eunoia JUNIOR COLLEGE

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17-02

The Cell & Biomolecules of Life – Stem Cells

1. Introduction

In order for a multicellular organism to function properly, it must diversify cell types, control their production, and eliminate aged/damaged cells.

Differentiation is a process by which a less specialised cell develops tissue-specific adaptation that enables it to become a more specialised cell type.

Many differentiated cell types have limited life spans. Disease can also lead to their loss. Since **differentiated cells do not usually divide**, their supply must be replenished and this is achieved through stem cells.

2. Learning Outcomes

(1) (2) (3)

(t) Describe the unique features of zygotic stem cells, embryonic stem cells and blood stem cells, correctly using the terms totipotency (zygotic stem cells which have ability to differentiate into any cell type to form whole organisms and so are also pluripotent and multipotent), pluripotency (embryonic stem cells which have ability to differentiate into almost any cell type to form any organ or type of cell and so are not totipotent but are multipotent) and multipotency (blood stem cells which have ability to differentiate into a limited range of cell type and so are not pluripotent or totipotent).

(u) Explain the normal functions of stem cells in a living organism (e.g. embryonic stem cells and blood stem cells).

(v) Discuss the ethical implications of the application of stem cells in research and medical applications and how human induced pluripotent stem cells (iPSCs) overcome some of these issues. (Procedural details of how iPSCs are formed are not required.)

3. References

Alberts B. Johnson, A., Lewis, J., Raff, M., Roberts, K. & Walter, P. (2002). Molecular Biology of The Cell. 4th Edition.
http://stemcells.nih.gov/info/basics/
http://www.isscr.org/

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4. Unique Features of Stem Cells

Notes to self

All stem cells have **three unique features**:

(a) Stem cells are a group of **undifferentiated** and **unspecialised** cells.

differentiated cells cannot divide but stem cells can.

- Specialised cells in tissues are differentiated and have tissue-specific structures that allow them to be adapted to perform unique roles.
  e.g. of specialised cells include red blood cells, muscle cells and nerve cells.
- Stem cells, however, **do not have** any **tissue-specific structures** and therefore **cannot perform tissue-specific functions**. Unlike a heart muscle cell, a stem cell cannot work with its neighbouring cells to pump blood through the body It also cannot carry oxygen though the bloodstream like a red blood cell. Stem cells are therefore undifferentiated and unspecialised cells.

(b) Stem cells can undergo **extensive proliferation** and are capable of **self-renewal** through **mitosis**.

There are 2 possible types of cell division a stem cell can undergo:

(i) **symmetrical division** (Fig. 1)
- The stem cell divides to produce **2 daughter stem cells** that have same characteristics as parent cell, and therefore possess the **same developmental** and **differentiation potential** as the parent cell.
- This enlarges the population of undifferentiated cells, **maintains a pool of stem cells for further division**.

(ii) **asymmetrical division** (Fig. 1)
- Asymmetrical division occurs when the stem cell is stimulated by **molecular signals** for differentiation.
- The stem cell divides to produce **1 daughter stem cell** that is identical to the parental stem cell and **1 progenitor daughter cell**:

  The **daughter stem cell** - to ensure a **constant pool** of stem cells

  The **progenitor daughter cell** is formed to **increase or renew** population of **specialised cells** in a **specific tissue.** The progenitor daughter cell is only capable of differentiating into related specialised cell type.

  e.g. progenitor daughter cells of hematopoietic (blood-forming) stem cells can only differentiate into various blood cells (e.g. white blood cells, red blood cells etc.) (Fig. 2). This replenishes cells that have a finite life span, such as red blood cells.

