An Introduction to Stem Cells

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This booklet aims to distinguish the hope from the hype, and to provide an introduction to the science, ethics, regulation and future applications of stem cell research. In particular, it focuses on progress in the development of stem cell therapies to treat Type 1 diabetes and Parkinson's disease.

The Galton Institute

Progress Educational Trust

October 2009

Galton Institute

Occasional Papers

Third Series, No.2

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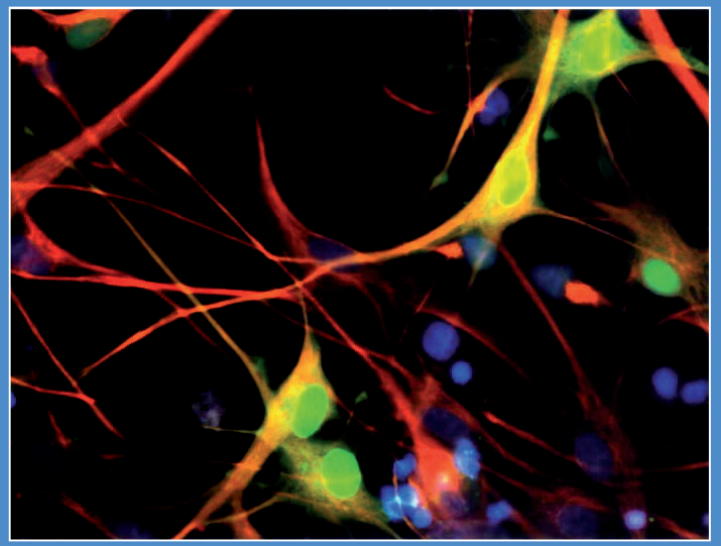
By Jess Buxton



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Published October 2009 by
The Galton Institute
19 Northfields Prospect
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In association with
Progress Educational Trust
140 Gray's Inn Road
London WC1X 8AX
www.progress.org.uk

Front cover image
Spinal cord cells grown in the laboratory, derived from human embryonic stem (ES) cells. *(provided by Dr Stephen Minger, King's College London)*

Print
CM Print
Website: www.cmprint.co.uk

Photographic credits
EuroStemCell

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Thanks to Stephen Minger, Fred Kavalier and John Galloway for reviewing drafts and expert comment, and to Sophie Pryor for providing diagrams.

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Executive summary

Stem cells hold much promise for the development of novel treatments for many serious medical conditions. However, several technical challenges must be overcome before new therapies based on stem cells become a reality. Stem cells can be obtained from a variety of sources: adult tissues, embryos, umbilical cord blood and via cell 'reprogramming'. It is not yet known which types of stem cell will be most useful for treating particular diseases, and most scientists agree that all avenues of research need to be explored. Research into stem cells obtained from early human embryos has triggered controversy, which in some countries has led to legislation either banning or restricting such work. Despite the practical and ethical difficulties that surround research into new stem cell therapies, the first clinical trials to test the safety of this approach are starting to emerge.

Introduction

Stem cells are the body's 'master cells', responsible for forming and replenishing all tissues and organs throughout life, from the newly fertilised egg through to old age. Scientists hope that by finding out how stem cells work in the body, and mimicking these processes in the laboratory, it will be possible to develop new treatments for a host of currently incurable diseases. Such cell lines could also be used to test out new drugs or other treatments, or to investigate the effect of potential toxins.

Research into stem cell therapies is part of the burgeoning field of regenerative medicine: the search for new treatments designed to permanently replace diseased or lost body parts and tissues. The astonishing potential of regenerative medicine was highlighted at the end of 2008, when scientists announced the successful transplantation of a tissue-engineered windpipe, created using the patient's own stem cells. In addition to providing replacement organs, stem cell therapies could eventually transform the treatment of conditions such as severe burns, spinal injury, some types of blindness, Parkinson's disease and Type 1 diabetes. It may even be possible to treat some forms of infertility, using egg and sperm-like cells derived from stem cells.

One type of stem cell therapy has been successfully used since the 1950s: bone marrow transplants, which can be used to treat a range of diseases that affect the blood. This is because bone marrow contains the stem cells that produce all the different types of white and red blood cells. In recent years scientists have discovered stem cells in several other adult tissues, which they hope can be used to treat certain other disorders.

But the ultimate goal of stem cell research is to develop cell therapies to replace *any* type of tissue. For this, scientists need to study stem cells that have the potential to produce any cell type, such as those present in an embryo just a few days after fertilisation. Scientists discovered how to get stem cells out of mouse embryos and grow them successfully in the laboratory over twenty years ago. Work on mouse embryos taught scientists much of what is known about embryo development, and the properties of embryonic stem (ES) cells. In 1998, researchers reported the first successful isolation of *human* ES cells, a discovery that heralded the start of research into new disease treatments based on stem cells grown in the laboratory.

In 2007, scientists showed it was actually possible to 'reprogramme' ordinary body cells in the laboratory to a **'pluripotent'** state so that they resemble ES cells derived from early human embryos. These cells, called **induced pluripotent stem (iPS)** cells, seem to be very similar to human ES cells, both in terms of cell behaviour and their ability to develop into a wide range of cell types. Therapies based on this technology would involve reprogramming the patient's own cells, so they would not be rejected by the body's immune system. However, more work needs to be done to understand how the reprogramming process affects the cells, and to establish whether iPS cells are as versatile as ES cells.

So despite the excitement surrounding stem cells, this area of medicine is still in its infancy. This booklet aims to distinguish the hope from the hype, and to provide an introduction to the science, ethics, regulation and future applications of stem cell research. In particular, it focuses on progress in the development of stem cell therapies to treat Type 1 diabetes and Parkinson's disease.

Stem cell science

What are stem cells?

Cells are the building blocks of all living things. Stem cells have two unique features that distinguish them from other body cells: they are capable of producing a range of more specialised types of cell, and they can also continually renew themselves to produce more stem cells.

These two properties are the reason for the excitement over the potential use of stem cells in medicine. If scientists can grow large quantities of stem cells in the laboratory, and then direct them to form specialised cell types, eg, muscle or heart, then they could use them to treat conditions in which such cells have been lost. But several important questions need to be answered first, such as discovering exactly which chemical signals trigger stem cells to produce particular cells. To translate these findings into safe, effective new therapies, scientists must then work out how to replicate these processes reliably in the laboratory, without introducing potentially harmful changes into the cells.

Stem cells are found throughout the body, at all stages of development and in several different tissues. Stem cells in the developing human embryo give rise to all the different types of cells needed in the body, which form over 200 different types of tissue, for example, liver, blood and nerves. Throughout life, adult stem cells - properly known as **'somatic'** stem cells - are responsible for replenishing cells lost to wear and tear or disease.

