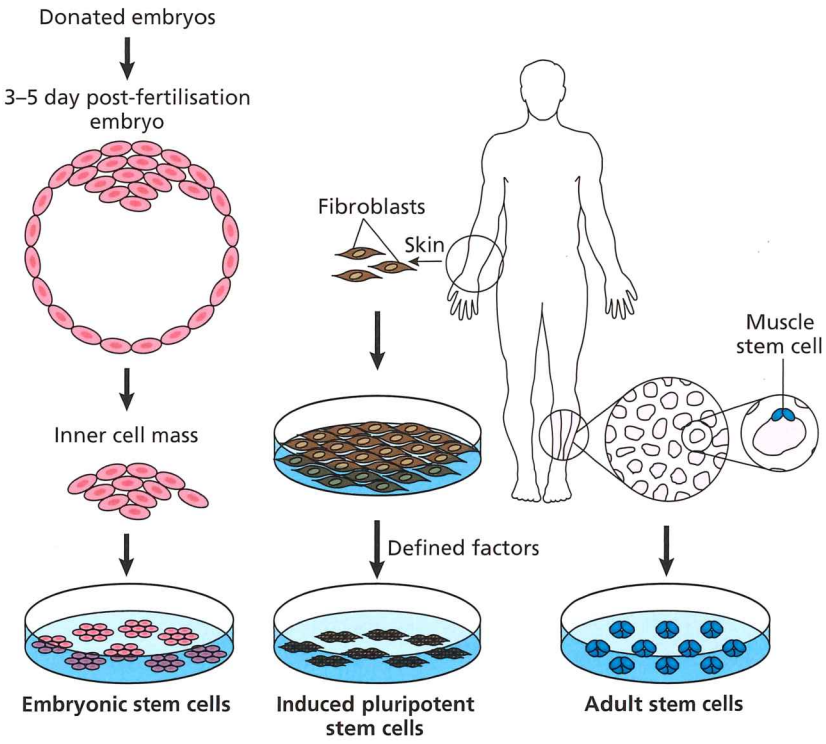


Figure 3 Types of stem cell and their origins

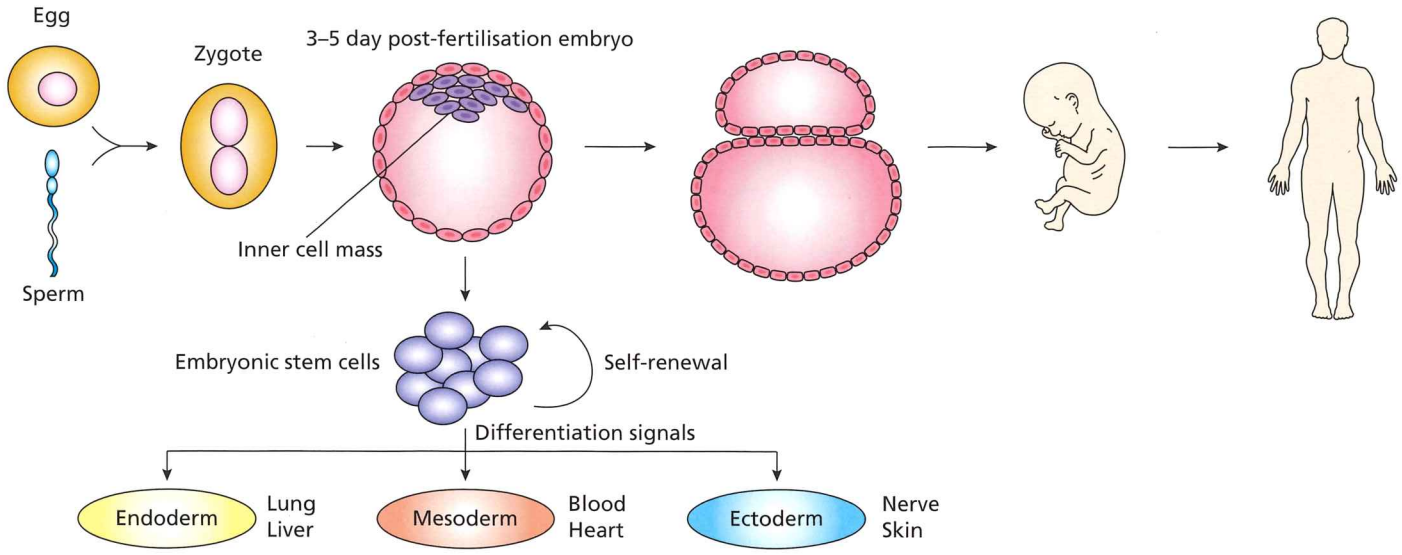


Figure 4 Human embryonic stem cells

can differentiate into only one type of specialised cell (see Biological Sciences Review, Vol. 29, No. 2, pp. 35–37). Some adult stem cells are multipotent — they can differentiate into several different types of cell, e.g. the blood stem cells described above.