Notes to self

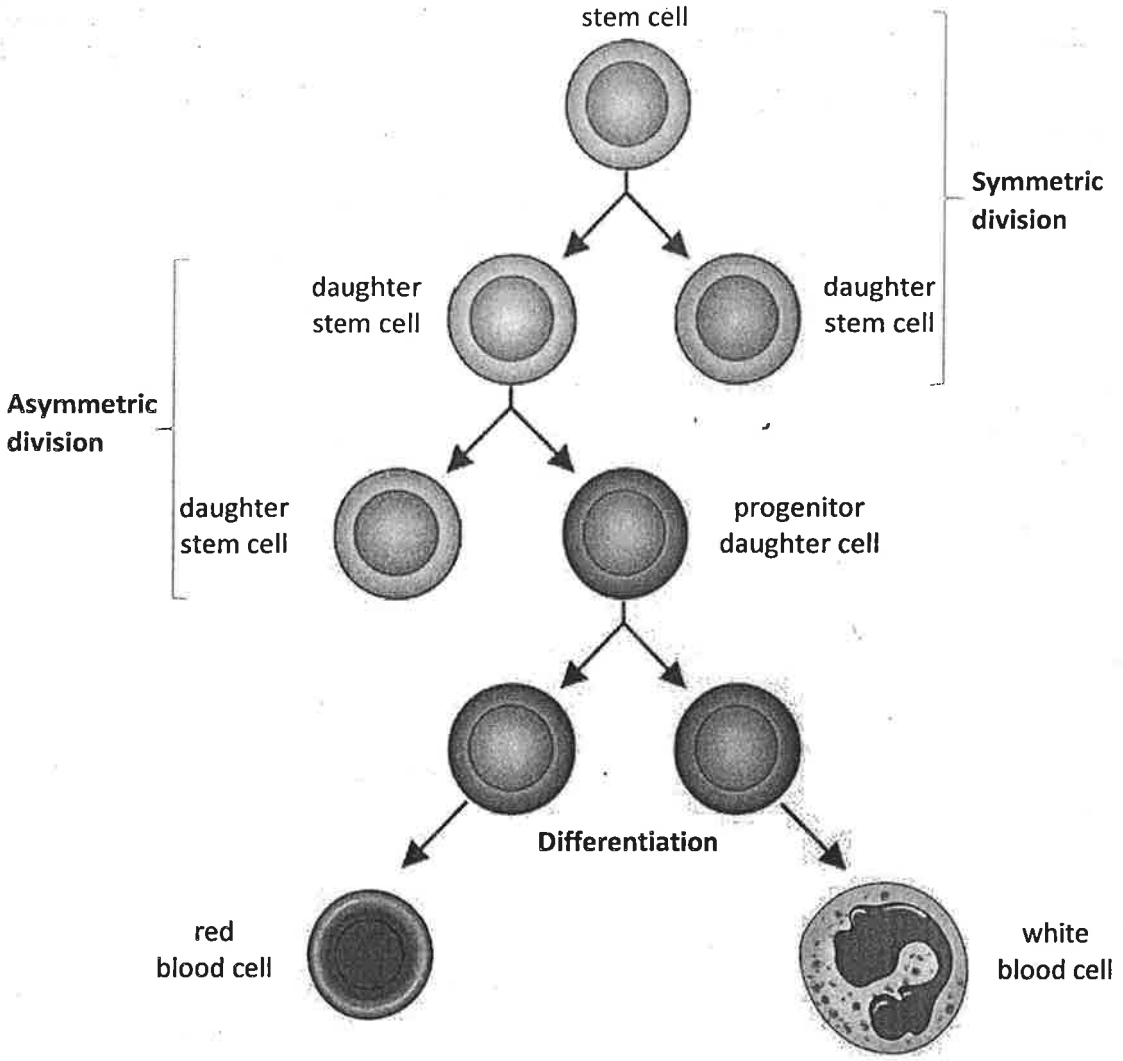

Fig. 1: **symmetrical and asymmetrical divisions.** In both scenarios, proliferation of cells (seen as an increase in number from 1 to 2 cells) occur. Developmental potential is maintained (where at least 1 of the daughter cells is a stem cell). Stimulus for each remains unclear.

Q: What happens if a stem cell division resulted in two progenitor cells all the time?

There will no longer be self-renewal and all stem cells will eventually be depleted

Notes to self

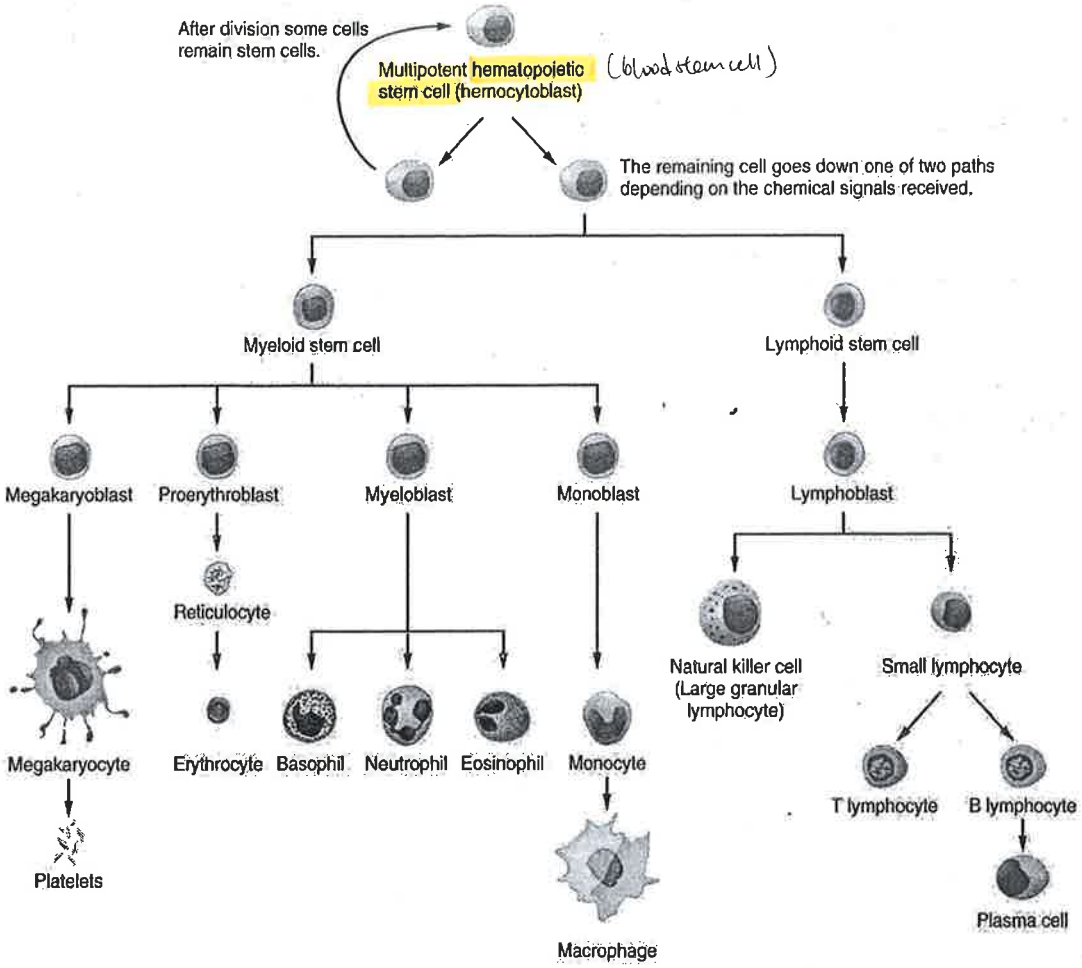

Fig. 2: **Hematopoietic stem cells give rise to a variety of specialised cells, but is restricted to blood cells only.**

Stem cells are essential in development, cellular regeneration & repair.

(c) Stem cells can **differentiate** into various specialised cell types upon stimulation by the appropriate **molecular signals**.

Molecular signals may activate or shut down expression of specific genes that result in synthesis of tissue-specific structures that enable these specialised cells to perform certain functions.

- These molecular signals switch some genes on, and others off.
- E.g. molecular signals: transcription factors, growth factors, hormones, cell-cell signals, properties of neighbouring cells.

Q: What is the difference between the genome of a stem cell and a specialised cell? What accounts for the difference in characteristics?

No difference. The difference in characteristics is a result of differences in gene expression.