Stem cells at different stages of development differ in their 'potency', that is, their potential to give rise to other types of cell. A newly fertilised egg is described as **totipotent**, because it has the potential to form an entire body. The stem cells present in very early embryos, called **embryonic stem (ES) cells**, are '**pluripotent**' – they cannot grow into a whole new body, but in the right environment they can grow into almost any of the body's cell types. Adult, or somatic stem cells, on the other hand, are '**multipotent**', as they give rise to a limited range of specialised cell types. For example, blood stem cells (haematopoietic stem cells, found in the bone marrow and umbilical cord blood), can make white and red blood cells, but not brain or muscle cells.

Recently, scientists discovered a way to make ordinary body cells pluripotent, by turning back the developmental clock so that they regain the ability to produce many different cells – much like returning a computer to its factory settings by removing all the software that has been loaded up. These **induced pluripotent stem (iPS) cells** seem to be very similar to ES cells, since they are capable of long-term self-renewal without becoming specialised. They can also be directed to form a wide range of specialised cell types, by treating them with particular chemical signals.

The following section looks at each of the different types of stem cell in more detail.

Embryonic stem (ES) cells

ES cells are derived from very early embryos, a few days after fertilisation, when they are just tiny clumps of cells invisible to the naked eye. Most of the human ES cells grown so far have been obtained from spare embryos donated by couples who have undergone *in vitro* fertilisation (IVF) treatment. To obtain ES cells, an IVF embryo is first grown in a laboratory dish for 3-5 days, by which time it has become a hollow ball called a **blastocyst**. Inside this ball is a cluster of around 30 cells, called the **inner cell mass**, which - if the embryo were returned to the womb and allowed to develop - would eventually produce all the different organs and tissues of the body (see **Figure 1**). To grow ES cells, the inner cell mass is carefully removed from the embryo, and placed into a plastic dish containing a cocktail of special nutrients and 'growth factors'. The embryo is destroyed in the process.

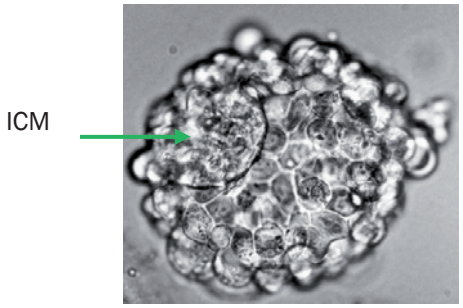

Fig. 1. Image of a human embryo at the blastocyst stage, showing inner cell mass (ICM). *Provided by EuroStemCell.*

The cells removed from the blastocyst will start to divide and multiply in the dish, eventually covering its surface, at which point they are gently removed and split into several more dishes. By repeating this process many times, after several months the original 30 cells will have produced millions more, potentially identical cells (see **Figure 2**). If the cells are all still unspecialised ES cells, capable of replenishing themselves, then at this point they are described as an **ES cell line**. As well as checking the physical appearance of the cells under a light microscope, there are other checks that scientists can do to verify that ES cells are still completely unspecialised – for example, measuring the activity of certain genes known to be switched on during very early embryo development.

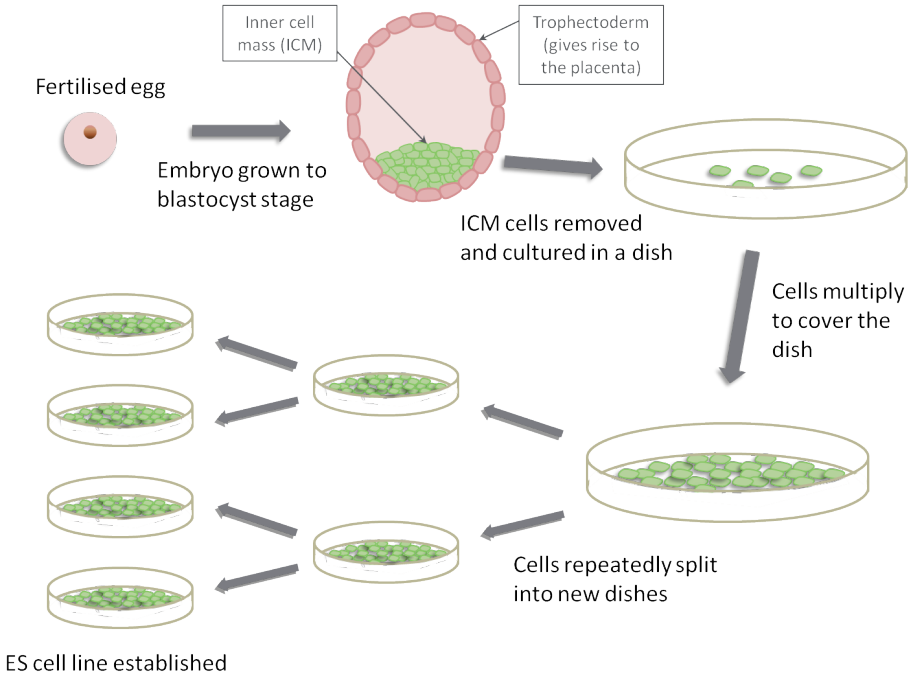

Fig. 2. Schematic diagram showing derivation of an embryonic stem (ES) cell line

Although it sounds straightforward, getting the growth conditions exactly right for obtaining and keeping human ES cells in the unspecialised, or **undifferentiated** state, has taken many years of experimentation. The success rate of the procedure is still low: despite technical improvements over the past decade, ES cells isolated from a human embryo only produce a stable ES cell line around 10% of the time. Once an ES cell line is established, further work is needed to coax the cells into producing a particular cell type, such as a heart or muscle cell. In the developing embryo, this process is controlled by the switching on and off of different **genes** in the cell, as well as chemical signals produced by neighbouring cells and other cues such as location. But in the laboratory, the process of getting ES cells to grow into a certain cell type is very much one of trial and error, and scientists have spent several years developing different methods to do so (see **Figure 3** for examples of cells derived from ES cells).

Many of the human ES cell lines produced so far have been grown on top of a 'feeder' layer of mouse-derived cells, which provides essential support and nutrients. These ES cells are invaluable for research, but will never be suitable for use in human treatments, since the risk of infection by animal viruses or rejection by the patient's immune system would be too great. Much effort has therefore gone into the creation of new 'clinical grade' human ES cell lines, grown under strictly controlled conditions and without using any animal products. It is estimated that over 400 different human ES cell lines have now been developed worldwide, many of which are suitable for developing new treatments. However, there are many challenges to overcome before ES cell therapies become a reality.