Embryonic stem cells are pluripotent — they can form any of the specialised cells in the body. Human embryonic stem cells (hESCs) are derived from the inner cell mass of a 3–5 day old embryo (see Figure 4). In the UK, the embryos used to make hESCs were originally created for the in-vitro fertilisation (IVF) process and their use for research is highly regulated. If embryos are not of high enough quality for IVF, or are no longer required, their owners can donate them for research rather than have them destroyed. Once a hESC line has been derived from an embryo, it can be cultured indefinitely and the cells remain pluripotent so long as they are maintained under the correct conditions.

Adult and embryonic stem cells hold huge promise for regenerative medicine, but they have limitations (see Table 1). When adult stem cells are cultured in the laboratory, they do not self-renew indefinitely as they do in the body. It is therefore difficult to get enough of them to be useful as a therapy, particularly for diseases where many people are affected and cells would be required in great numbers, for example in treatments of heart or liver disease. In contrast, hESCs can self-renew indefinitely in culture, so there is a potentially endless supply to meet patients' needs. There are, however, considerable ethical issues over the derivation of stem cells from human embryos. This is why some countries and institutions will not allow research using these cells.

A further problem with therapies derived from hESCs is that they will always be **allogeneic** (from a donor and therefore non-self) and so may be attacked by the recipient's immune system. Adult stem cell therapies can be either allogeneic or **autologous** (where the recipient is given back their own cells).

Table 1 Advantages and disadvantages of the different types of stem cells

	Embryonic stem cells	iPS cells	Adult stem cells
Origin	Allogeneic	Autologous *or* allogeneic	Autologous *or* allogeneic
Expansion	Limitless (?)	Limitless (?)	Poor
Differentiation	All cells of the body = pluripotent	All cells of the body = pluripotent	Limited lineages = unipotent or multipotent
Ethics	Pro-life concerns	Few issues	Few issues

Induced pluripotent stem cells

In 2006, a Japanese researcher came up with a new way of making pluripotent stem cells without using embryos. He took skin cells called fibroblasts and reprogrammed them to make them pluripotent. He did this by treating them with four transcription factors (Oct-3/4, SOX2, c-Myc and KLF4) which switched on the genes that induce and maintain pluripotency (see Figure 5). Expression of these genes seemed to turn back the clock in the skin cells so that they went back to a pluripotent state. The cells were named 'induced pluripotent stem (iPS) cells'.

iPS cells represent a huge advance in the field of stem cell biology. They have all the advantages of hESCs without the ethical concerns raised by using embryo-derived cells. Additionally, there is the possibility of making patient-specific therapies using iPS cells derived from the patient's own tissue. Reprogramming has revolutionised the field of regenerative medicine and it is likely that iPS-derived therapies will soon reach clinical trials.

New blood

The Novosang collaboration is a group of UK researchers working on an exciting project that aims to use pluripotent stem cells to make RBCs for transfusion. Making RBCs from cells that have an infinite capacity to self-renew could provide a potentially unlimited supply of infection-free blood. If the blood was type O negative (universal donor) then it would be a suitable match for over 95% of the population (see Table 2). In developing countries that lack blood donation systems, this would benefit many lives. This new source of RBCs would also be

Table 2 The major blood groups and donor matching. O– blood can be given to almost everyone so is called the universal donor

Type	You can give blood to	You can receive blood from
A+	A+, AB+	A+, A–, O+, O–
O+	O+, A+, B+, AB+	O+, O–
B+	B+, AB+	B+, B–, O+, O–
AB+	AB+	Everyone
A–	A+, A–, AB+, AB–	A–, O–
O–	Everyone	O–
B–	B+, B–, AB+, AB–	B–, O–
AB–	AB+, AB–	AB–, A–, B–, O–

advantageous in developed countries, where ageing populations require more therapies such as joint replacement surgery and cancer treatment, which in turn increases demand for blood transfusions. It would also eliminate the risk of transfusion-transmitted infections.