5. Types of Stem Cells & Their Normal Functions in Living Organisms

Notes to self

In mammals, stem cells are commonly categorised according to their ability to differentiate/differentiation potential, i.e. totipotent, pluripotent or multipotent.

(a) Totipotent stem cells

Has the ability to **differentiate** into **all of the cell types** that make up an **entire** organism **including extra-embryonic tissue** such as **placenta**, which nourishes the embryo.

Via multiple cellular divisions, totipotent stem cells have the ability to give rise to an entire organism.

Totipotent cells are said to be also **pluripotent** and **multipotent**.

Examples

Zygotic stem cell that is derived from a **fertilised egg, zygote**, as well as cells that are **produced within the first 3 mitotic divisions** (i.e. **up to 8 cell stage**) after the egg is fertilised (Fig. 3).

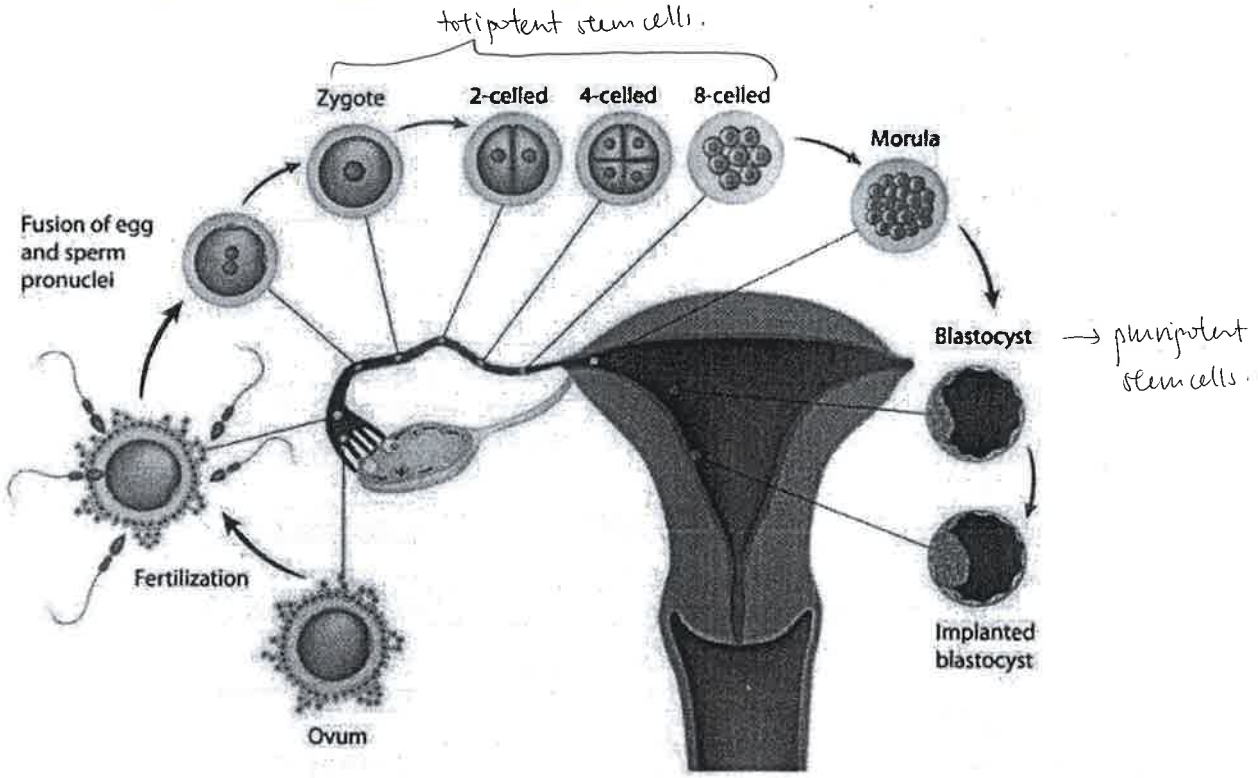

Fig. 3: **Stages of development of fertilised egg in human female**. Following fertilisation, the zygote develops into an early embryo via mitosis. After 4 to 5 days post-fertilisation, a ball of cells (consisting of 200 to 300 cells) known as blastocyst is formed.

(b) Pluripotent stem cells

Notes to self

Has the ability to **differentiate into all of the cell types** that make up an organism **except extraembryonic tissue** such as the **placenta**.

Pluripotent stem cells alone **cannot form the entire organism** as extraembryonic tissues such as the placenta is required for foetal nourishment and development.

Pluripotent cells are also said to be **multipotent**.

Examples

Embryonic stem cells (ESCs) are derived from **cells of inner cell mass of blastocyst** (200-300 cells), which forms at about 4 to 5 days post fertilisation (Fig. 3 and 4).

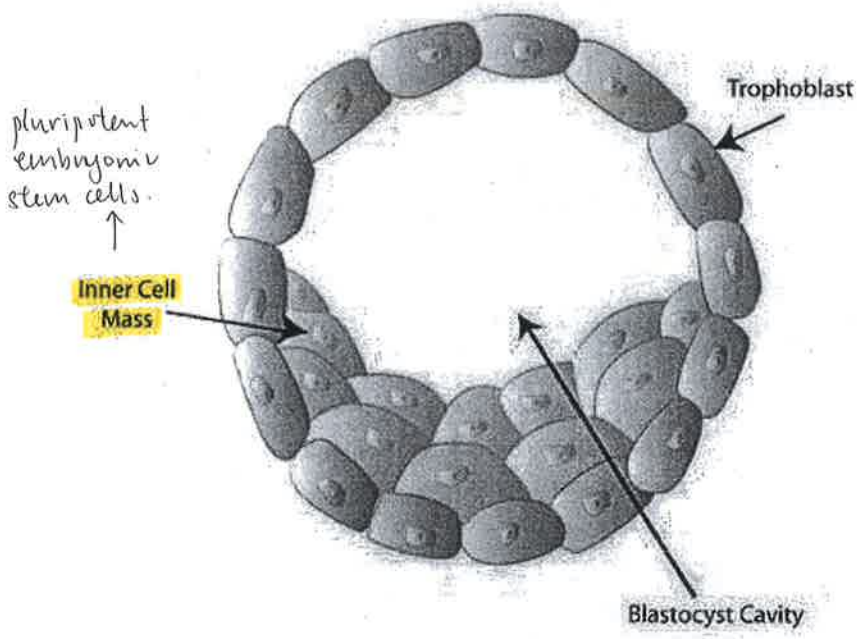

Fig. 4: **The three parts of a blastocyst:** inner cell mass, trophoblast and blastocyst cavity. The inner cell mass of the blastocyst will eventually develop into the foetus, while the trophoblast will develop into the placenta. The inner cell mass alone cannot form an entire, intact organism as the embryo cannot survive without the placenta. As such, the cells of the inner cell mass are pluripotent and not totipotent.

