Can/Should We Grow Our Body Parts?

Key Stage 4 Programme

Pupil Name

Handbook Designed by Priyanka Makwana

Timetable and Assignment Submission

Timetable – Tutorials

Tutorial	Date	Time	Location
1			
2			
3			
4			
5			
6 (Feedback)			
7 (Feedback)			

Timetable – Homework Assignments

Homework Assignment	Description	Due Date
Tutorial 1		
Tutorial 2		
Tutorial 3		
Tutorial 4		
Tutorial 5		

Assignment Submission – Lateness and Plagiarism

Lateness	
Submission after midnight on 9th August	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

Uni Pathways – Pupil Feedback Report

Grade	Marks	Guidance
1st	70+	
2:1	60-69	
2:2	50-59	
3rd	40-49	
Working towards a pass	0-39	
Did not submit	DNS	No assignment received by The Brilliant Club

Lateness	
Any lateness	10 marks deducted
Plagiarism	
Some plagiarism	10 marks deducted
Moderate plagiarism	20 marks deducted
Extreme plagiarism	Automatic fail

Name of PhD Tutor			
Title of Assignment			
Name of Pupil			
Name of School			
ORIGINAL MARK / 100		FINAL MARK / 100	
DEDUCTED MARKS		FINAL GRADE	

If marks have been deducted (e.g. late submission, plagiarism) the PhD tutor should give an explanation in this section:

Learning Feedback Comment 1 – ***Enter Key Learning Priority Here***	
What you did in relation to this Key Learning Priority *Enter feedback here*	How you could improve in the future *Enter feedback here*
Learning Feedback Comment 2 – ***Enter Key Learning Priority Here***	
What you did in relation to this Key Learning Priority *Enter feedback here*	How you could improve in the future *Enter feedback here*
Learning Feedback Comment 3 – ***Enter Key Learning Priority Here***	
What you did in relation to this Key Learning Priority *Enter feedback here*	How you could improve in the future *Enter feedback here*
Resilience Comment	
How you showed learning resilience during the course *Enter feedback here*	How you could build learning resilience in the future *Enter feedback here*

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Course Rationale

Regrowing a missing limb is no big deal — to a starfish or salamander, creatures that are well-known for using regenerative "superpowers" to replace missing arms and tails. But they aren't the only animals that can rebuild body parts that are destroyed or damaged. Deer can re-sprout as much as 30 kilograms of antlers in only three months. Zebrafish can regrow their hearts, while flatworms have demonstrated that they can regenerate their own heads.

For humans, though, what's lost is lost — or is it?
Individual cells in your body are constantly being replaced as they wear out, a process that slows with aging but continues throughout the human lifetime. You can even observe this frequent and visible regeneration in one of your organs: your skin. In fact, humans shed their entire outer layer of skin every two to four weeks, losing about 18 ounces (510 grams) of skin cells per year.

In this course you will introduce new concept of Regenerative Medicine and Tissue Engineering that has open new of possibilities of generating tissues or organs. As we progress through the tutorial variety of scientific jargons and terminology will be studied. You will be able to see how our body is cleverly design to perform various tasks and is it possible to replicate similar task outside the body. You will develop research skills and more importantly critical thinking. There will be opportunity to discuss your opinion with other team mates and explore your curiousness.

Mark Scheme

STEM Mark Scheme

Skills	1st (70-100)	2:1 (60-69)	2:2 (50-59)
Knowledge and Understanding	All content included is relevant to the general topic and to the specific question/title Good understanding of all the relevant topics. Scientific terms are defined and used accurately throughout Clear justification on how the content included is related to the specific issues that are the focus of the assignment	Most of the content included is relevant to the general topic and to the specific question/title Good understanding of most the relevant topics Scientific terms are used accurately but not always clearly defined. Adequate justification on how the content included is related to the specific issues that are the focus of the assignment	Some of the content included is relevant to the general topic and to the specific question/title Good understanding on some of the relevant topics but occasional confusion on others. Scientific terms are used mostly accurately with occasional confusion and often not defined. Some justification on how the content included is related to the specific issues that are the focus of the assignment

Structure and Presentation	Ideas are presented in paragraphs and arranged in a logical structure that is appropriate for the assignment The introduction clearly outlines how the essay/report will deal with the issues The conclusion summarises all the main points clearly and concisely Tables and graphs are effectively constructed including appropriate headings, units and scales. All sources are referenced correctly in an agreed format	Ideas are presented in paragraphs and arranged in a structure that is mostly appropriate for the assignment The introduction adequately describes how the essay/report will deal with the issues The conclusion summarises most of the main points clearly Most tables and graphs are well constructed Most sources are referenced correctly in an agreed format	Ideas are presented in paragraphs and arranged in a structure The introduction mentions how the essay/report will deal with the issues The conclusion summarises some of the main points clearly Some tables and graphs are well constructed but contains some errors Some sources are referenced correctly in the agreed format with occasional errors

Critical thinking	Critical Evaluation	Moved beyond description to an assessment of the value or significance of what is described Evaluative points are consistently explicit/systematic /reasoned/justified Effective critiques on the reliability of sources provided	Mostly description but some assessment of the value or significance of what is described Evaluative points are mostly explicit/systematic /reasoned/justified Some evidence of critiques on the reliability of sources provided	Only description with minimal assessment of the value or significance of what is described Evaluative points are at times explicit/systematic /reasoned/justified Limited evidence of critiques on the reliability of sources provided
Written communication	Structure and Presentation	Ideas are presented in paragraphs and arranged as a logical sequence of ideas The introduction clearly outlines how the essay will deal with the issues The conclusion summarises all the main points clearly and concisely All sources are referenced correctly in the agreed format	Ideas are presented in paragraphs with some structure The introduction adequately describes how the essay will deal with the issues The conclusion summarises most of the main points clearly Most of the sources are referenced correctly in the agreed format 	Ideas are presented in paragraphs and are loosely structured The introduction mentions how the essay will deal with the issues The conclusion summarises some of the main points clearly Some sources are referenced correctly in the agreed format with occasional errors
	Language and Style	No spelling, grammar or punctuation errors Writing style consistently clear, tone appropriate and easy to follow Accurate and consistent use of technical language and vocabulary	Minimal spelling, grammar or punctuation errors Writing style mostly clear, tone appropriate and easy to follow Some attempts of using technical language and vocabalary, but not always accurate	Some spelling, grammar or punctuation errors Writing style moderately clear, tone appropriate and easy to follow Use of simple language and vocabulary effectively but struggles to use technical language

Subject Vocabulary

Word	Definition
Differentiation	☐ Process by which less specialised cell becomes a more specialised cells.
Embryonic stem cells	☐ Pluripotent stem cells derived from the inner cell mass of a blastocyst
Morpholoy	☐ From and structure of cells
Mitosis	☐ A type of cell division that results in two daughter cells each having the same number and kind of chromosomes as the parent nucleus, typical of ordinary tissue growth
Phenotype	☐ Observable physical properties of cells
Pluripotent	☐ Cell capable of giving rise to several different types of cells

Knowledge Organiser

Section 1

What are Stem Cells?

- Stem cells provide new cells for the body as it grows, and replace specialised cells that are damaged or lost. **Different types of stem cell**

- There are three main types of stem cell:
  - embryonic stem cells
  - adult stem cells
  - induced pluripotent stem cells

Embryonic stem cells

- cells supply new cells for an embryo? as it grows and develops into a baby. They are pluripotent, which means they can change into any cell in the body.

Adult stem cells

- Cells supply new cells as an organism grows and to replace cells that get damaged. They are said to be multipotent, which means they can only change into some cells in the body, not any cell.

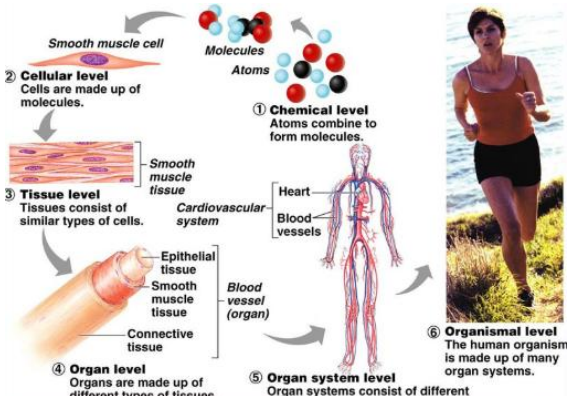
Induced pluripotent stem cells (iPS cells)

- iPS cells are stem cells that scientists make in the laboratory.
- 'Induced' means that they are made in the lab by taking normal adult cells, like skin or blood cells, i.e. reprogramming them to become stem cells.
- Just like embryonic stem cells, they are pluripotent so they can develop into any cell type

Section 2

Levels of Structural Organization of the Human Body

The organization of the body often is discussed in terms of six distinct levels of increasing complexity, from the smallest chemical building blocks to a unique human organism.