The use of cultured blood would be of particular benefit to those with blood disorders such as beta-thalassemia. Patients with beta-thalassemia require regular blood transfusions, which can lead to a toxic build-up of iron in their bodies. Cultured blood would consist of freshly made cells of the same age, rather than a mixture of ages as in donated RBCs. This should mean that patients would not need to be transfused as often, thereby reducing the iron overload and toxicity it causes.

RBCs are particularly good candidates for stem cell therapy. A major consideration with regenerative medicine is how to deliver cellular therapies to the body and get the cells to stay where they are needed, rather than migrating away or being washed away in the bloodstream. Many researchers are investigating the use of artificial scaffolds to grow stem cells into the correct three-dimensional structure. This would allow these cells to replace, for example, parts of the heart or liver, but would still need to be transplanted

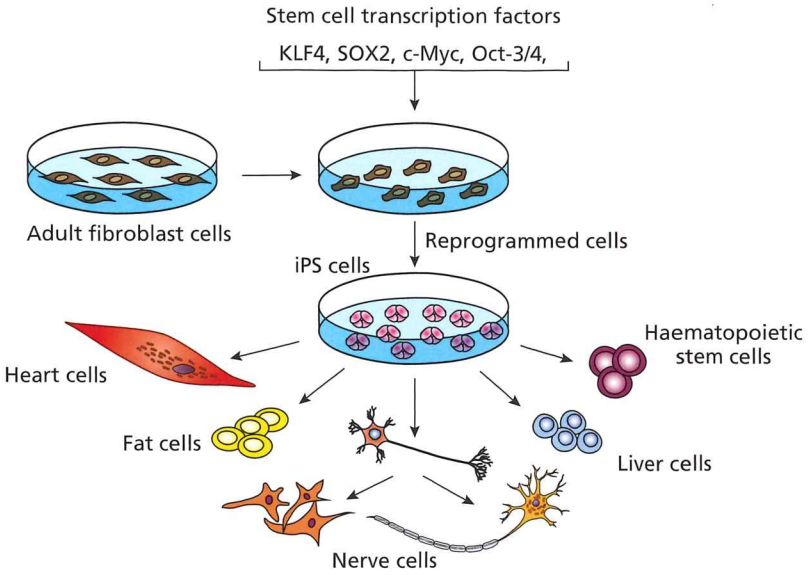


Figure 5 Reprogramming of fibroblasts to induced pluripotent stem cells (iPS cells)

and integrated with the recipient's own tissue. Using stem cell-derived RBCs is much more straightforward — RBCs do not have to form a three-dimensional structure, do not have to be delivered to a specific site and can be introduced into the body via a vein.

Cell therapies derived from hESCs or iPS cells must be completely free of any remaining pluripotent cells, as there is a risk that these could cause tumours if given to a patient while undifferentiated. As RBCs have no nucleus, the final product could be treated to ensure that any remaining pluripotent cells are destroyed and therefore unable to cause tumours. Additionally, as RBCs only have to be blood group matched rather than tissue matched, there is a much lower risk of rejection for a blood transfusion than for a heart or kidney transplant.

Successes and challenges

RBCs cultured from pluripotent stem cells are not artificial or synthetic — they are living cells which have become RBCs by going through the same stages as in the body. Differentiation in culture is achieved by treating pluripotent stem cells with mixtures of chemicals and proteins that instruct the cells to modify gene expression so that they gradually change from pluripotent stem cells into multipotent, haematopoietic stem cells and eventually into highly specialised RBCs (see Figure 6). The process takes around 30 days, with different mixes of