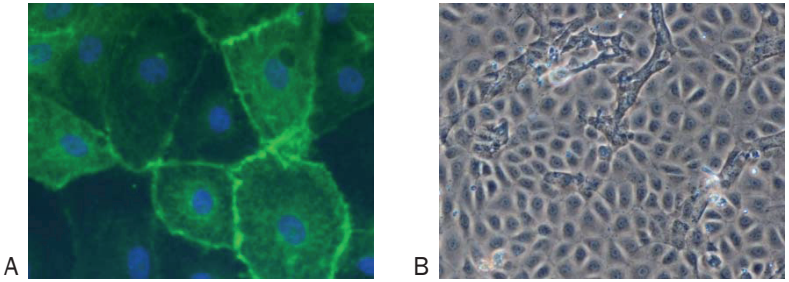

Fig. 3. Images of cells that form the lining of the lung (A) and nerves (B) grown in the laboratory using human ES cells. *Provided by Dr Stephen Minger, King's College London.*

Therapeutic cloning

One of the major hurdles facing scientists hoping to develop new stem cell therapies is overcoming the body's immune response, which would cause a patient to reject cell transplants from a genetically unrelated individual. Although this can potentially be overcome by taking lifelong immunosuppressive drugs, a better solution would be to develop genetically matched cell therapies. This is the aim of an area of research popularly known as '**therapeutic cloning'**, since it uses the same technique used to clone animals. The first cloning experiments were actually carried out over 50 years ago, by researchers aiming to understand the complex process of embryo development.

Scientists used to think that the specialisation of cells during embryo development was a one-way process: that is, a specialised body cell in a fetus, child or adult could never be returned to an unspecialised state. But this belief was overturned in a classic set of experiments designed to answer a different question: do the cells of an adult animal specialise by *losing* all their genes except the ones they need to carry out their specific function (eg. to be a liver or skin cell), or do they simply *switch off* the genes they don't need? One way of finding out is to remove the **nucleus** (the structure at the centre of a cell that contains nearly all of its genetic information) from a body cell, and place it into an egg cell that has had its own nucleus had been removed – a technique known as **somatic cell nuclear transfer (SCNT).** If the resulting cell can still develop into an embryo, then it must still have the complete set of genes required to grow a whole animal.

In 1952, US scientists Robert Briggs and Thomas King carried out the first successful SCNT experiments, using frogs. They took a single cell from a leopard frog embryo in the early stages of development, and removed its nucleus using a type of fine glass tube called a pipette. They then inserted it into a frog egg cell from which the nucleus had been removed (described as being **enucleated**). The resulting cells sometimes grew into whole new embryos and, in later experiments, tadpoles. These tadpoles were **clones** of the original embryo from which the cell nucleus was taken, as their genetic material was identical. But despite their success, Briggs and King were convinced that SCNT would only work using cell nuclei from early embryos, and not

cells taken from adult tissue, which are fully 'differentiated' - that is, they have committed to a being a particular type of cell.

In 1958, work by UK scientist John Gurdon proved Briggs and King wrong. Using a different species of frog, he managed to clone tadpoles using the nuclei of fully differentiated cells taken from the intestinal lining of adult frogs. Although none of the cloned tadpoles grew into frogs themselves, Gurdon's work showed that nuclei from fully specialised cells still contained all the genetic information necessary to make a complete new animal. Further experiments by Gurdon, in which he created adult cloned frogs using eggs with genetically 'tagged' nuclei taken from tadpole cells, proved conclusively that cell specialisation is a *reversible* process – genes stay the same, it's the gene 'switches' that vary in different cell types.

For a long time, it was thought that it would be impossible to repeat the frog cloning results using cells from adult mammals. But this belief too was eventually overturned, with the birth of Dolly, a cloned sheep, on 5 July 1996. Dolly was created using SCNT by scientists working at the Roslin Institute in Scotland. They took the nucleus from an udder cell of an adult sheep, and inserted it into an unfertilised sheep egg cell that had been enucleated. They applied an electrical shock to the new cell, and it began to divide and grow into an embryo, which they transferred into the womb of a ewe so it could grow into a lamb. The cloning process is very inefficient - Dolly's birth followed 276 failed attempts - but nevertheless scientists have since managed to clone many other species, including mice, rats, pigs, cats and dogs.

But what does cloning have to do with stem cell therapies? In 1998, two years after Dolly's birth, a US team of researchers at the University of Wisconsin announced that they had managed to grow the world's first human ES cells in the laboratory. Scientists were quick to realise that by combining cloning technology with human ES cells, it might be possible to develop cell therapies tailored to individual patients: so-called 'therapeutic cloning'. The idea would be to remove the genetic material from a patient's body cell and insert it into a hollowed-out unfertilised human egg, and then to start development in a lab dish. But rather than return the resulting cloned embryo to the womb, as in animal reproductive cloning, for therapeutic cloning it would be used to create a cloned ES cell line (see **Figure 4**). These cells could then be used to produce large quantities of more specialised cells such as nerve or muscle. If it works,

this approach could bypass the problem of immune rejection of stem cell transplants, since such cell-lines would be genetically identical to the patient.

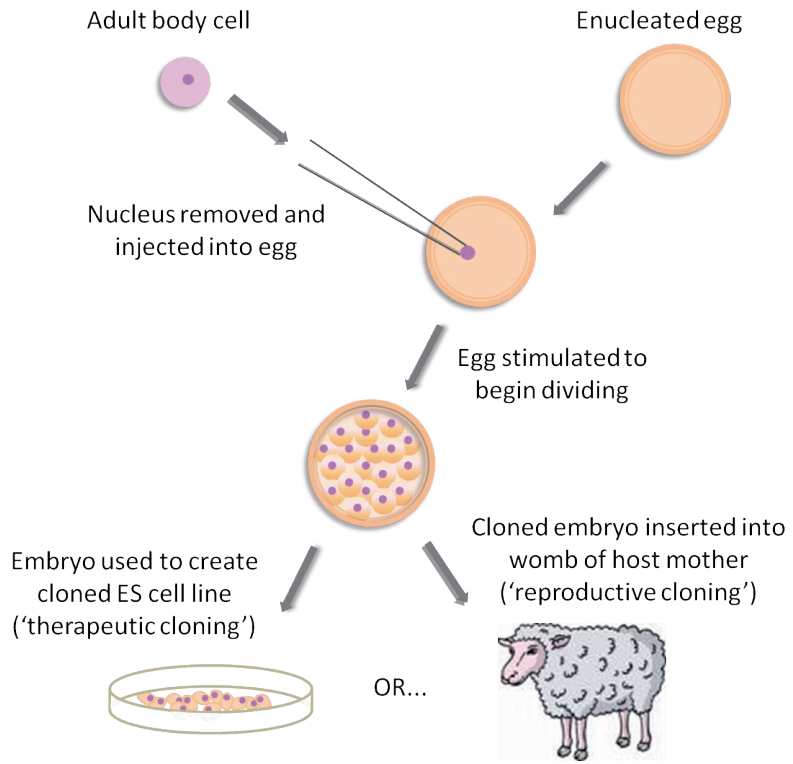

Fig. 4. Schematic diagram of somatic cell nuclear transfer, or 'cloning' technique

However, there are many practical problems associated with obtaining such cells for treatments. The SCNT technique central to therapeutic cloning research is technically very difficult, with a low success rate, and is also dependent on a plentiful supply of unfertilised healthy human eggs. Obtaining eggs from women's ovaries is an invasive, potentially risky procedure, and as such there is a shortage of donated eggs for fertility treatment, as well as for basic medical research.