For stem cell studies, **embryonic stem cells** have to be isolated from inner cell mass of **blastocyst**, which are subsequently cultured in the laboratory (Fig. 5).

Notes to self

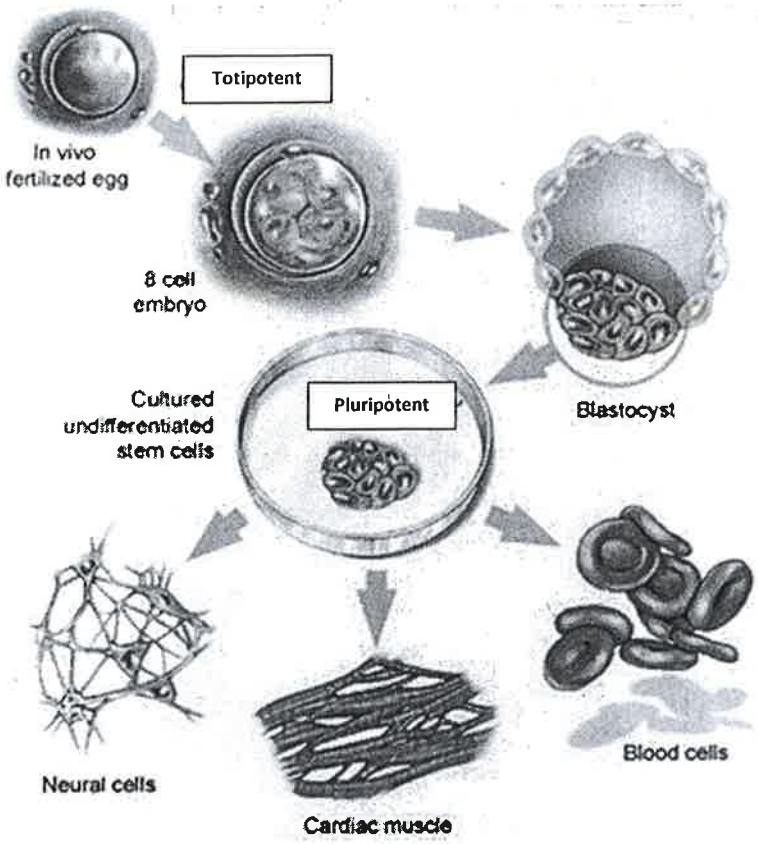

Fig. 5: Various types of specialised cells can be derived from pluripotent stem cells; taken from inner cell mass of a blastocyst.
http://www.stemcellresearchfoundation.org/WhatsNew/Pluripotent.htm

(c) Multipotent stem cells

Has the ability to **differentiate into several related specialised cell types** but far **fewer types** than the pluripotent embryonic stem cell.

→ blood stem cell cannot give liver cell.

The main purpose of multipotent adult stem cells is to **produce specialised cells** for **growth and development**, and for **replacement of cells** that are lost due to **cell death and injury**.

Examples

Stem cells found in a juvenile or adult animal is usually multipotent **adult stem cells**. These stem cells are found in **different tissues** of an organism after **embryonic development**. They are found in small numbers (1 in 10,000 – 15,000) in diverse tissues such as bone marrow, blood, cornea and retina, teeth, intestine, liver, muscles, nervous system and brain, pancreas and skin. Examples of adult stem cells include the following:

- **Blood/ haematopoietic stem cells**, which are found primarily in **bone marrow**, can differentiate to all type of blood cells, including red blood cells, white blood cells, and platelets, but not other cell types such as kidney cells.

Notes to self

Blood is a tissue which contains many different types of specialised cells, each of which has a different function: (Fig. 2)
- red blood cells – transport oxygen
- various types of white blood cells (e.g. lymphocytes, monocyte) – fight infection
- platelets – blood clotting

On average, the life span of red blood cells and white blood cells are about 112 days and 3-4 days, respectively. Haematopoietic stem cells constantly **proliferate** and **differentiate** into various **specialised** blood cell types to **replace** the blood cells lost through normal cell death. This maintains the immune and transport function of blood.

- **Neural stem cells** can differentiate into nerve cells and neural support cells called glial cells.

Q: Adult stem cells are found only in adults – true or false?

False. Adult stem cells are not confined to adults who are 21 years of age. Infants have them as well.

Q: What is the differentiation potential of umbilical cord stem cells?

Multipotent.