Section3

Tissue Engineering and Regenerative Medicine

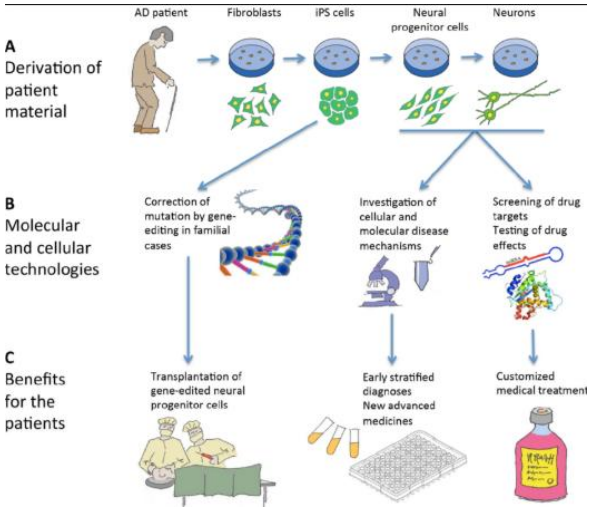
Tissue engineering is designing and manufacturing a functional constructs that can restore, maintain, or improve damaged tissues or whole organs in human body. Artificial skin and cartilage are examples of engineered tissues.

Regenerative medicine

- Regenerative medicine is the branch of medicine that develops methods to regrow, repair or replace damaged or diseased cells, organs or tissues.
- Regenerative medicine includes the generation and use of therapeutic stem cells, tissue engineering and the production of artificial organs.

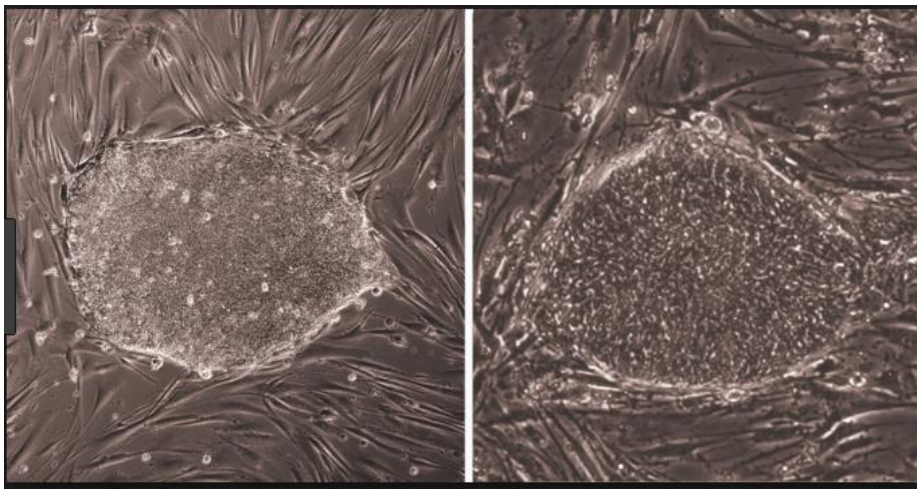
Section 4

Application of iPS cells



Schematic illustration of the use of induced pluripotent stem (iPS) cells in relation to Alzheimer's disease. (A) iPS cells are derived from a skin biopsy of AD patient and differentiated into neural progenitor cells and neurons; (B) disease-causing mutation can be corrected by gene-editing of the iPS cells, (C) The patients can in the long run benefit from these activities by cell therapy, better diagnostic procedures, customized treatments and novel medical approaches.

Tutorial 1 – What are stem cells?



What is the Purpose of Tutorial 1?

- Objective 1 To understand difference between somatic cell and stem cell
- Objective 2 To appreciate different types of stem cells
- Objective 3 To consider usage of embryonic stem cells
- Objective 4 To consider ethical issues associated with embryonic stem cells

Stem Cells

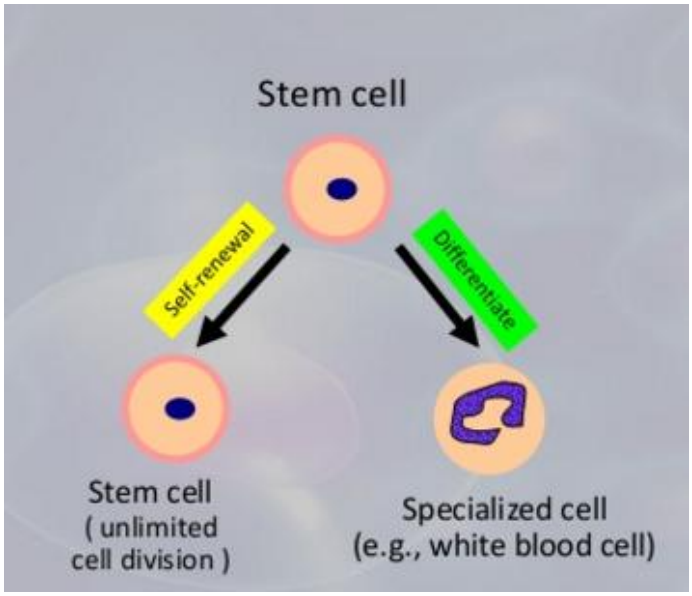


Figure 1: Stem cell division.

Cells in the body have specific purposes, but stem cells are cells that do not yet have a specific role and can become almost any cell that is required. Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell.

Stem cells are distinguished from other cell types by two important characteristics.

- First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity.
- Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions.

Stem cells are important for living organisms for many reasons. In the 3- to 5-day-old embryo, called a blastocyst, the inner cells give rise to the entire body of the organism, including all of the many specialized cell types and organs such as the heart, lungs, skin, sperm, eggs and other tissues. In some adult tissues, such as bone marrow,

muscle, and brain, discrete populations of adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease.

Researchers categorize stem cells, according to their potential to differentiate into other types of cells. The full classification includes:

Totipotent: These stem cells can differentiate into all possible cell types. The first few cells that appear as the zygote starts to divide are totipotent.

Pluripotent: These cells can turn into almost any cell. Cells from the early embryo are pluripotent.

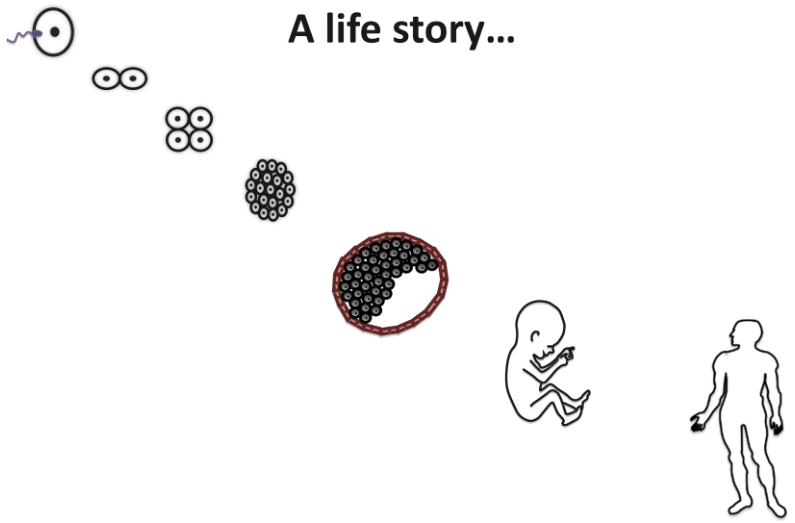
Multipotent: These cells can differentiate into a closely related family of cells. Adult hematopoietic stem cells, for example, can become red and white blood cells or platelets.

Oligopotent: These can differentiate into a few different cell types. Adult lymphoid or myeloid stem cells can do this.

Unipotent: These can only produce cells of one kind, which is their own type. However, they are still stem cells because they can renew themselves. Examples include adult muscle stem cells.