In February 2004, it seemed that a team based at Seoul National University in South Korea had managed to overcome these difficulties, when they reported that they had obtained one cloned ES cell line from 30 cloned human embryos, after more than 200 tries. Just over a year later the same team, lead by Woo Suk Hwang, announced they had succeeded at therapeutic cloning, making 11 'tailor-made' cell-lines from individual patients. But following an investigation into unethical methods used to obtain the eggs for the research, the Korean team's findings were discovered to be fraudulent, causing a scandal that rocked the scientific community and sent shockwaves through the field of stem cell research.

At the time of writing, no-one has yet managed to produce a cloned human ES cell-line - although previous fears that the technique would never be successful in primates were allayed in 2007, when a US team managed to produce cloned ES cell-lines from rhesus monkeys. The main barrier to cloning human ES cells continues to be the shortage of human eggs available for research. Some stem cell researchers feel that asking women to donate eggs for cloning research is unreasonable until the efficiency of the process can be improved – at the moment, it is likely that hundreds of eggs would be needed to create just a few cloned ES cell lines. So another possibility is to use eggs from another mammal, for example cows or rabbits, to allow scientists to perfect the technique and develop cell lines for research (though not for treatment). This would involve placing the genetic material from a human body cell into an animal egg that had its own nucleus removed. So-called human 'admixed' embryos triggered much debate during the passage of the UK's new legislation last year covering fertility treatment and human embryo research (see the section on regulation for more details).

If scientists eventually succeed in their quest for cloned human ES cell lines, it may be that public banks of cloned ES cell lines - similar to blood banks – will offer a more realistic hope for cell therapies than individual, genetically-matched cells. It could even be that cloned ES cells will never be used therapeutically, but instead will be used in research aimed at shedding light on poorly-understood conditions such as motor neurone disease (MND). Another possibility is that cloned human ES cells will become unnecessary, at least in terms of developing new therapies, following the

recent discovery that adult body cells can simply be 'reprogrammed' so that they behave like ES cells.

Reprogrammed stem cells (iPS cells)

The discovery that an adult body cell can be returned to an embryonic state without the need to create an embryo was hailed as 'Breakthrough of the Year' by the US scientific publication *Science* in 2008. Such **induced pluripotent stem (iPS) cells** appear to hold all the promise of ES cells, but without the technical challenges and ethical controversy that surrounds human ES cell research. In 2006, Japanese researchers announced that they had managed to turn specialised cells taken from adult mice into pluripotent cells very similar to ES cells. Along with a US team, they achieved a similar feat in 2007, this time using human adult cells. The speed of progress in this area continues unabated, and in 2008 the same team unveiled the creation of 'patient-specific' cell lines that could be used to study disease processes in the laboratory. Other groups have showed that like ES cells, iPS cells can be coaxed into forming more specialised cells, such as brain and muscle.

Scientists first created iPS cells by inserting four genes that are very active in the developing embryo into an adult cell, using a virus. Although no-one is exactly sure how the process works, the effect of these genes on the adult cell is to 'turn back' its developmental clock, just as the cloning technique does. Several modifications have since been made to the original iPS cell method, with the aim of making the cells more suitable for use in therapies. For example, one of the genes used originally is known to be involved in triggering cancer, so new methods have substituted another gene that doesn't have this effect. Another group has developed a way of removing the key genes altogether after the cells have been reprogrammed. Scientists are also trying to develop new ways to deliver the genes to the cell, since the viruses originally used insert themselves into the cell's own genetic material – which again could potentially cause the cell to grow in an uncontrolled way, leading to tumours.

Whilst many scientists are very excited about the potential use of iPS cells in new cell therapies, some have also sounded a note of caution, expressing concerns over the rush to get iPS cells into the clinic before basic biological questions have been answered. For example, it is not known how the reprogramming process works, or

how similar iPS cells are to ES cells, and whether they will be capable of generating such a wide range of different tissues.

ES cells are still regarded as the 'gold standard' for understanding embryo development and how cells change when affected by disease. Indeed, without years of research on human and mouse ES cells, scientists would not have known which genes are active in the early embryo, and iPS cells would never have existed. Many stem cell researchers have therefore stressed that continuing with ES cell research is essential for understanding the reprogramming process and harnessing the therapeutic potential of iPS cells.

Adult stem cells

An 'adult' stem cell is any self-renewing cell that can also produce a limited range of more specialised cells, whether it is present in the fetus, child or adult. For this reason, a more accurate name for them is 'somatic stem cells', rather than adult stem cells. They are found throughout the body, where they are responsible for replenishing different groups of cells. For example, bone marrow contains two types of stem cell: haematopoietic cells, which make all the different types of blood cell, and stromal (sometimes also referred to as mesenchymal) stem cells, which form bone, cartilage, fat and connective tissue.

Doctors have been carrying out transplants using blood stem cells from bone marrow for many years, to treat blood disorders such as leukaemia, lymphoma, anaemia and immune deficiencies. A successful transplant depends on the availability of a bone marrow donor who is a close 'tissue-match' to the recipient, to minimise the chances that the new bone marrow cells will be rejected by the patient's immune system. Sometimes a close family member is able to donate bone marrow, otherwise a suitable matched, unrelated donor may be found via organisations that match blood stem cell donors and patients, such as the Anthony Nolan Trust. However, despite the estimated 11 million tissue-typed individuals currently registered on bone marrow donor registries, finding a full match for some patients remains a challenge. It is estimated that although matches for unrelated donors can be found for up to 75% of Western European patients, the figure for many other ethnic groups is closer to 20-30%.

An alternative to transplants using bone marrow stem cells is to use blood stem cells taken from the umbilical cords of newborn babies. However, cord blood does not yield as many stem cells as bone marrow, so is used more often for operations on children, rather than adults. Like those from bone marrow, cord blood stem cells can be used to treat conditions that affect the blood. An advantage of using cord blood cells is that they are more tolerant of tissue-type mismatches, since they are immature cells that don't have the same ability to trigger rejection by the patient's immune system. This means that samples stored in public cord blood banks can potentially be used treat a wider pool of patients than those available through bone marrow registries. At the time of writing, donated cord blood samples are stored in an international network of 36 public banks in 23 countries.