Notes to self

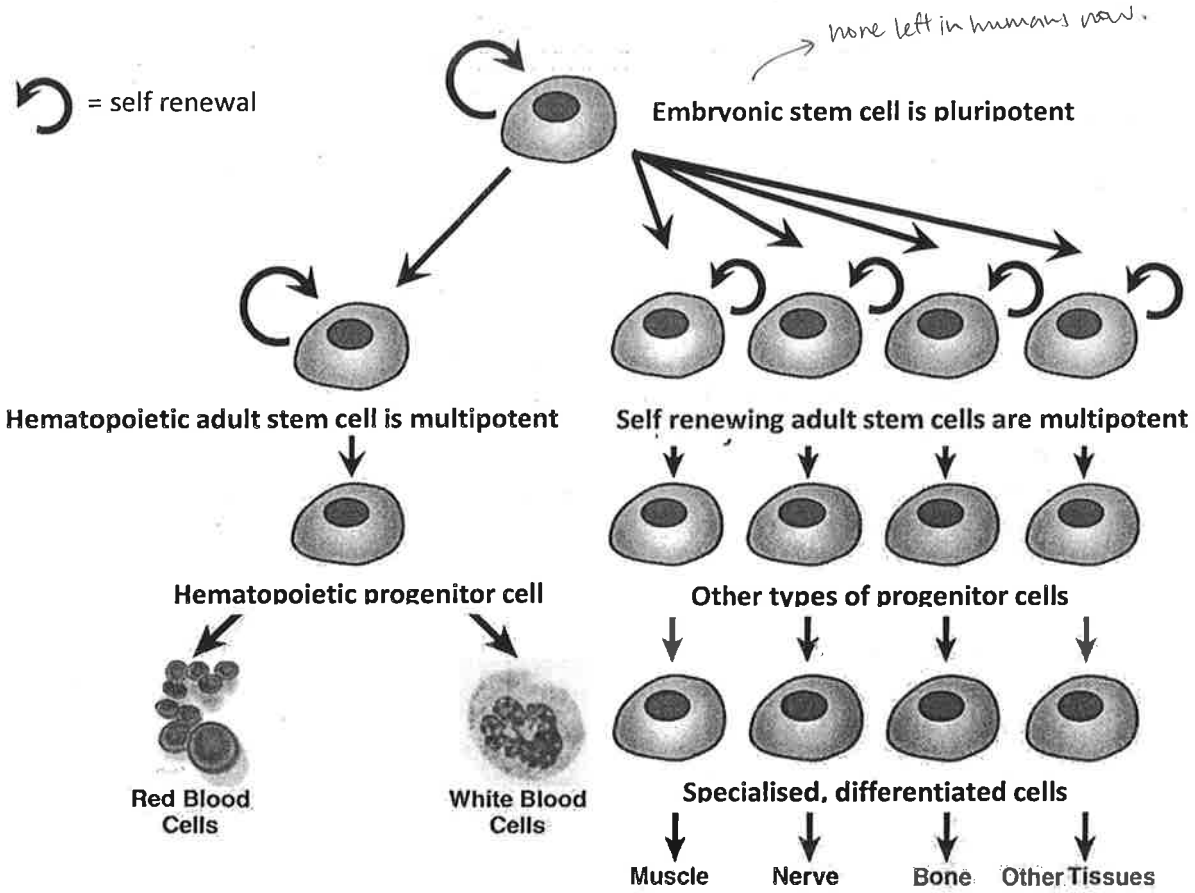

Fig. 6: Adult stem cell differentiation process is similar to embryonic stem cells, except that the variety of specialised cells generated is smaller. E.g. A hematopoietic stem cell can generate a variety of blood cells, but not nerve cells or other types of cells. This is because adult stem cells are not pluripotent, but multipotent. Progenitor cells are more differentiated than stem cells and cannot renew themselves.

In summary:

Type of Stem Cell / Differentiation Potential	Characteristics	Examples
Totipotent	Has the ability to **differentiate** into **all of the cell types** that make up an **entire** organism **including extra-embryonic tissue** such as **placenta**, which nourishes the embryo. **Totipotent** cells are said to be also **pluripotent** and **multipotent**.	▪ **Zygotic stem cell** that is derived from a **fertilised egg, zygote.** ▪ Cells that are **produced within the first 3 mitotic divisions** (i.e. **up to 8 cell stage**) after the egg is fertilised.
Pluripotent	Has the ability to **differentiate into all of the cell types** that make up an organism **except extraembryonic tissue** such as the **placenta**. **Pluripotent** stem cells alone **cannot form the entire organism** as extraembryonic tissues such as the placenta is required for foetal nourishment and development. **Pluripotent** cells are also said to be **multipotent**.	▪ **Embryonic stem cells (ESCs)** are derived from **cells of inner cell mass of blastocyst** (200-300 cells), which forms at about 4 to 5 days post fertilisation.
Multipotent	Has the ability to **differentiate into several related specialised cell types**, but far **fewer types** than the pluripotent embryonic stem cell.	**Adult stem cells** such as **blood/ haematopoietic stem cells**

Normal functions of stem cells in a living organism are:
(a) **self-renewal** to ensure a constant pool of stem cells,
(b) **growth and development**,
(c) **differentiate** into **specialised cells** to **replace** cells that are lost due cell death and injury.

6. Medical Applications of Stem Cells - Stem Cell Therapy

Notes to self

Multipotent and self-renewing nature of adult stem cells has been harnessed to provide therapy and treatment to a range of diseases. On the other hand, the use of embryonic stem cells remains controversial.

Stem cell therapy involving adult stem cells has general advantages:

- **multipotent** nature of adult stem cells ensures that the adult stem cells differentiates into the respective **specialised cell type**, thus **restoring function** of damaged or diseased tissue.
- **self-renewing** nature of stem cells ensures that transplanted stem cells constantly **replicate** in the patient to **maintain a constant pool** of stem cells. Hence, repeated stem cell transplants are not necessary to sustain the therapeutic effects.

There are two general approaches to stem cell therapies:

- **Stem cell transplant**
- **Genetically modified stem cell transplant**

Stem cell transplant

Limitations

① Adult stem cells found in small numbers and can be difficult to isolate.

② three limited cell types cannot treat all diseases.

Adult stem cells can be obtained directly from the donor organ or tissue in which they are found, and have provided many different therapies for illnesses such as Parkinson's disease, leukemia, multiple sclerosis, lupus, sickle-cell anaemia, and heart damage. These treatments carry a risk that donated cells will be rejected. Also, unlike pluripotent embryonic stem cells which have the ability to differentiate into all cell types that make up an organism (except for extra-embryonic tissues), adult stem cells are limited to differentiating into different cell types of their tissue of origin.

Example:
Transplant of bone marrow haematopoietic stem cells from normal healthy bone marrow donors to leukaemia patients (Fig. 7).