Embryonic stem cells are considered pluripotent instead of totipotent because they cannot become part of the extra-embryonic membranes or the placenta.

Activity 1.1 Label the following diagram.



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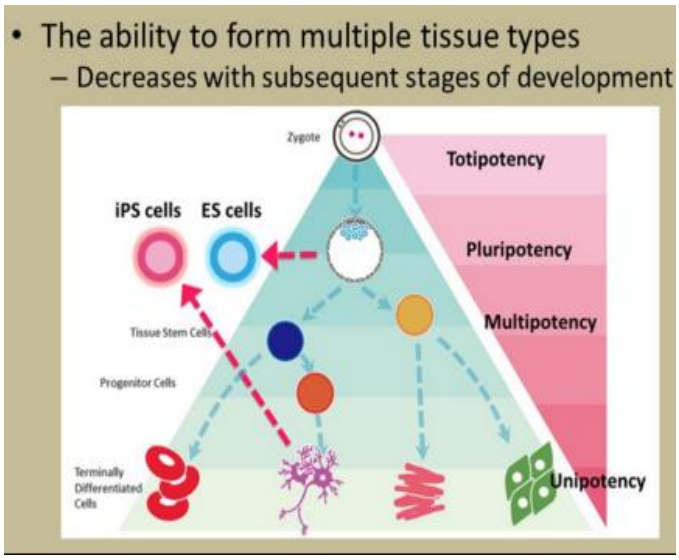
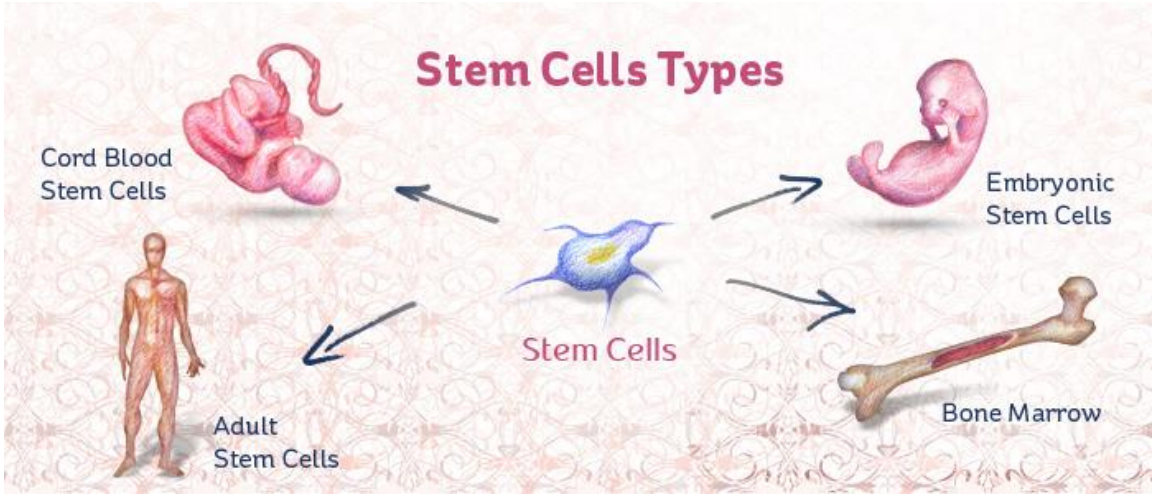
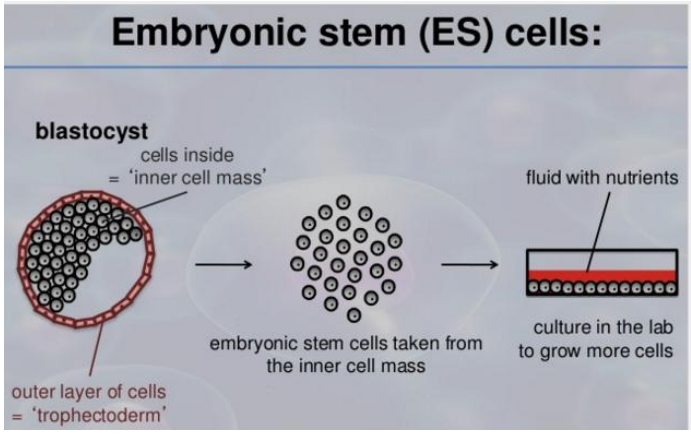
Cells' ability to differentiate into other cell types is classified into cell potency. Therefore more cell types cell able to differentiate into, the greater its potency.