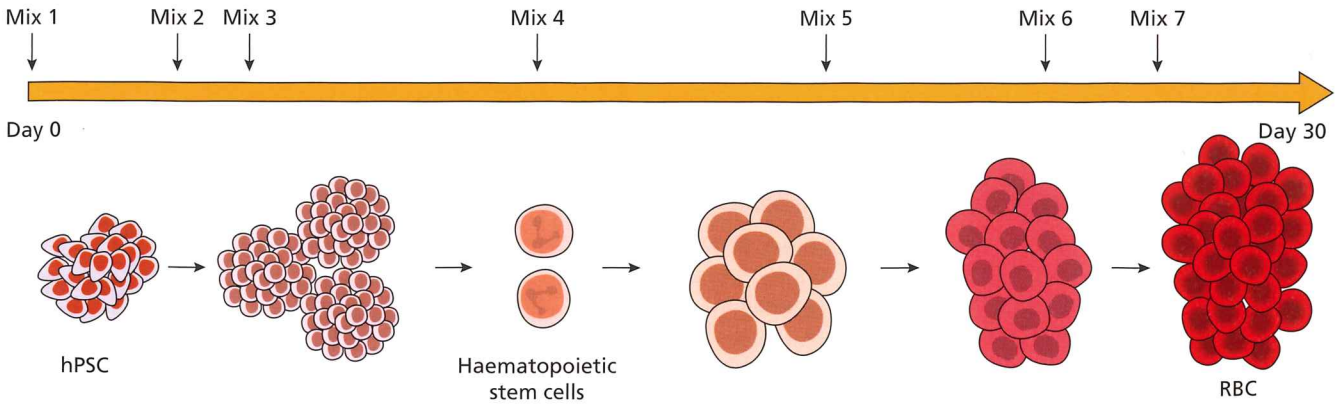
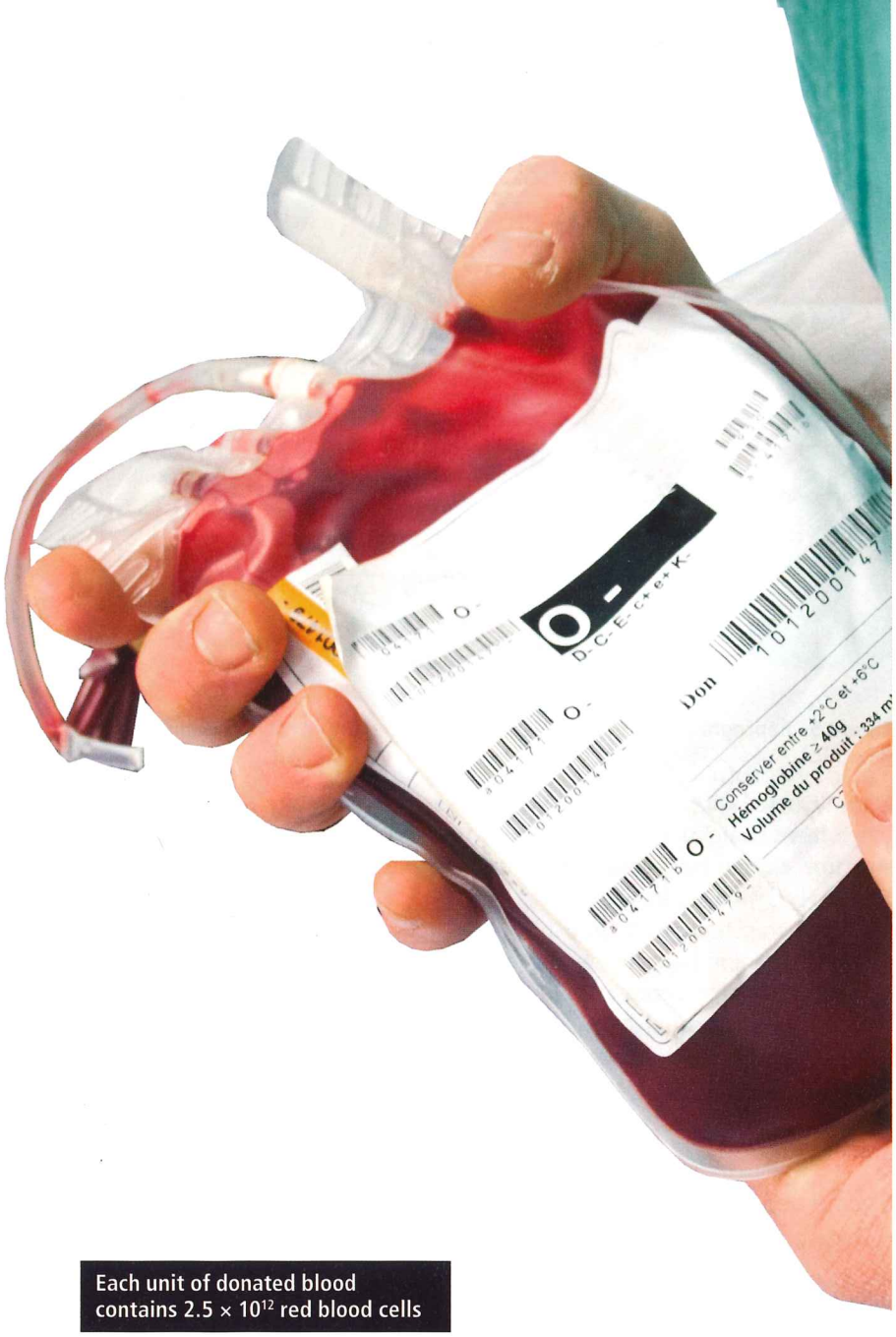