Leukaemia is a type of cancer of the blood or bone marrow characterised by abnormal increase in the number of immature white blood cells called blast cells. These abnormal immature blast cells are not functional, frequently crowd the bone marrow and prevent the production of functional white blood cells.

The treatment of choice is a **bone marrow haematopoietic stem cell transplant.** The patient is first irradiated to remove all existing haematopoietic cells and white blood cells from the body. Bone marrow haematopoietic stem cells from **normal, healthy bone marrow donors** are multiplied, and **infused** into the patient. These transplanted haematopoietic stem cells will then populate the bone marrow and **differentiate** into **normal blood cells.** This treatment thus **restores function of blood.** As stem cells are capable of **self-renewal**, the stem cells will replicate and ensure a **constant pool** of stems cells which will differentiate into the various blood cells subsequently when required, **without the need for repeated transplants.**

Notes to self

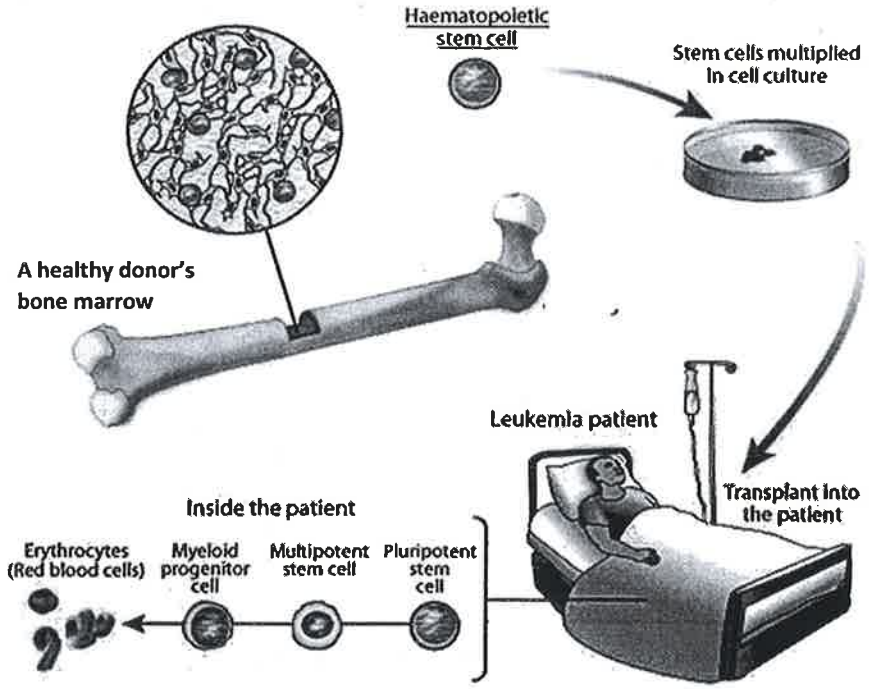

Fig. 7: Stem cells from a healthy donor. The stem cells, are first cultured *in vitro* to increase their numbers. If successful, they produce a large number of red and white blood cells, to help the patient recover.

Genetically modified stem cell transplant

Example:
Genetically modified stem cells can be used in **gene therapy** to treat **genetic diseases** (e.g. genetic blood disorders).

This strategy involves **removing stem cells from the patient, genetically modifying** the genome of the stem cells by **inserting a normal, functional allele** and then **reintroducing** the modified stem cells back into the patient (Fig. 8).

The genetically modified stem cells are capable of **self-renewal**, and thus will **proliferate** to form more genetically modified stem cells that contain the normal, functional allele. These genetically modified stem cells will **differentiate** into specialised cell which **restores the functions** of the diseased tissues.

Notes to self

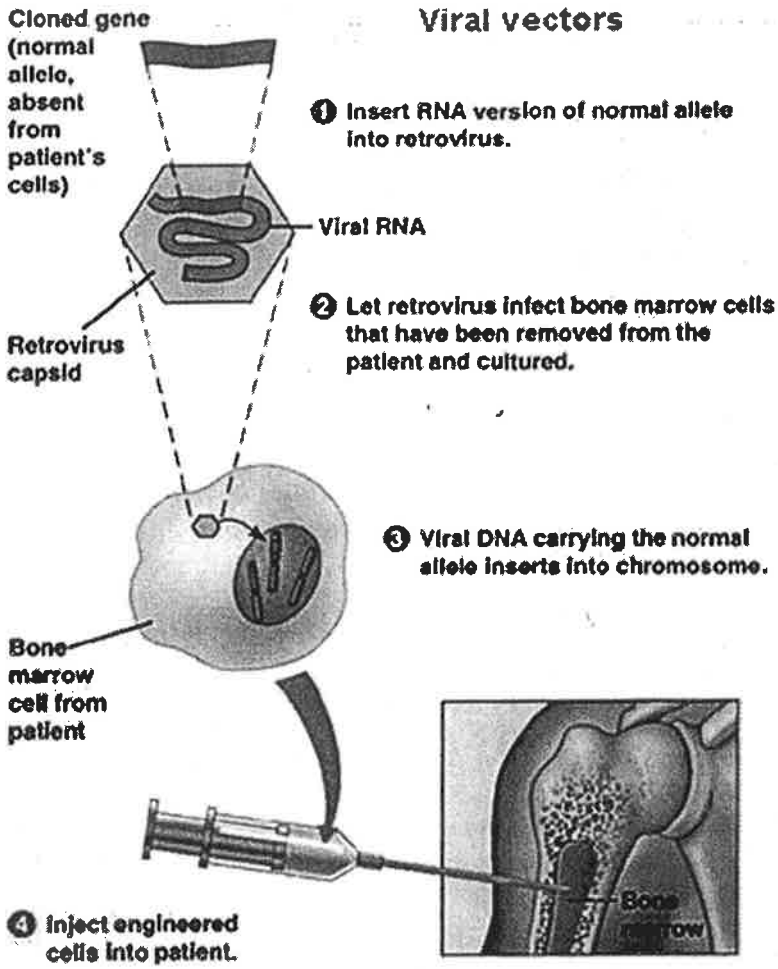

Fig. 8: Stem cells (labelled as bone marrow cells) from a patient can be removed and genetically modified, before re-introduction into the patient. This method takes advantage of the stem cell's ability to keep dividing and to differentiate into the specialised cells needed by the patient. A virus that cannot cause disease is used to deliver the normal, functional allele into the stem cell.