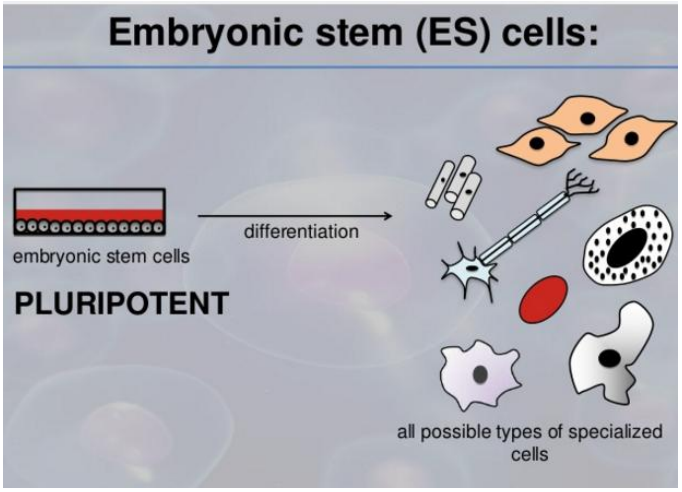


Types of Stem Cells



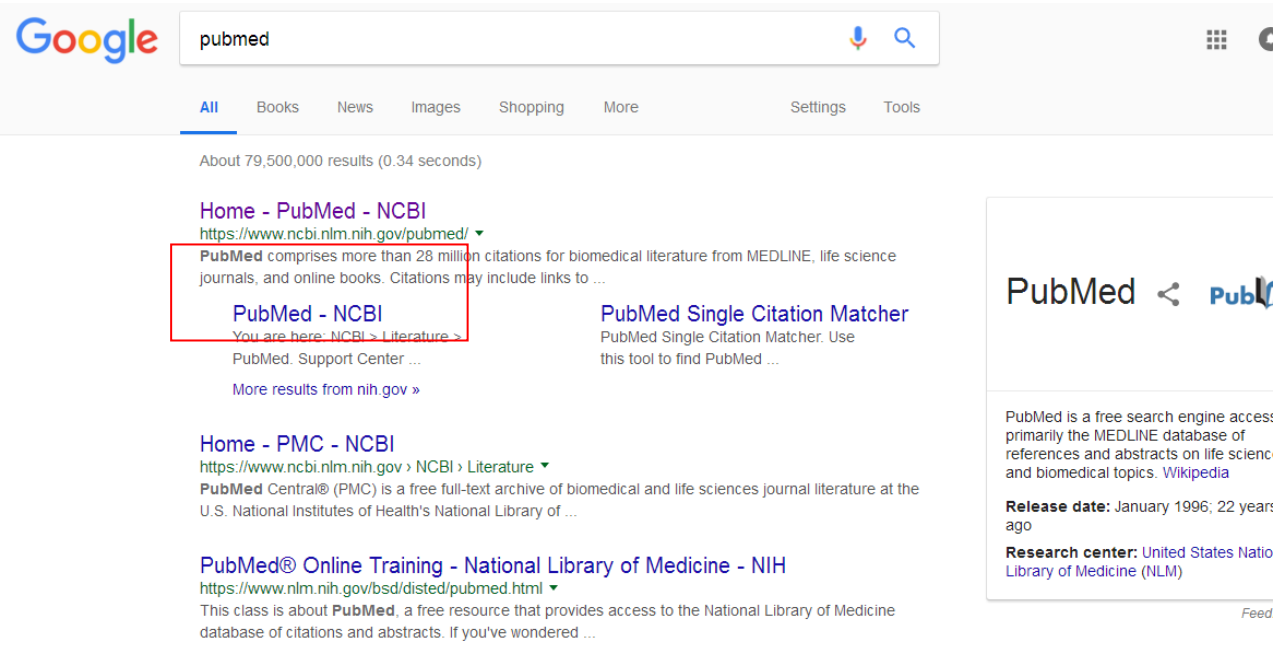


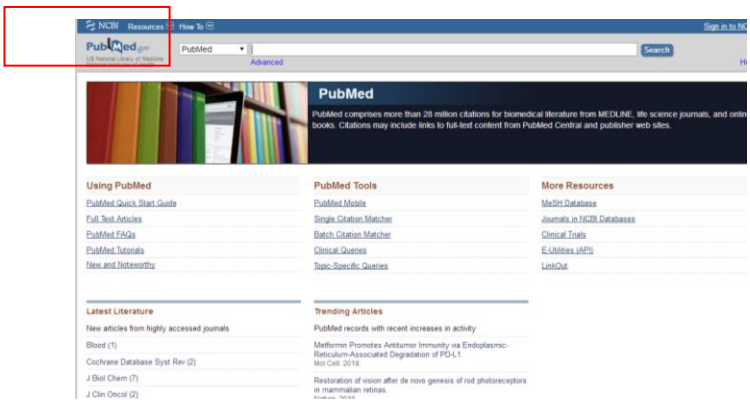
Embryonic Stem Cells

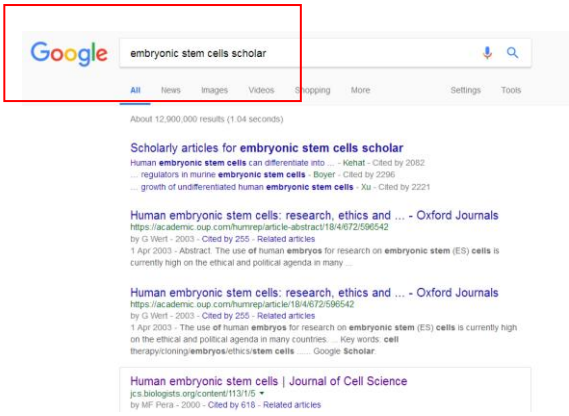


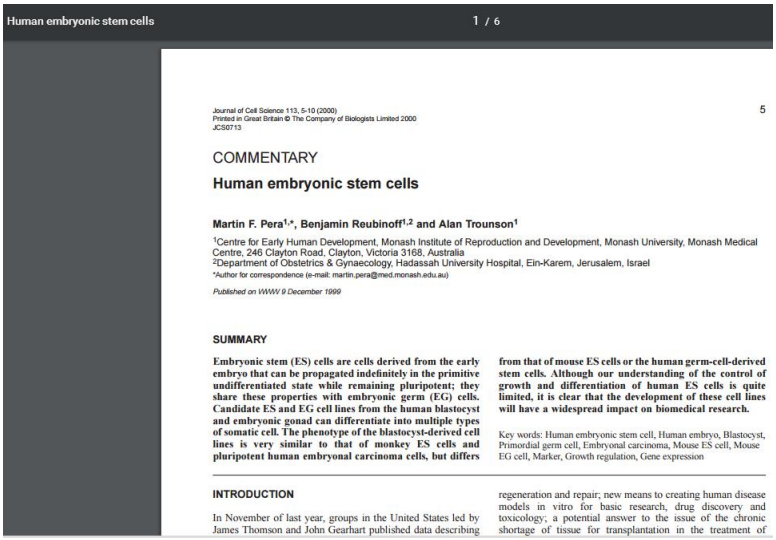
Usage and advantages of embryonic stem cells.

Activity 1.2 Finding a journal article.









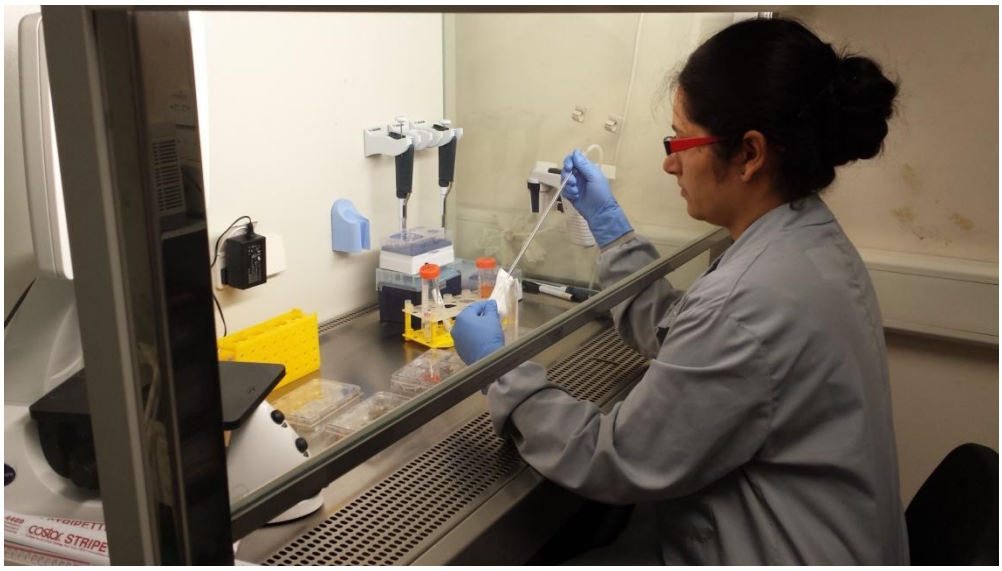
Baseline Assignment

Write an essay on the embryonic stem cells application considering ethical issues. (500 word limit).

1. What are the stem cells?
2. State types of stem cells.
3. What are embryonic stem cells?
4. Advantages of using embryonic stem cells.
5. What are the ethical issues associated with its applications of treating various human diseases?

Additionally prepare a 2 minute talk to present your views in the next tutorial.

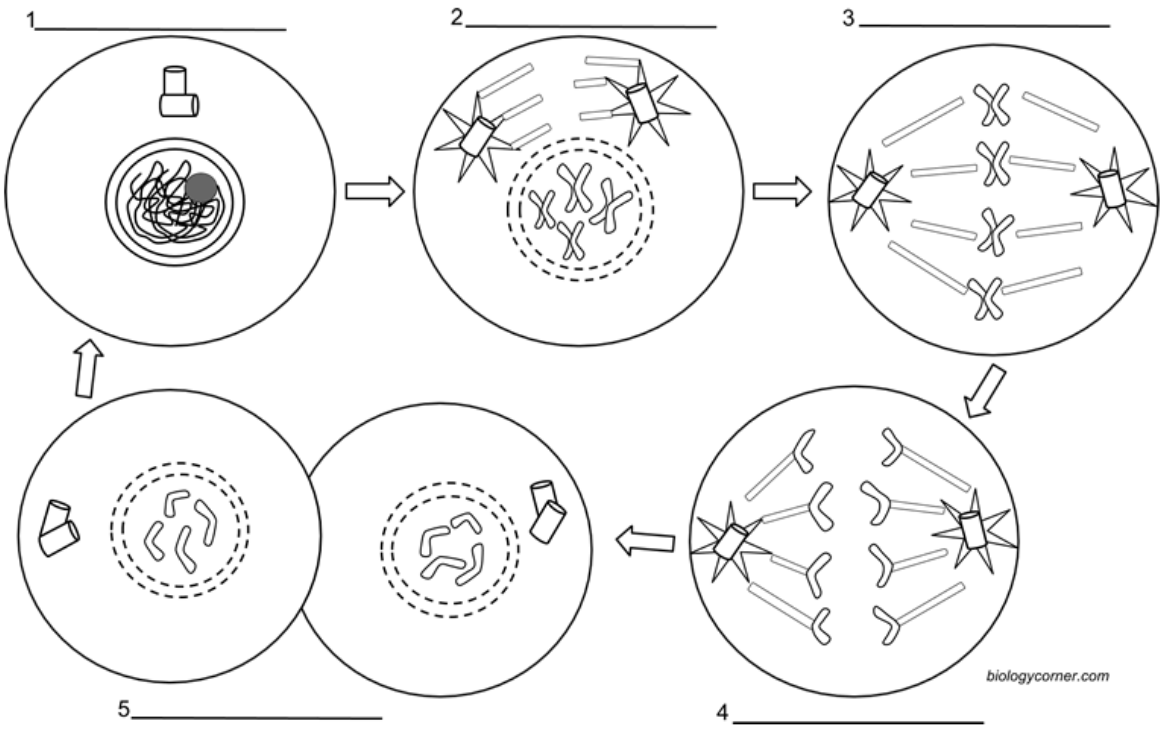
Tutorial 2 – Cell Culture – Growing living cells in the lab



What is the Purpose of Tutorial 2?