In recent years, a number of private cord blood banks have also sprung up, which charge parents a few hundred pounds to store cord blood from their newborn babies, for possible future use solely by their own child. Critics say that such companies play on parents' fears, as they offer future speculative stem cell treatments for many conditions not currently treatable with cord blood. In 2006, the UK's Royal College of Obstetricians and Gynaecologists (RCOG) said that there was little evidence to recommend private cord blood collection for low-risk families, and it called for increased funding into the NHS public cord blood bank. Also, even if a child does fall ill with a condition such as leukaemia, which may be treated with their own stored cord blood, the disease may already have been present from birth. This means that using the patient's own blood cells carries a risk of the disease recurring, even if the transplant is initially successful. For such children, there may be more chance of a long-term cure using donated bone marrow or cord blood cells from a healthy, matched donor.

As well as the blood stem cells found in bone marrow and cord blood, adult stem cells are thought to be present in some other (but not all) tissues of the body. However, their numbers are small, so getting hold of them is technically challenging. And even once they are sitting in a laboratory dish, adult stem cells are difficult to grow in the large numbers that would be required for therapy. As with stem cells from embryos, scientists are trying to find ways to coax adult stem cells into producing specific tissues. One startling discovery made in recent years is that some adult stem cells

may be able to produce specialised cells of other tissues. There have been reports, for example, of blood stem cells growing into brain, muscle and liver cells. It's not yet clear whether this phenomenon - called **'transdifferentiation'** or plasticity - is something adult stem cells do naturally in the body, or if it only happens under certain laboratory conditions. In fact, recent studies suggest that in most cases, the blood stem cells are fusing with existing cells in other tissues, rather than transforming into completely different cell types.

Some promising early results have been claimed from giving patients with heart failure injections of their own stem cells into heart muscle, but further trials are needed before this becomes a proven therapy. In this case, it seems that the beneficial effects are down to the injected blood stem cells promoting the growth of a new blood supply to the damaged heart.

Stem cell medicine

The promise of stem cells

Whether from embryos, cord blood, adult tissues or reprogrammed cells, before stem cells can be used in new therapies, scientists need to find out how to grow them in large numbers in the lab and how to reliably direct their growth into the specific type of cell required. They then need to find ways of making sure they survive in a patient after transplantation, and do the job they are supposed to do. Most importantly, they need to be sure that stem cell therapies will not have any serious side effects, such as cancer or infection.

If these major technical challenges can be overcome, there is huge potential for stem cells to transform modern medicine. Injuries or conditions in which cells and/or tissues have been lost or rendered useless could be routinely treated with a matched cell transplant. For example, paralysis caused by spinal injuries could be treated with a transplant of nerve cells grown from stem cells in the laboratory. Once in the patient's body, the new cells could forge new nerve connections, restoring movement and other lost body functions. Scientists have already succeeded in reversing paralysis in rats and mice using stem cell injections, and the first clinical trial testing a stem cell treatment in people paralysed from the chest down was given the go-ahead in the US in early 2009. This will be tested on patients who have very recent spinal cord damage – one or two weeks after injury – and will probably not be suitable for people who have been paralysed for longer.

In the UK, researchers are currently seeking approval for a new trial to test a stem cell therapy for age-related macular degeneration (AMD), the most common cause of adult blindness. Both this and the spinal injury trial will use cells grown using embryonic stem cells, but several trials using adult stem cells are also underway, including several testing the treatment of heart disease using the patient's own cardiac stem cells.

Hopes are also high for new treatments in which entire organs are replaced by new ones grown in the laboratory. This approach uses a protein 'scaffold' in the shape of the organ upon which the patient's own cells are grown, before the entire new organ is returned to the body. Seven US patients with a serious bladder disease have already been successfully treated with replacement bladders grown in this way, and in a blaze of publicity, a 30 year-old Spanish woman received a new lab-grown windpipe after her own was destroyed by tuberculosis. Other organs such as the heart and liver will pose more of a challenge, since they require a complex network of different cell types, blood vessels and nerves to work properly. Nonetheless, US scientists took another step towards providing lab-grown hearts for patients awaiting transplants in 2008, when they unveiled a beating rat heart grown in the laboratory.

The challenges of developing stem cell treatments: Type 1 diabetes and Parkinson's disease

Two conditions for which research into stem cell therapies offers hope in the near future are Parkinson's disease and Type 1 diabetes. Both are caused by the loss of one particular kind of specialised cell, so in theory, they present less of a challenge than diseases which affect several different tissues or types of cell. However, in practice, many technical difficulties remain to be overcome before stem cell treatments become a reality for people affected by these conditions. Many of the issues faced by researchers working on new cell therapies for these diseases are shared by those aiming to develop treatments for other conditions.

Type 1 diabetes is caused by the complete loss of cells that produce **insulin**, the hormone that enables the body to use energy from food, and so controls blood sugar levels. As a result, people with this form of diabetes require several daily injections of insulin to survive. The disease usually appears in childhood, and in later life patients are at risk of secondary complications of diabetes such as kidney disease, blindness and nerve damage. The cells that make insulin are found in specialised structures in the pancreas called the islets of Langerhans (see **Figure 5**), which consist of insulin-producing beta cells and other types of cells, which all work together to regulate blood sugar levels. Doctors have had some success in treating Type 1 diabetes by transplanting whole islets from a donated pancreas. However, the islets from at least two donor organs are needed to treat just one patient, and even if the transplant is successful, patients need lifelong treatment with immunosuppressive drugs to

prevent rejection of the transplanted cells. So, much research is currently focussed on developing new cell-based transplant therapies for this condition.

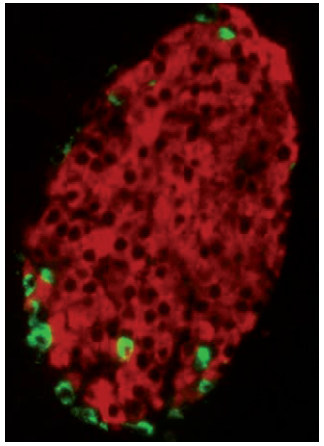

Fig. 5. An islet of Langerhans from a mouse pancreas. The insulin-producing beta cells are coloured red. *Provided by Chen Li, Professor Peter Jones and Professor Shanta Persaud, King's College London*.

To develop a stem cell therapy for Type 1 diabetes, scientists must first find a way of growing insulin-producing cells in the laboratory, and then ensuring they survive and continue to make insulin when injected into the patient's body. Whether they use stem cells from embryos or reprogrammed iPS cells, the main challenge remains the same. In the developing embryo, stem cells give rise to insulin-producing cells by responding to a complex cascade of chemical signals, passing through several intermediate cell types along the way. So to grow cells that make insulin in the laboratory, scientists must devise a way of mimicking this process, making sure the cells get the right signals at the right time.