Q: Why are retroviruses used as vectors for gene therapy?

Has integrase to integrate normal, functional allele into ~~genome~~ genome of target cell

Embryonic stem cell vs Adult stem cell in Stem Cell Therapy & Research

Feature of comparison	Embryonic stem cells	Adult stem cells
Differentiation potential	Pluripotent: capable of differentiating into any cell in the entire organism except extraembryonic tissue, e.g. placenta.	Multipotent: typically only give rise to the cells of the tissue in which they are found.
Role in human body	To develop the embryo into an entire human.	To replace specific cells in the body which die throughout life due to wear and tear or injury and disease.
Sources	Unused IVF embryos which have been donated, or embryos created for the purpose from donated eggs and sperm.	Any tissue with adult stem cells: e.g. bone marrow, muscles and skin; and from the fetus, umbilical cord, placenta and amniotic fluid.
Advantages in research and therapy development	▪ Embryonic stem cells make up a significant proportion of a developing embryo and are easier to isolate and grow *ex vivo* than adult stem cells ▪ Have a strong ability to self-renew in the laboratory and divide more rapidly than adult stem cells, making it easier to maintain of a constant supply of ES cells. ▪ Pluripotency means that ES cells have the potential to produce any cell type in the body, potentially allowing them to be used for the treatment of a wider range of diseases.	▪ If taken from the patient's own body for use in therapies, cells would be genetically identical to that of the patient, avoiding the problem of immune rejection. ▪ There are less ethical considerations compared with using embryonic stem cells.
Disadvantages in research and therapy development	▪ ES cells are genetically different to cells of potential patients, so immune rejection could occur. ▪ Ethical issues over embryo destruction.	▪ They usually only produce a limited number of different cell types. ▪ Conditions supporting self-renewal in the laboratory have only been identified for a few tissue stem cell types, for instance skin and cornea. ▪ Are found in small numbers and can be difficult to isolate. ▪ Adult stem cells from the patient's own body will not be effective in treatment of genetic disorders, as the DNA of these stem cells also carry the genetic mutations.

7. Bioethics

Notes to self

Ethics is a field of study that looks at the moral basis of human behaviour ("***Why do we act as we do?***") and attempts to determine the best course of action in the face of conflicting choices ("***How do we decide what to do when people disagree about a complex issue?***"). It is a key component of living within a society in a civilized way.

Bioethics is a subfield of ethics that explores ethical questions related to the life sciences. Bioethical analysis helps people make decisions about their behaviour and about policy questions that governments, organizations, and communities must face when they consider how best to use new biomedical knowledge and innovations. Examples are:

"***How should we decide who receives organ transplants?***" or, "***Should a terminally ill patient be allowed to end his/her life with physician-prescribed medication?***"

Principles of Bioethics

(a) Respect for Persons
(Respecting the inherent worth of an individual and their autonomy)
Respect for Persons emphasizes the inherent worth and dignity of each individual, and acknowledges a person's right to make his or her own choices. It means not treating people as a means to an end.

(b) Maximize Benefits/Minimize Harms (Beneficence/ Non-maleficence)
Maximizing Benefits and Minimizing Harms asks how we can do the most good and the least amount of harm. It considers how one would directly help others and act in their best interests, while "doing no harm."

(c) Justice (Being Fair)
Justice considers how we can treat people fairly and equitably. It involves the sharing of resources, risks, and costs according to what is "due" to each person.

In addition to the Principles of Bioethics introduced above, ethicists use a number of different ethical perspectives and theories to defend their positions, including:

- Moral Rules and Duties
- Virtues
- Outcomes
- Care

8. Ethical Implications of Stem Cell Therapy and Research

Notes to self

Argument against using embryonic stem cells

(a) Some assert that the embryo has the status of a human being as it has the potential to become one. They believe that embryonic stem cell research violates the sanctity of life and is tantamount to murder. This is against the principles of respect for persons and beneficence/ non-maleficence.

(b) Some object to extracting stem cells from an embryo to make replacement body cells is treating the embryo as just a source of spare parts. Embryonic stem cell research takes a purely utilitarian view of the embryo. This is against the principles of respect for persons and beneficence/ non-maleficence.

(c) Justice and equity:
- Adult stem cell treatment is established and there are fewer ethical issues involved. For instance, adult stem cells from sources such as umbilical cord blood, have already produced some results. Thus adult stem cell research may be able to make greater advances if more money and resources were channeled into it instead of embryonic stem cell research.
- As embryonic stem cell research is expensive, funds can be channeled to treat other more treatable diseases, thereby maximising justice.

(d) Claims of the benefits of embryonic stem cell research are over-rated / few (if any) examples of success in medical applications.

(e) Current benign applications may lead to abuse in the future. Once human status is denied to embryos, this precedent may extend to other categories of human beings such as the profoundly disabled or the elderly infirm.

(f) Possibility of maleficence due to unforeseen consequences in treated patients, such as possible risks of–tumor formation, immunological reactions, unexpected behavior of the cells, and unknown long-term health effects.

(g) Donation and consent - For those people involved in donating eggs, embryos or tissues, there are issues of informed consent, understanding of research aims and privacy.

Argument for using embryonic stem cells

Notes to self

(a) Embryonic stem cells can potentially treat a wide range of diseases as they have the potential to grow indefinitely in a laboratory environment and can differentiate into almost all types of bodily tissue. Treatments that have been proposed include treatment for physical trauma, degenerative conditions (e.g. Parkinson's disease), and genetic diseases (in combination with gene therapy). As such, use of embryonic stem cells can potentially help to maximise benefits.