- Objective 1 To understand cell culture principles
- Objective 2 To consider growing cells in the petri dish
- Objective 3 To monitor/visualise cell growth

Activity 2.1: Cell division – A division of cell into two daughter cells with same genetic material.



Activity 2.2: Animal cell culture

Read the following passage and highlight key points. Share with your partner and discuss it.

What is Cell Culture?

Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established.

Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (i.e., reach **confluence**). At this stage, the cells have to be **subcultured** (i.e., passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

Finite vs Continuous Cell Line

Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as **senescence**; these cell lines are known as **finite**. However, some cell lines become immortal through a process called **transformation**, which can occur spontaneously or can be chemically or virally induced. When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a **continuous cell line**.

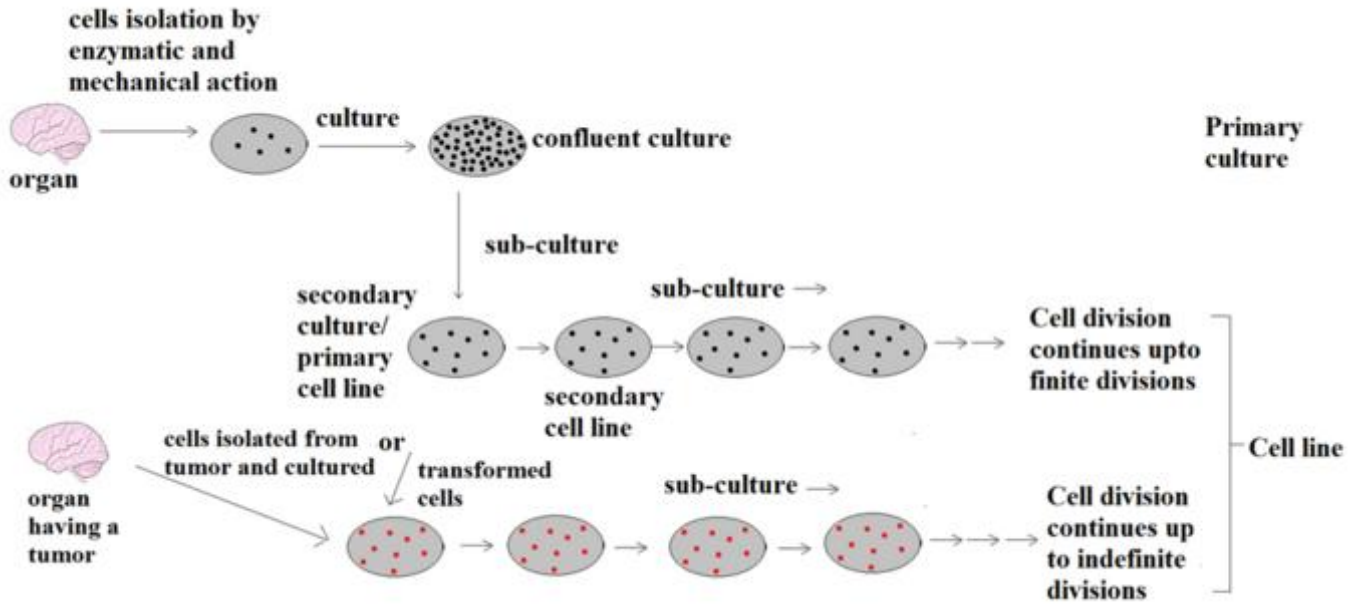


Figure 2.1: Primary cell culture and generation of cell line.

Culture Conditions

Culture conditions vary widely for each cell type, but the artificial environment in which the cells are cultured invariably consists of a suitable vessel containing the following:

- A substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals)
- Growth factors
- Hormones
- Gases (O_2, CO_2)
- A regulated physico-chemical environment (pH, osmotic pressure, temperature)

Most cells are **anchorage-dependent** and must be cultured while attached to a solid or semi-solid substrate (**adherent**or **monolayer culture**), while others can be grown floating in the culture medium (**suspension culture**).

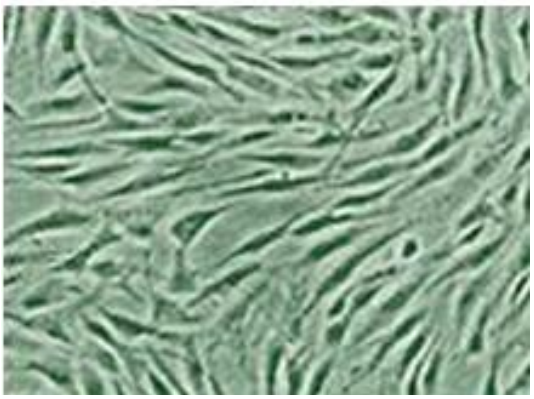
Cryopreservation

If a surplus of cells are available from subculturing, they should be treated with the appropriate protective agent (e.g., DMSO or glycerol) and stored at temperatures below −130°C (**cryopreservation**) until they are needed.

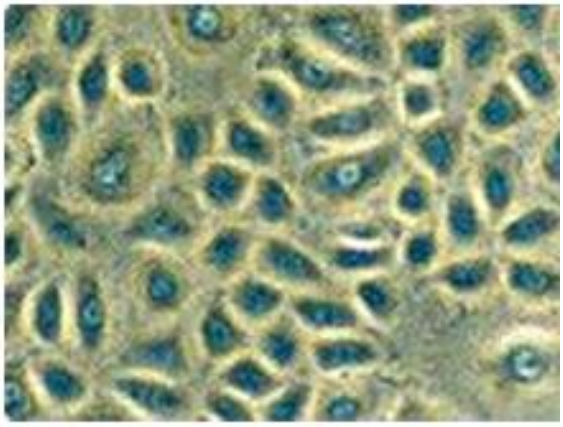
Morphology of Cells in Culture

Cells in culture can be divided into three basic categories based on their shape and appearance (i.e., **morphology**).

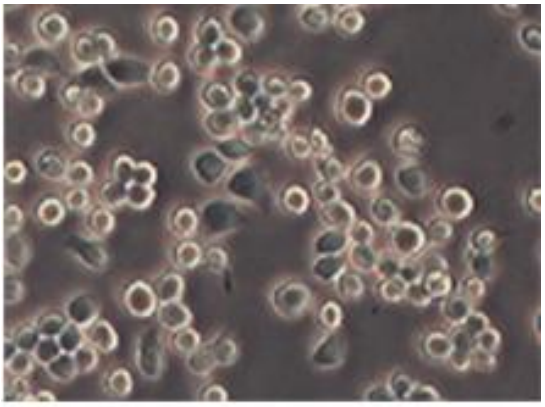
Fibroblastic (or fibroblast-like) cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate.



Epithelial-like cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.



Lymphoblast-like cells are spherical in shape and usually grown in suspension without attaching to a surface.



Applications of Cell Culture

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of drugs and toxic compounds on the cells, and mutagenesis and carcinogenesis. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins). The major advantage of using cell culture for any of these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

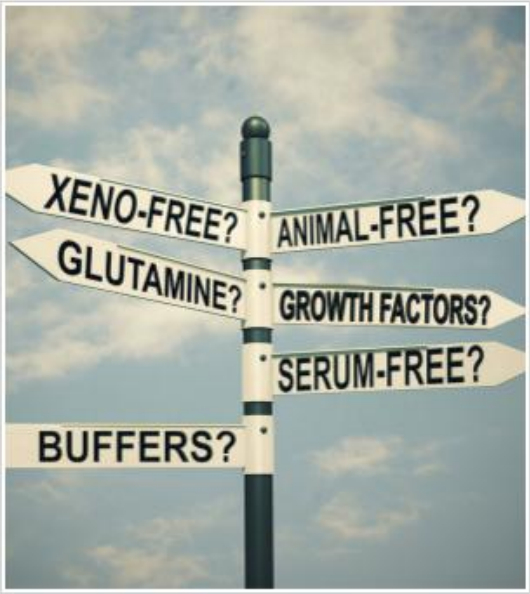
Activity 2.3: Read the passage on stem cell culture media components and extract couple of main points from each section.