Figure 6 Differentiation of red blood cells (RBCs) from human pluripotent stem cells (hPSC) is a stepwise process. Each mix contains a different combination of media, chemicals and proteins



Each unit of donated blood contains 2.5×10^{12} red blood cells

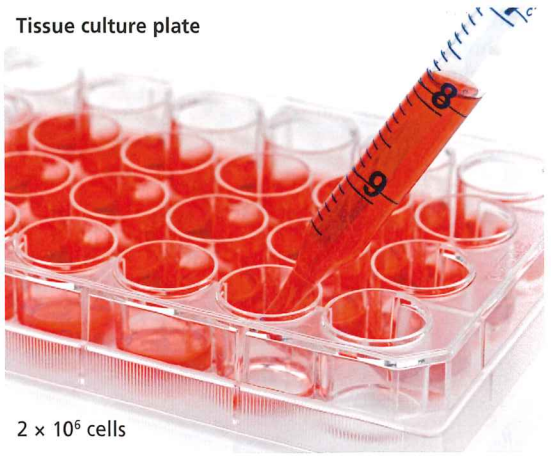


Figure 7 Scale-up of the differentiation of red blood cells from pluripotent stem cells

chemicals and proteins being added every few days. The cells multiply as they differentiate, so that one pluripotent stem cell can produce up to 100 000 RBCs. Mature RBCs normally have no nucleus because they expel it in a process called enucleation. The signals that instruct the cells to undergo enucleation are not well understood, so it is difficult to get cells to replicate this process in culture. As a result, many RBCs still have a nucleus at the end of the 30 day culture period and improving the enucleation rate remains a major challenge.

Box 1 Blood facts

- The average human body has 2.5×10^{13} RBCs. In comparison, there are about 1×10^{11} stars in the Milky Way.
- It would take 500 RBCs standing on their side to reach a height of 1 mm.
- Half the body's red blood cells are replaced every 7 days.
- The body produces 2 million new RBCs every second.
- 1 unit of donated blood contains about 2.5×10^{12} RBCs.
- 2 million units of blood are transfused every year in the UK and 100 million worldwide.

Terms explained

Allogeneic transplant A transplant using cells from a donor.

Autologous transplant A transplant using cells from the recipient's own body.

Bioprocessing The production of a biological material for commercial use.

Haematopoietic To do with the formation of blood or blood cells.

Novosang plans a first-in-human clinical study on cultured RBCs in 2017 to test their survival and recovery following transfusion. This could pave the way for the mass production of RBCs, so it is important that the production process is amenable to scale-up. In the laboratory, the differentiation of RBCs from hESCs was first performed in small tissue culture plates to produce a few million cells but has since been scaled up to produce around 10^{10} RBCs. One unit of donated blood contains 2.5×10^{12} RBCs, so a further scale-up to large industrial culture vessels — bioreactors — will be required to produce RBCs in the enormous numbers required (see Figure 7).

The process will have to be performed at a clinical standard so that the blood produced is safe to be given to recipients. These are huge challenges but they are currently being addressed by experts in **bioprocessing**, cell manufacturing and clinical regulation. With this multi-disciplinary approach, Novosang hopes that RBCs cultured from stem cells will one day help to meet the worldwide demand for transfusable blood.

Dr Angela McCahill is a research associate in the Stem Cell Biology Group at the University of Glasgow. She is interested in exploiting the properties of stem cells to make new medicines and treatments for human ailments.