(b) Embryos are not equivalent to human life:

- Embryos are not conscious, cannot feel and cannot survive outside the womb. i.e. a early embryo that has not yet been implanted into the uterus does not have the psychological, emotional or physical properties that we associate with being a person. Something that can potentially become a person should not be treated as if it actually were a person. An early embryo therefore does not have any interests to be protected, and we can use it for the benefits of patients, who *are* people.
- The embryo cannot develop into a child without being transferred to a uterus.
- Blastocysts are a cluster of human cells that have not differentiated into distinct organ tissue, making cells of the inner cell mass no more "human" than a skin cell.
- Some believe life only begins when the heartbeat develops (during the fifth week of pregnancy) or when the brain begins developing (at 54 days after conception).

As such, use of embryos do not go against the principle of non-maleficence.

(c) There is legislation on the period when ES cells can be extracted and this may help to minimise harm.
e.g. Current UK legislation does not allow use of embryos that are more than 14 days old. In fact, ES cells are obtained earlier from blastocyst (between 3-8 days after fertilization).
e.g. under Singapore legislation, only embryos less than 14 days can be used.

A source of embryos would be surplus human embryos from created for fertility treatment, or from existing ES cell lines which originated from ES cells derived from embryos less than 14 days old.

(d) Surplus embryos created via *in vitro* fertility treatments are destroyed, or stored for long periods of time, long past their viable storage life. These can be used for creating new stem cell lines for research which would otherwise be destroyed. This helps to maximise benefits while minimising harm at the same time.

(e) Instead of destroying new human embryos to establish new stem cell lines, there are existing stem cell lines that can be used. This thereby minimises harm.

Induced pluripotent stem cells (iPSCs) – An Alternative to ES Cells

Notes to self

Differentiated and specialised adult somatic cells (e.g. skin cells) can be reprogramed to become pluripotent stem cells called **induced pluripotent stem cells (iPSCs)**. The iPSC technology was pioneered by Shinya Yamanaka's lab in 2006; the introduction of four specific genes encoding transcription factors could 'reprogramme' some specialised cells to become pluripotent so that they lose their specialist functions and behave in virtually the same way as embryonic stem cells.

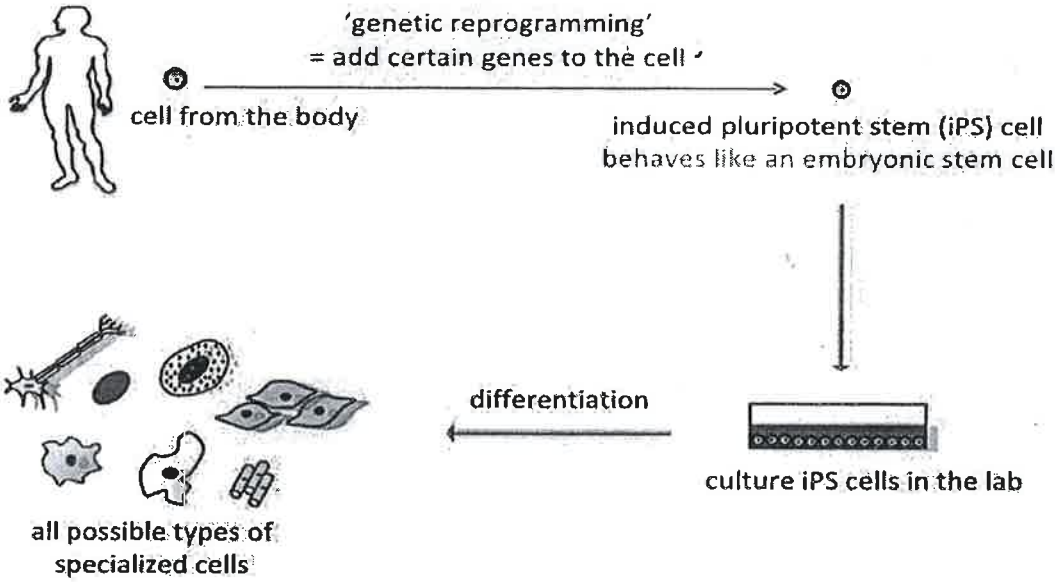

Fig. 9: Formation of iPSCs from adult somatic cell. In Yamanaka's method, retroviruses are used to introduce four reprogramming genes into adult somatic cells to derive iPSCs.

Advantages of Using iPSCs

Induced pluripotent stem cells are perhaps the most prominent alternative source of stem cells proposed for therapy and research. It offers several advantages:

(a) Since iPSCs can be derived directly from adult tissues, it does not generate or destroy any human embryos.
(b) iPSCs can be easily obtained from any type of adult/specialised somatic cell (e.g. skin cell) without risk to the donor.
(c) In contrast to ES cells extracted from human embryos, iPSCs derived from a patient's own cells would open the possibility of generating lots of patient-specific cells, which will not be rejected by the immune system upon transplantation.
(d) Further, it also allows the generation of pluripotent stem cell lines from patients with inherited diseases, in order to better understand why the disease develops and use in personalized drug discovery efforts.
(e) An additional reproductive technology that may be enabled by iPSCs is the generation of sex cells (sperm and eggs) for treating infertility.

Possible Problems with Using iPSCs

Notes to self

(a) Low efficiency: in general, the conversion to iPSCs has been incredibly low. For example, the rate at which somatic cells were reprogrammed into iPSCs in was 0.01–0.1%.

(b) Genetic modification of adult cells to obtain iPSCs using retroviruses to deliver the reprogramming genes may pose cancer risk to the patient. Cancer may result due to overexpression of proto oncogenes (one of the four reprogramming genes is *Myc*, a proto-oncogene) or insertional inactivation of tumour suppressor genes.

(c) There are ethical concerns (e.g. lack of consent, long term unexpected consequences) related to the creation of embryos and children from iPSC-derived sex cells.

Summary

Ethical complications are related to the means of procuring stem cells (e.g. techniques involving the destruction of human embryos), human cloning and the exploitation of embryo and egg donors. Alternative methods of obtaining stem cells need to be discussed along with scientific challenges to ensure that stem cell research is carried out in an ethically appropriate manner. The use of iPSCs still appears to overcome many ethical issues and provides viable solutions related to stem cell research.