Cell Culture Basics: Stem Cell Media – The "What" and "Why"

By bsargent on February 3, 2016

Like 0 Tweet Share G+

A guest blog by Lia Kent, Scientific Training, Biological Industries (BI)



Introduction

For over 30 years, Biological Industries has been developing specialized cell culture media for many cell types, including primary cells and stem cells. Over time we have seen a significant evolution of stem cell media as well as our ability to understand, evaluate, and optimize cell culture conditions. Cell culture is one of the most common and complex techniques used in the life sciences, and the media itself is a critical element for maintaining healthy, proliferating stem cells in culture. When you break it all down, all stem cell media essentially contain the same basic components: a basal medium, buffer system, glutamine, serum (or serum alternative), specific growth factors, and additional supplements. Here we look at a few of the main media components to better understand their importance and influence on cell cultures and how working with a highly pure and defined stem cell medium is key for regenerative medicine applications.

Basal Media

Classical basal media (DMEM, MEM, RPMI, etc.) are chemically defined formulations, each developed to support a particular cell line or culture condition. The main differences between the various classical basal media are the identity and quantity of buffers, salts, and growth supplements.

Many of the basal media were originally developed using mouse fibroblasts, HeLa, or CHO cell lines, and modifications over time have established basal media suitable for a wide range of cell types. DMEM/F12 is a popular stem cell basal medium, and a good example of this type of modification, as it is a 1:1 mixture of a complex medium (Ham's F12) and a medium with higher amino acid and vitamin concentrations (DMEM).

Interestingly, the names of classical basal media represent the researcher or the institute who developed them. (In the examples below, you can also see how basal media can be modified over time by multiple researchers.)

Examples:

- RPMI = Roosevelt Park Memorial Institute
- BME = Eagle's Basal Medium (Eagle = developing researcher)
- MEM = Modified Eagle's Medium (Eagle = developing researcher)
- DMEM = Dulbecco's Modified Eagle's Medium (Dulbecco = developing researcher)

Buffers

Stem cell media must match not only the basic nutrient needs of the cells in culture, but also be appropriate for the culture conditions being used. Most mammalian cells grow well at a pH of 7.4, and maintaining this pH is critical for stem cell cultures. When using vented flasks and culture dishes, the CO_2 gas in the atmosphere dissolves into the cell culture medium and establishes equilibrium with HCO_3-. Because CO_2 is acidic, the pH of the medium is lowered. Although salts and amino acids within the medium can provide some buffering capacity, additional buffering compounds are added to ensure that a proper physiological pH is maintained.

Sodium Bicarbonate

To stabilize changes in pH within a CO_2 incubator, **sodium bicarbonate** ($NaHCO_3$) is a non-toxic natural buffer commonly included in stem cell medium. As a general rule of thumb, 1.5 g/L of $NaHCO_3$ is needed to keep the medium at pH 7.4 when cultured in 5% CO_2 incubator.

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

$$NaHCO_3 \leftrightarrow Na^+ + HCO_3^-$$

Figure: CO_2 in cell culture media yields acidic conditions. Sodium bicarbonate buffers this reaction.

HEPES

HEPES is a zwitterionic organic buffer that is also used to maintain physiological pH of cell culture media. HEPES is recommended when the cell culture system is very sensitive, increasing the buffering capacity and stabilizing the pH within the range of 7.2 to 7.6, and is not dependent on CO_2 levels.

Phenol Red

In addition to CO_2 levels, cell metabolites, media additives, and a variety of other factors can also impact the pH of stem cell media. For this reason, **phenol red** is commonly included in stem cell media as a visual pH indicator. Media containing phenol red will appear red at a pH of 7.4, and will range from bright yellow to deep purple as the pH fluctuates. Media that appears yellow or pink/purple is generally unsuitable for stem cells, and indicates that the culture requires immediate attention (medium exchange, passaging, incubator gas adjustment, etc.). Choose a medium without phenol red if your cells are particularly estrogen-sensitive, as this compound mimics the actions of some steroid hormones.

pH

Figure 1: Phenol red pH chart — Media containing phenol red will appear red with a pH of 7.4, and will range from yellow to purple as the pH changes. Making a pH standard for a particular basal medium can be helpful and used in the lab as a more accurate visual reference.

Glutamine

Cells require nitrogen to build nucleotides, amino acids, and vitamins. **L-glutamine** is an essential amino acid that is a required additive to most cell culture media, facilitating the storage and transfer of nitrogen to the cells in culture. Glutamine is also one of the most readily available amino acids for use as an energy source when glucose levels are low and energy demands are high, especially for rapidly proliferating cell types or cells cultured under hypoxic conditions, where glucose metabolism is less efficient.

However, when dissolved in liquid media, free L-glutamine is quite unstable. At temperatures above 4°C, the L-glutamine within cell culture media non-enzymatically degrades into toxic ammonium and pyroglutamate by-products. This short half-life is why stock solutions of L-glutamine must be stored frozen at -20°C, and why L-glutamine should be added to freshly prepared media just prior to use.

L-alanyl-L-glutamine

In order to combat the rapid breakdown and increase glutamine stability within cell culture media, L-glutamine dipeptides are often substituted. **L-alanyl-L-glutamine**, for example, is a dipeptide that is stable in cell culture medium over longer periods of time, even at 37°C. The strong bonds within L-alanyl-L-glutamine stabilize the compound from degradation, while the L-glutamine itself is still readily accessible to the cells. Supplementing stem cell media with L-alanyl-L-glutamine can extend the shelf-life of the media at 4°C and greatly reduce the problems associated with the breakdown of glutamine into ammonia waste.

Growth Factors

Growth factors, **cytokines**, and **chemokines** are the chemical messengers of cells. They are small protein molecules naturally secreted by cells that induce a physiological effect (proliferation, growth, or differentiation) on the cells receiving the signal. Each stem cell type requires media supplemented with specific growth factors in order to maintain or direct the culture's health, proliferation, and differentiation. For example, **bFGF** (fibroblast growth factor-basic) is the key growth factor involved in maintaining pluripotency for human embryonic stem (ES) and induced pluripotent stem (iPS) cell cultures, while **LIF** (leukemia inhibitory factor) is the main regulator for mouse ES and iPS cells.

The quality of the growth factors used in stem cell media can have a profound impact on the culture. Growth factors used in cell culture media are generally recombinant proteins, and can be synthesized in multiple types of cells and species, including *E. coli*, CHO, and human lines. Always use growth factors with high biological activity (ED_{50}), high purity, and proper protein folding and other post-translational modifications for the best results. Growth factors and cytokines are stable when stored as lyophilized powders, but have a short shelf-life once added to media. To avoid degradation of these components, prepare small aliquots of concentrated stock solutions, and add them to media just before use.

Amino Acids

In addition to essential amino acids supplied in the basal medium and supplemented glutamine, **non-essential amino acids (NEAA)** are also often added to culture medium to stimulate proliferation and prolong the viability of the cells in culture. Although cells can naturally synthesize NEAA, supplementing them in the media reduces any potential side-effects that can affect cell growth when amino acid levels are low.

Serum

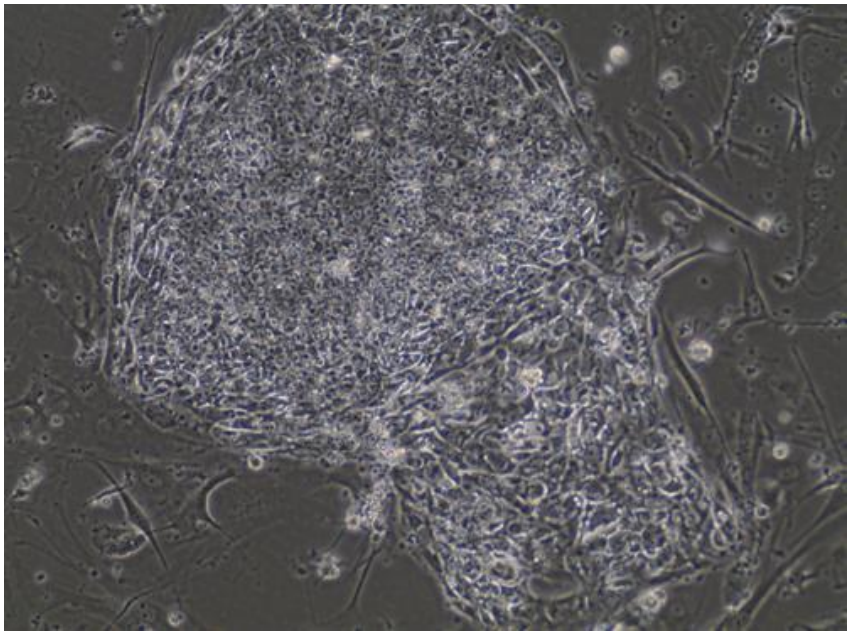
Most mammalian cell cultures require the addition of animal or human serum to the media. The basic definition of serum is "the liquid component of clotted blood." Serum for cell culture is a complex organic mixture of proteins, peptides, growth factors, amino acids, lipids, carbohydrates, hormones, vitamins, and many other components. Some cell cultures depend on serum to provide not only nutrients, but compounds that promote cell proliferation and attachment.