Further reading

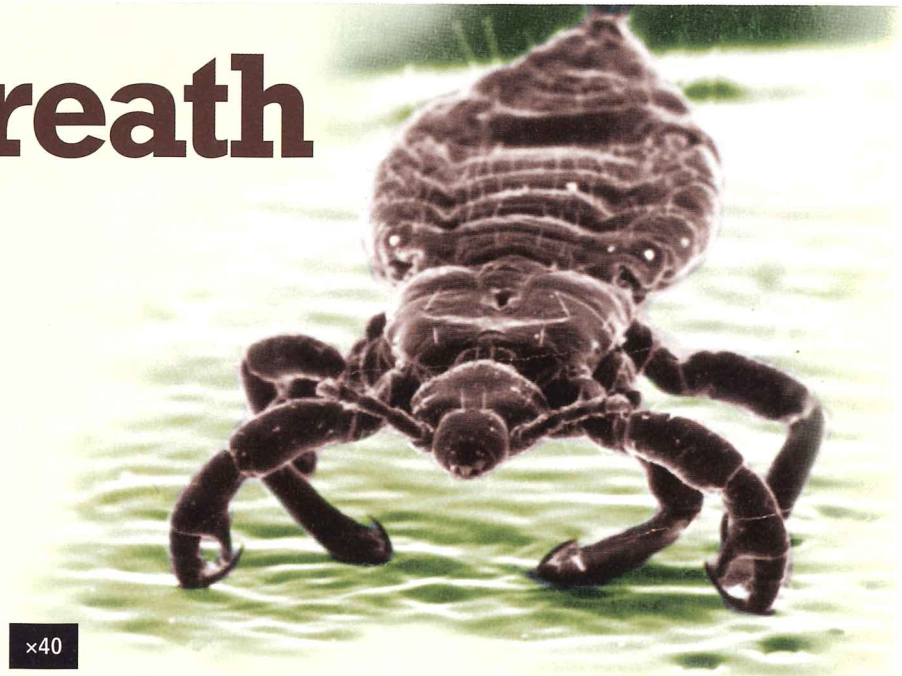
Find out more about creating red blood cells from stem cells: **www.novosang.co.uk**

For more about stem cells and their impact on society: **www.eurostemcell.org**

Key points

- Stem cells are unspecialised cells which can self-renew indefinitely and can differentiate into specialised cells.
- Adult stem cells can be unipotent or multipotent.
- Human embryonic stem cells and induced pluripotent stem cells are pluripotent.
- Stem cells are useful tools for research and development of regenerative medicines.
- Pluripotent stem cells are being used to make red blood cells for future blood transfusions.

Insect breath



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Like us, insects take in and expel air via internal tubes — tracheae. Instead of one, however, insects have many openings along their bodies, often referred to as spiracles. These structures are frequently depicted as simple openings to the outside, and gas exchange is described in terms of insects making pumping movements with their thorax. 'When the body expands, air is sucked in through the spiracles and into the tracheae' (from an A-level textbook) is certainly true, but the openings are considerably more impressive than simple holes. Each has a mechanism that gives the insect muscular control over its opening and closing (see Figure 1). Dust is filtered out of the air on its way in towards the valve, but the valve can be closed — sealing the insect from the surrounding atmosphere. Insects can do this if they detect harmful molecules (e.g. insecticides aimed at ridding humans of head lice, such as the one shown here), and also to prevent dehydration. In dry environments, the loss of water vapour that necessarily happens when animals breathe out can be avoided if the spiracles are closed.

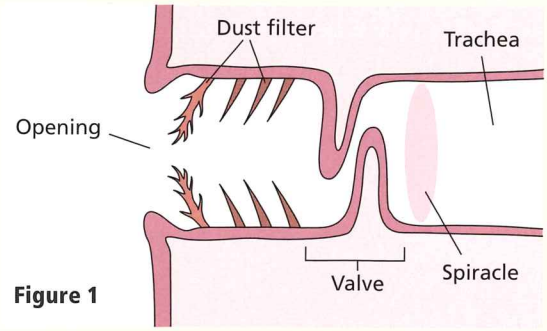

Figure 1

The image of the head louse is a scanning electron micrograph. I placed a live head louse in the freezer for a few minutes to immobilise it, covered a stub (which is like the head of a drawing pin) with sticky tape, and placed the insect on the surface. The specimen was coated with a very thin layer of gold and put into a scanning electron microscope, which sends a focused beam of electrons across the specimen. As the beam hits the gold atoms, electrons are emitted and collected to form the image. All this happens in a vacuum. A few minutes after I removed the stub from the microscope, the louse calmly walked off the stub. So if you see someone with gold head lice, you have evidence of the power of insect spiracles!

Liz Sheffield