Researchers have so far tried two different approaches to making insulin-producing cells from ES cells. One method starts with **embryoid bodies** - ES cells grown as a 'ball', rather than flat on the surface of a laboratory dish - which can potentially form all the tissues of the body. However, it is difficult to get a pure population of insulin-producing cells when starting with embryoid bodies, since by their nature they are more likely to give a mixture of different cells. Not surprisingly, animal experiments have shown that cells obtained in this way are prone to forming **teratomas**: benign tumours made up of several different types of tissue. A more promising approach may be to start with just one of the three main layers of cells found in the early

embryo, called the **endoderm**, thus cutting down the number of different cell types that could arise. Human insulin-producing cells made in this way have already been successfully used to treat mice with Type 1 diabetes. Scientists have also shown that this method can be used to grow islet-like clusters of cells using reprogrammed skin cells, rather than ES cells.

But getting stem cells to grow into cells that make insulin is just the first step in developing new cell therapies for diabetes. Despite the successes described above, current methods are too expensive, time-consuming and labour-intensive for routine medical use. And they are inefficient, too, with usually just a fraction of the stem cells eventually growing into insulin-producing cells. One way around this is to develop 'pancreas-like' cell-lines that naturally produce some of the chemical signals needed for making cells that produce insulin. Some progress has already been made in this area. There is also a need for better ways of separating insulin-producing cells from a mixture of cell types growing together – unlike, for example, heart cells that can be seen 'beating' in a dish, cells that make insulin are not so easy to identify. Much more research is also needed into the other types of cell found in islets, and how they work together with the insulin-producing beta cells. Finally, scientists need to find a way of making sure transplanted cells can be adequately connected up to the body's blood supply, to ensure their long-term survival in the patient.

What about progress in developing new stem cell therapies for Parkinson's disease? This condition is cause by a loss of nerve cells, or **neurons**, in a part of the brain known as the **substantia nigra**. These cells are responsible for producing a chemical known as dopamine, which allows messages to be sent to the parts of the brain that co-ordinate movement. As these cells are progressively lost, people with Parkinson's disease gradually lose their ability to control their muscles. Scientists hope that by replacing the lost dopamine-producing cells with new ones, they may be able to treat the symptoms of this currently incurable disease. Such an approach is far more likely to be successful in Parkinson's disease - where one particular type of a cell in a small area of the brain is affected - than in conditions such as Alzheimer's disease, in which large numbers of neurons in numerous areas of the brain are eventually affected.

But like the quest for insulin-producing cells to treat diabetes, obtaining enough dopamine-producing cells to treat Parkinson's disease is not straightforward. Trials

using transplants of nerve cells from aborted fetuses have shown mixed results, and are not likely to become a routine treatment due to the large number of fetuses that are required to obtain any clinical benefit. So scientists hope that stem cells - either from embryos or reprogrammed iPS cells - will provide an alternative source of specialised nerve cells. Like other potential stem cell therapies, the major challenge currently facing researchers is to get enough of the specific cells required, without contamination from other cell types. In the case of Parkinson's disease, the cells of interest are nerve cells that can make a substance called **tyrosine hydroxylase (TH)**, which is necessary for making dopamine.

Several groups of scientists have already managed to coax human ES cells to grow into TH-producing cells by simply growing them on a 'feeder' layer of supporting cells. However, the feeder cells used are often obtained from mice, so cells grown in this way will never be suitable for use in humans, due to the risk of infection with animal viruses. Work is ongoing to identify exactly which chemical signals produced by the feeder cells are making the stem cells develop into TH-producing cells. This would mean that the chemicals required could be added directly to the stem cells, cutting out the need for feeder cells altogether. Another approach is to genetically alter the stem cells so that they are more likely to develop into TH-producing cells, but again, this raises safety concerns when treating patients. Currently the most efficient method for getting TH-producing nerve cells from embryo stem cells involves both a feeder layer *and* genetic alteration, so much work remains to be done to develop cells that can be used in clinical trials.

Nevertheless, the TH-producing cells obtained so far have already been used in animal experiments to see if they can alleviate the symptoms of Parkinson's disease. Researchers have had some good results using cell transplants to treat mice, rats and monkeys with the disease, using cells obtained from either mouse ES or reprogrammed iPS cells. However, they have had less success using transplants of human cells to treat these animals. A key factor in the success of stem cell transplants is the timing of when the cells are 'harvested' from the laboratory dish. Too soon, and not enough of the cells will be of the required type. Too late, and many will die after transplantation, or will stop producing TH. As with cell therapies for Type 1 diabetes, researchers also need to ensure that no unwanted cells are transplanted along with

the TH-producing cells. Further work will also be needed to find out how to get transplanted cells to integrate themselves into the patient's brain, to ensure their long-term survival.

In summary, despite encouraging progress, it is likely to be several years before stem cell therapies become a reality for either Type 1 diabetes or Parkinson's disease. Major challenges facing researchers working on both conditions include developing cost effective, reliable and safe methods for growing the cells required in large enough quantities; ensuring that transplanted cells survive in the patient; and minimising the risk of serious side effects such as tumours. The issue of transplant rejection by the patient's immune system will also need to be addressed, either by using immunosuppressive drugs, or perhaps by developing tissue-matched cell therapies tailored to individual patients.

Ethics and regulation of stem cell research

The moral debate over ES cell research

The ethical issues surrounding stem cell research concern the use of human embryonic stem (ES) cells, which involves the destruction of the embryos from which they are obtained. From a scientific point of view, work on human ES cells is essential to fully understand the processes involved in embryo development and cell specialisation. From a medical perspective, despite the recent advances made in cell reprogramming, it is not yet clear whether reprogrammed adult cells will have the same therapeutic potential as stem cells obtained from embryos. In addition, there is a need for further research on human embryos to find out the best way to grow them in the laboratory, both for stem cell research and for improving the success rates of fertility treatments that involve IVF (in vitro fertilisation).

Such work is controversial because of different views about the moral status of the human embryo. For those who believe that life begins at conception, destroying a human embryo - even a five-day old blastocyst created in a laboratory - is wrong. Others feel that while an embryo has the *potential* for life, it is not sentient, and so cannot be regarded as person. They argue that it would be more unethical to block research that could potentially prevent human suffering. So the moral debate over

human ES cell research can be summed up by the question 'does the means, i.e. using human embryos in research, justify the end, i.e. potential new cures for diseases?'

Religious views about when life begins have influenced this debate in many countries, sometimes in unexpected ways. For example, the official teaching of the Roman Catholic Church is unequivocal in its opposition to any research on human embryos. However, there are other interpretations of the Catholic tradition which hold that carrying out research on embryos that will never enter a woman's womb is justifiable. In this argument, it is deemed acceptable to use embryos left over after IVF treatment for medical research, since they would otherwise be destroyed.