Stem cell media that includes serum commonly contains between 10 to 20% **fetal bovine serum (FBS)**. Although serum used in stem cell media generally undergoes numerous modification and purification steps, the end product will always hold the same key disadvantages for stem cell applications, notably that the material is undefined, highly variable between batches, and derived from an animal source.

Disadvantges of Serum in Media

- Risk of contamination (mycoplasma, viruses, etc.)
- Animal source material
- Undefined
- Variable in quality and composition
- Price

Tutorial 3 – iPS cells and reprogramming: turn any cell of the body into a stem cell



What is the Purpose of Tutorial 3?

- Objective 1 To understand how induced plutipotent stem cells are generated
- Objective 2 To consider reprogramming of the cells
- Objective 2 To understand transfection of cells

Induced Pluripotent Stem

Induced pluripotent stem cells (also known as iPS cells or iPSCs) are a type ofpluripotent stem cell that can be generated directly from adult cells by process know as reprogramming. Reprogramming allows us to turn any cell of the body (any age) into a pluripotent stem cell. Its discovery in 2006 by nobel laureate Shinya Yamanaka has opened up exciting possibilities for studying and treating disease.

How are these iPS cells made?

Yamanaka added four genes to skin cells (fibroblast) from a mouse, starting a process inside the cells called reprogramming. Within 2 – 3 weeks, the skin cells were transformed into induced pluripotent stem cells.

Activity 3.1 Generation of Induced Pluripotent Stem (iPS)Cells

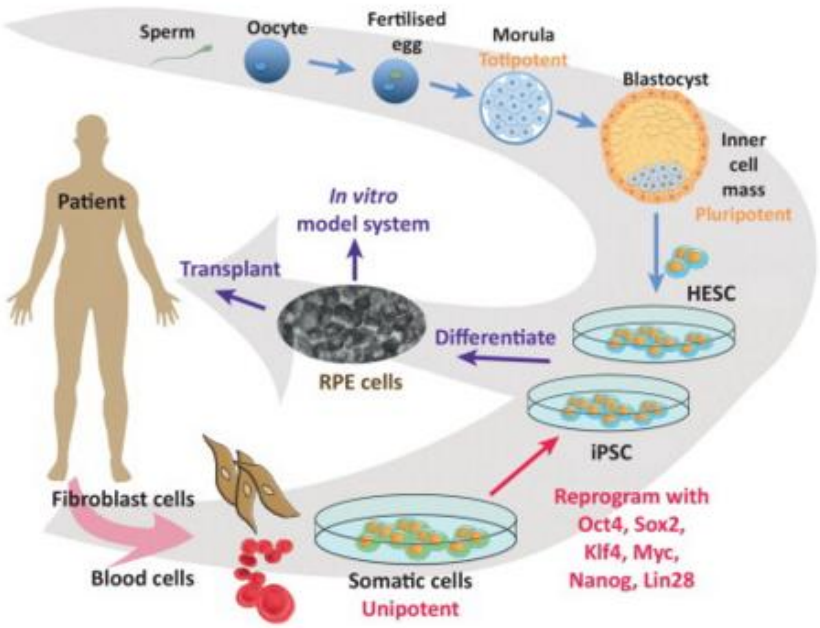


Figure 3.1 Schematic of the generation of the embryonic and induced pluripotent stem cells

1. Describe the generation of induced pluripotent stem cells?

2. State the differences between iPS cells from embryonic stem cells.

3. Describe phenotype and morphology of iPS cells.

Activity 3.2 Read the passages on the principles and mechanisms of mammalian cell transfection and highlight the scientific jargon, any ideas you are not sure of and finally summarise main points.

Mammalian cell transfection is a technique commonly used to express exogenous DNA or RNA in a host cell line (for example, for generating RNAi probes). There are many different ways to transfect mammalian cells, depending on the cell line characteristics, desired effect, and downstream applications.

Transient vs. stable transfection

Transient transfections are most commonly used to investigate the short-term impact of alterations in gene and protein expression. Plasmid DNA (pDNA), messenger RNA (mRNA), short-interfering RNA (siRNA), short-hairpin RNA (shRNA) and microRNA (miRNA) are commonly used in transfection experiments. During transient expression, however, the nucleic acid sequence is not integrated into the host cell genome. Therefore, the effect on target gene expression is temporary (24-72 hours for RNA probes, 48-96 hours for DNA probes).

On the other hand, when long-term expression of the sequence is desired, stable transfection protocols are used to generate permanent expression in cell lines. In this case, the transfected genetic material is integrated into the host cell genome. A selection marker is used to identify cells that have successfully integrated the sequence of interest. The selecting agent enriches and enables the growth of a subpopulation where the exogenous genetic material has been incorporated into the genome.

Transfection of DNA or RNA molecules into cultured mammalian cells can be accomplished using various methods and reagents. These methods include chemical (liposome-mediated, non-liposomal lipids, polyamines, dendrimers), physical (electroporation, microinjection) or viral-based (retrovirus, adeno-associated virus, lentivirus) delivery systems.

Transient transfection methods

- *Liposome-mediated transfection*

Liposomes are synthetic analogues of the phospholipid bilayer, the building block of the cellular membrane. These transfection compounds share a number of characteristics with their natural counterparts, including the presence of hydrophobic and hydrophilic regions of each molecule which allow for the formation of spheroid liposomes under aqueous conditions. In the presence of free DNA or RNA, liposomes encapsulate the nucleic acids to create an efficient delivery system. The charge, composition and structure of the liposome defines the affinity of the complex for the cellular membrane.

Under specific conditions, the liposome-nucleic acid complex is able to interact with the cell membrane to gain access into the cell by endocytosis and subsequently release the nucleic acids into the cytoplasm. There are several factors that determine the successful

delivery of nucleic acids by liposome into mammalian cells, including particle size, lipid formulation, charge ratio and the method of liposome preparation.

- *Non-liposomal transfection agents (lipids and polymers)*

Alternatives to liposomes include non-liposomal lipids and polymers capable of forming complexes with nucleic acid to form micelles, or tiny encapsulating droplets. The transfection is usually performed under aqueous conditions, which enables the lipophilic portion of the amphiphilic compound, or the part of the droplet that displays affinity for fatty-acid compounds such as the cell membrane, to form the micelle capsule that encases the exogenous nucleic acids.

- *Electroporation*

Electroporation is a highly efficient technique for delivering exogenous nucleic acids to suspension cells and non-adherent primary cells (like lymphocytes). This technique uses electricity to create transient pores (electropores) in the cellular membrane to enable the uptake of charged nucleic acid molecules (RNA or DNA) into the target cells.

Stable transfection methods

- *Microinjection*

In certain cases, permanent expression of a probe or gene of interest is desirable in your experimental system. This requires targeting specific cells within a population for gene delivery by microinjecting specific DNA sequences into the nuclei of target cells. The limitation of this method is that the number of cells that can effectively be transfected by this method is limited by the skill of the person performing the microinjection.