Regulation of ES cell research

Differing attitudes towards the use of human embryos have led to a variety of legislative approaches to ES cell research worldwide. Some countries have very restrictive laws that either ban human ES cell research altogether, or permit only limited work, for example on imported ES cell lines. Countries with a restrictive approach include Austria, Germany, Ireland, Italy and Poland. Another type of approach permits ES cell research on IVF embryos donated by couples who have undergone fertility treatment, but bans the creation of embryos specifically for research. Brazil, Canada, France, Iran, South Africa and others have laws that fall into this category. Finally, countries including Australia, Belgium, China, India, Israel, Japan, Singapore, South Korea, Sweden and the UK have a permissive approach to human ES cell research. They allow the creation of human ES cells using a variety of methods, including SCNT (the technique used to create 'cloned' embryos), subject to licensing and regulation.

In the UK, stem cell research benefited from the regulations in place which already permitted the use of human embryos for research into infertility and contraception, provided they are destroyed before they reach 14 days old. Such research is licensed by the Human Fertilisation and Embryology Authority (HFEA), which was given statutory backing in 1990 with the passing into law of the Human Fertilisation and Embryology (HFE) Act. The *Human Fertilisation and Embryology (Research Purposes) Regulations 2001* widened the permitted experimental uses of human embryos

beyond those originally specified, in order to permit research into human ES cells and exploring their therapeutic potential.

In 2008, a new HFE Act passed into law, following a complete overhaul of the legislation governing infertility treatment and human embryo research in the UK. It was felt that new scientific and ethical developments in these areas meant the HFEA was being asked to issue licenses for treatments and research projects that were not covered by the original 1990 Act. In terms of ES cell research, this included research using animal eggs to overcome the shortage of human eggs available for SCNT experiments. This would involve the creation of a type of **'admixed'** human embryo, in which the **nucleus** from the egg of a cow or rabbit is removed, and replaced with that of a human cell. When allowed to develop in a laboratory dish, the resulting embryo can then be used to generate cloned human ES cells. However, they would still contain a tiny amount of animal DNA in the **mitochondria**, structures found in every cell that make energy, which have their own genetic material. The admixed embryo issue was hotly debated during the passage of the Bill through Parliament, with strong opposition led by the Catholic Church. But following a concerted effort by patient support groups, scientists and other organisations to engage with politicians and the public, the Bill was eventually passed with the provision for licensed research on human admixed embryos left intact.

The successful passage of the HFE Act 2008 illustrates the importance of involving the scientific community in political and media debates on controversial areas of new science. Scientists need to engage with both policy makers and the public to explain their work, their reasons for taking a particular approach, and the potential benefits that might flow from the research. A recent project carried out by two UK research councils sought to engage with the public specifically on the topic of stem cells, in order that their views could be taken into account by scientists and policy makers. The ensuing report, entitled 'Stem cell dialogue', found that there was widespread public support for stem cell research and therapies, though ethical and social concerns were raised. One of the recommendations was that future dialogue between scientists and the public over stem cells should focus on the practice of research within institutions. It also stressed the need to communicate the uncertainties in stem cell science, to avoid public debate being dominated by hype.

In the US, the regulatory environment for ES cell research has been very different to that in the UK. Government funding of research on human embryos is not permitted in the US, and this ban was extended to human ES cell research by former President George Bush, although he later permitted federally-funded stem cell scientists to work on a limited number of ES cell-lines already in existence in 2001. There was strong opposition to this restriction by many scientists and patient groups, backed by celebrity campaigners who stood to benefit from the research. They included the now deceased Superman actor Christopher Reeve, who was paralysed following a riding accident; Nancy Reagan, whose husband Ronald died of Alzheimer's disease in 2004; and star of the 'Back to the Future' films Michael J Fox, who has Parkinson's disease.

The efforts of the pro-ES cell research lobby eventually led to a bill that would have lifted restrictions on federally-funded research, allowing scientists to derive new cell-lines from spare IVF embryos. Although two different versions of this bill were passed by the House of Representatives and the Senate, on both occasions Bush exercised his presidential right to veto any bill passed by Congress. In the meantime, a number of US states – notably California – passed legislation allowing *state* funds to be used in ES cell research, including the creation of new cell lines. Work on ES cells also continued in the private sector in the US.

President Bush's ban came to an end in 9 March 2009, when newly elected US President Barack Obama signed an executive order legalising the use of federal funding for research into human ES cell lines, accompanied by legislative backing from Congress and new guidelines from the National Institutes for Health (NIH). There have been calls for the new NIH guidelines to be revised, to address donor consent issues that may otherwise prevent scientists from working on ES cell-lines obtained before the ban came into place. But overall, the US policy change was welcomed by stem cell scientists both in the US and elsewhere. It is hoped the increased funding will boost global collaboration in the field of stem cell research, and so hasten the speed of new discoveries.

Looking forward

In summary, there is much to do before stem cell therapies move from bench to bedside. In addition to the many technical challenges, much investment is needed to ensure research advances can be effectively translated into new medical treatments. There is a continued need for supportive legislation, to allow scientists to pursue all ethical avenues of inquiry. Guidelines setting out standard procedures for developing and using new stem therapies will also be needed, to ensure that clinical trials around the world are carried out safely and reproducibly. But if all these obstacles can be overcome, stem cell therapies have the potential to transform medicine, perhaps in just a decade or two.

Appendix

Cells and embryo development

Everybody starts life as a single cell: a fertilised egg that got half its genetic information from the father's sperm, and half from the mother's egg. This divides into two cells, then four, then eight – and so on – eventually forming a body made up of around 100 million, million cells, which all communicate and cooperate with each other to make different tissues and organs. The fate of a particular cell in the developing embryo - whether it becomes a nerve, liver or blood cell - depends on its location, the instructions it receives from other cells and those it receives from its own genes. Although every cell (except egg and sperm cells) has a complete copy of the embryo's genetic information, each cell only uses the particular *set* of genes it needs to do its job. So the set of genes that are active in a nerve cell will partly differ from that in a liver or blood cell, for example. Different genes are 'switched on' at different stages of development, to guide the formation of particular tissues and organs.

Each cell in the growing embryo must end up in the right place, doing the right job. To do this, the cells have to multiply, move around and specialise. The embryonic stage of development lasts for nine weeks in humans, after which it is known as a fetus. During this time the basic body shape is laid down and all the major organs are established. How does a single cell, the fertilised egg, develop into a fetus made up of

around 200 different types of cell? By studying the development of animal embryos, scientists have identified the main stages of this process, outlined below. In some cases, the key genes involved in triggering the formation of a particular tissue or cell type are known, but much remains to be discovered. In particular, the events that take place in the very early human embryo are still shrouded in mystery. Below is an overview of what *is* known about the process. Since it is impossible to study early embryonic development in humans, much of this information has come from studying early mouse development.

In the first two days of life, the newly fertilised human egg travels along the mother's fallopian tube to the womb. On its way, it divides to make a clump of 32 cells – the **morula** stage. As the cells of the embryo continue to divide and multiply, they begin to specialise. As development proceeds, the cells become increasingly more specialised, until they end up as part of a particular body tissue (see **Figure 6**). By five days old, the embryo, now called a blastocyst, still consists of only a few hundred cells. Inside the blastocyst is a clump of undifferentiated **pluripotent** cells called the 'inner cell mass', which will eventually give rise to all the different organs and tissues of the body. It is these cells which, when removed from the **blastocyst** and grown in a laboratory dish, can give rise to human **embryonic stem** cells.

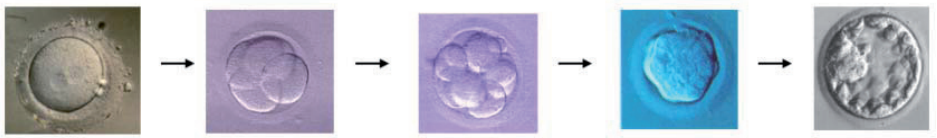

Fig 6. Stages of early human embryo development, shown with approximate timescale. Day 1 = newly fertilised egg; Day 2= four-cell embryo; Day 3 = eight-cell embryo; Day 4 = morula stage; Day 5 = blastocyst stage. *Provided by Professor Peter Braude, King's College London.*

The cells on the inside of the blastocyst will eventually grow into the embryo, and those on the outside will grow into the placenta. Within the next couple of days, the embryo burrows into the lining of the womb, a process known as implantation. The

cells closest to the placenta are destined to become the front of the body. The top and bottom ends of the embryo are also defined very early on. The other axis laid down in the early embryo is the left-right asymmetry of the body – although the human body looks roughly symmetrical from the outside, much of the inside is asymmetrical.

By 14 days the embryo looks like a flat disc, with a visible groove called the **primitive streak** running through the middle. The primitive streak marks the beginning of nervous system development and thus is important in bioethics and law, where some argue it marks the beginning of a unique, potential human. For this reason, research on human embryos in the laboratory is only permitted up to this stage, in countries where such work is allowed.

Scientists hope that identifying the events and gene triggers involved in early embryo development will enable them to find ways of coaxing ES cells in the laboratory to develop into particular tissues. In the third week of human development, three different layers of cells form in the embryo, known as the **ectoderm, endoderm and mesoderm**. Between them, these cells will develop into all of the different tissues of the embryo. The outer layer (ectoderm) grows into skin, brain and nerves. The middle layer (mesoderm) grows into muscle, the heart and lungs, blood vessels, bones and many of the organs. The inner layer (endoderm) grows into the gut, stomach, pancreas and the liver.

To shape the body, a structure called the **neural plate** forms on top of the embryo, which folds up to form the **neural tube**. This will become the brain and spinal cord. The early embryo is then divided up into blocks called **somites**, which will later form the body and limb muscles, ribs and backbone. Which tissues are made in which somite is dependent on its position in the embryo. Limbs grow between the fourth and eighth weeks of development, starting off as tiny bumps called limb buds. Organs also form during this time, as layers of unspecialised cells are coaxed down different developmental pathways. By nine weeks after fertilisation, the process of cellular differentiation is mostly finished and the fetus is on course to becoming a fully formed human being.

Further Resources

The field of stem cell research is advancing at an astonishing speed, with new findings being announced on a weekly basis. For this reason, the best place to get up-to-date information on the latest developments in the science, clinical applications and regulation of stem cells is via the internet. Below is a selection of websites offering accurate, reliable information on this topic.

1. The International Society for Stem Cell Research

The ISSCR website has a wealth of resources, including information on the science, ethics and regulation of stem cell research. Its 'Patient Handbook on Stem Cell Therapies' provides answers to questions about clinical trials involving stem cells, and how to judge if a new trial is being conducted safely and responsibly.

http://www.isscr.org/public/index.htm

2. EuroStemCell

The European stem cell portal EuroStemCell has a wide range of accessible resources for non-scientists, including award-winning films freely available to view online and critical analysis of the latest developments in stem cell research.

http://www.eurostemcell.org/

3. Nature Web Focus: Reaching for regenerative medicine

This collection of articles from Nature journals covers the basic science, and the challenge of making medicine from stem cells, whether derived from adult tissue, reprogrammed cultured cells or embryos.

http://www.nature.com/nature/focus/regenerativemedicine/index.html

4. NIH Stem Cell Information
The National Institutes of Health resource for stem cell research, with details of US federal and state policy in this area, as well as scientific information.

http://stemcells.nih.gov/

5. BioNews

BioNews is a free web and email-based source of news, information and comment in human genetics, embryo research and assisted reproduction, produced by the UK charity Progress Educational Trust. It covers both the ethical and scientific developments in these areas, including progress in stem cell research.

http://www.bionews.org.uk

Glossary

Adult stem cell: A type of cell found in adult tissues that is capable of dividing and multiplying to produce a limited range of different specialised cells.

Blastocyst: A mammalian embryo in the first stage of development, when the fertilised egg has grown into a hollow ball made up of a few hundred cells.

Cell: Cells are the building blocks of all living things.

Embryo: A stage of development which, in humans, lasts for eight weeks after the fertilised egg first starts to divide.

Embryonic stem cell: A type of unspecialised cell derived from early embryos, which is capable of developing into all (multipotent) or a wide range (pluripotent) of body tissues.

Fetus: A stage of development which, in humans, lasts from nine weeks after fertilisation until birth.

Gene: An inherited instruction that tells the body how to make proteins, or molecules that control other genes. Humans have around 25,000 different genes.

iPS cell: iPS (induced pluripotent stem) cells have similar properties to embryonic stem cells, but are created by 'reprogramming' cells from adult tissues, such as skin cells.

IVF: IVF (in vitro fertilisation) is a treatment for infertility, in which eggs are removed from a woman's ovaries, fertilised with sperm in a laboratory, then placed in the womb shortly afterwards to continue developing.

Nucleus: A structure found in the centre of eukaryotic cells (eg plant and animal cells), which contains the vast majority of its genetic material. (plural: nuclei)

Reproductive cloning: The production of an exact genetic replica of an animal, either by splitting an early embryo, or by using a technique known as somatic cell nuclear transfer to clone a body cell taken from an adult or embryo.

SCNT: SCNT (Somatic cell nuclear transfer) is a technique in which the genetic material from an egg cell is removed and replaced with that of an adult or embryo body cell of the same animal species. Also known as cell nucleus replacement (CNR), or 'cloning'.

Therapeutic cloning: A popular name for the proposed use of embryo stem cells, derived using somatic cell nuclear transfer technology, to develop genetically-matched cell therapies for a range of diseases.

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