- *Virus-mediated gene delivery (transduction)*

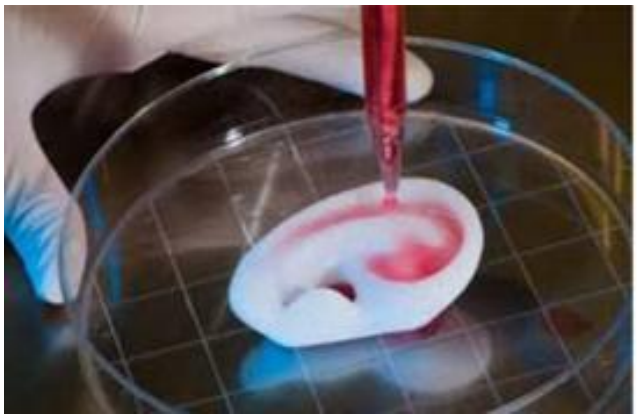
Exogenous genes or probes can be introduced into cultured mammalian cells by viral transduction techniques that use viruses as carriers. Viral delivery is useful for transfecting primary cell cultures, and there are numerous *in vivo* gene delivery approaches that allow the

delivered genetic material to be integrated into the target cell genome using the viral machinery. Often, a selection marker (either antibiotic-based or GFP-based) is used to select for cells that have been successfully transduced with the virus. Once the genetic material is incorporated into the host genome, it relies on the host transcriptional machinery for expression. In the case of shRNA/miRNA probes, RNAi machinery is required for processing. These gene therapy approaches, however, have limited clinical and laboratory uses because the viral vector integrates randomly into the genome and could produce significant bio-hazardous risks.

Homework Assignment 3

Find a journal article that describes the study of induced pluripotent stem cells. Summarise the findings of the chosen article and make some notes. You will be asked to present your work in the next tutorial.

Tutorial 4 – Tissue Engineering and Regenerative Medicine



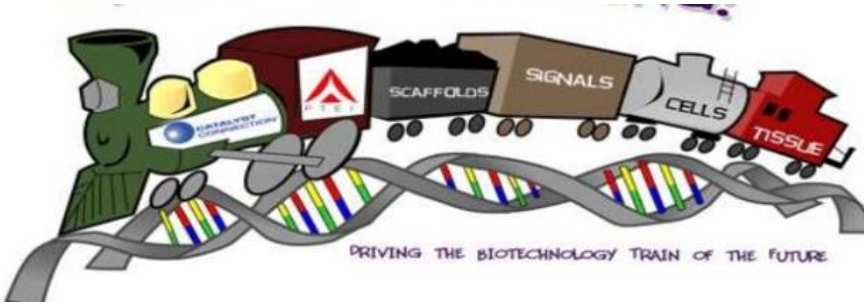
What is the Purpose of Tutorial 4?

Objective 1 To appreciate the concept of regenerating tissues in the lab

Objective 2 To understand tissue engineering

Objective 3 To consider different cell types used in tissue engineering – Stem cells

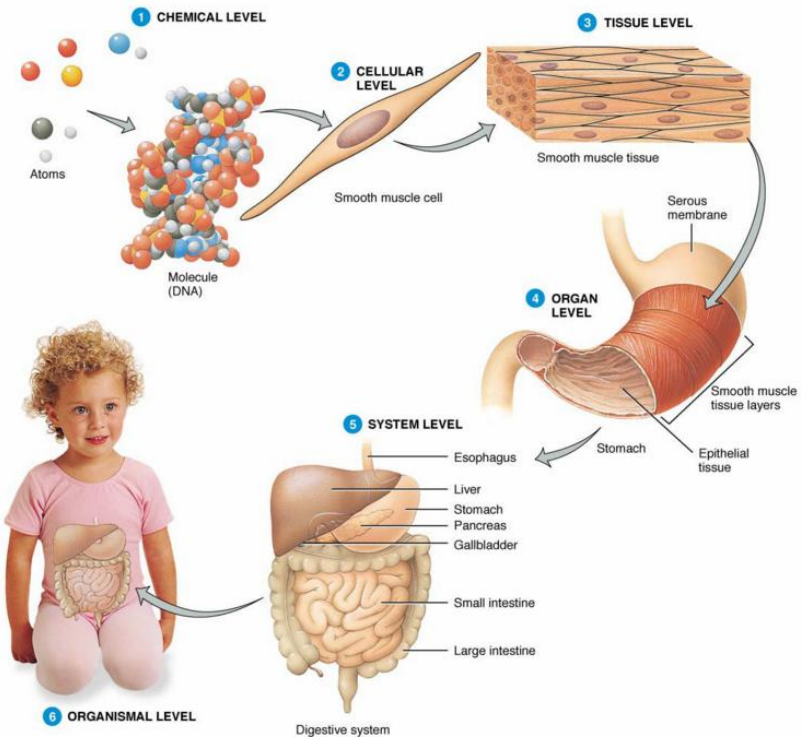
Tissue Engineering



Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue function.

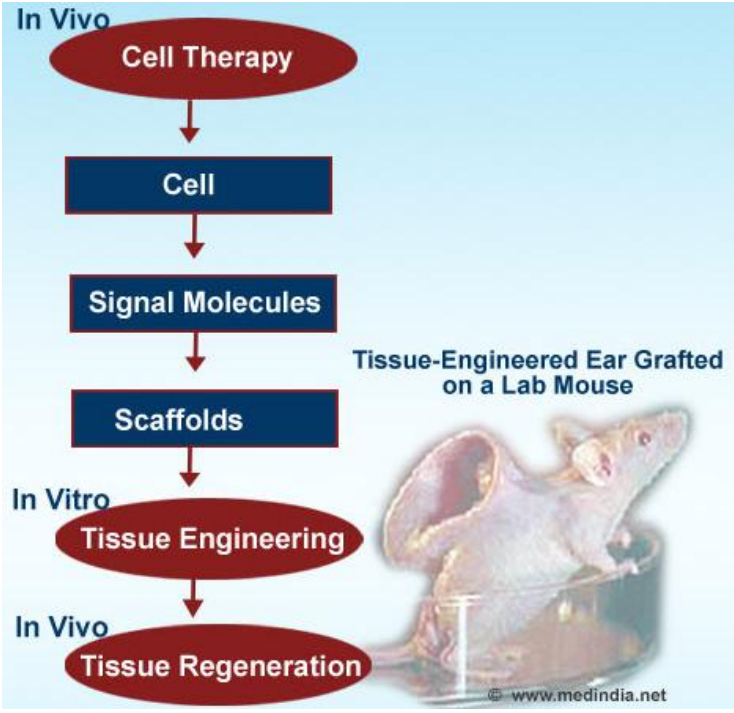
What do you understand by the term "Tissue Engineering"?

The hierarchy of human structure.

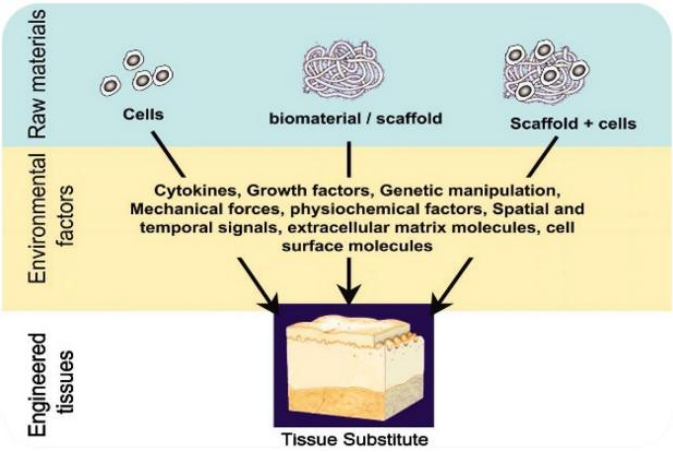


State the differences between invivo and invitro?

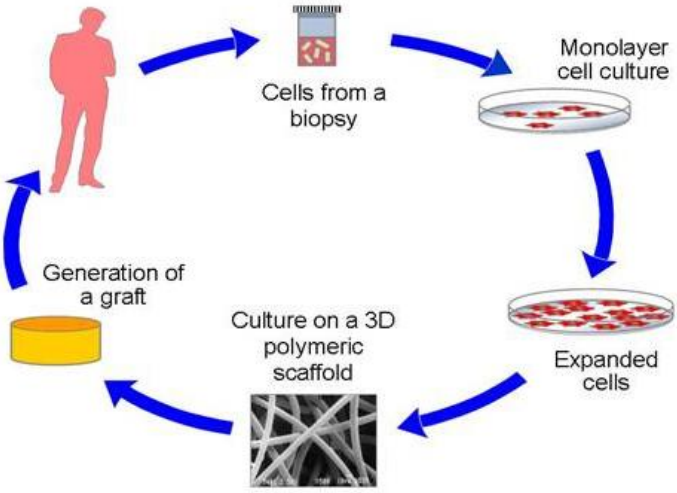
Strategies of Tissue Engineering



Prerequisite for Tissue Engineering



Basic principles of tissue engineering

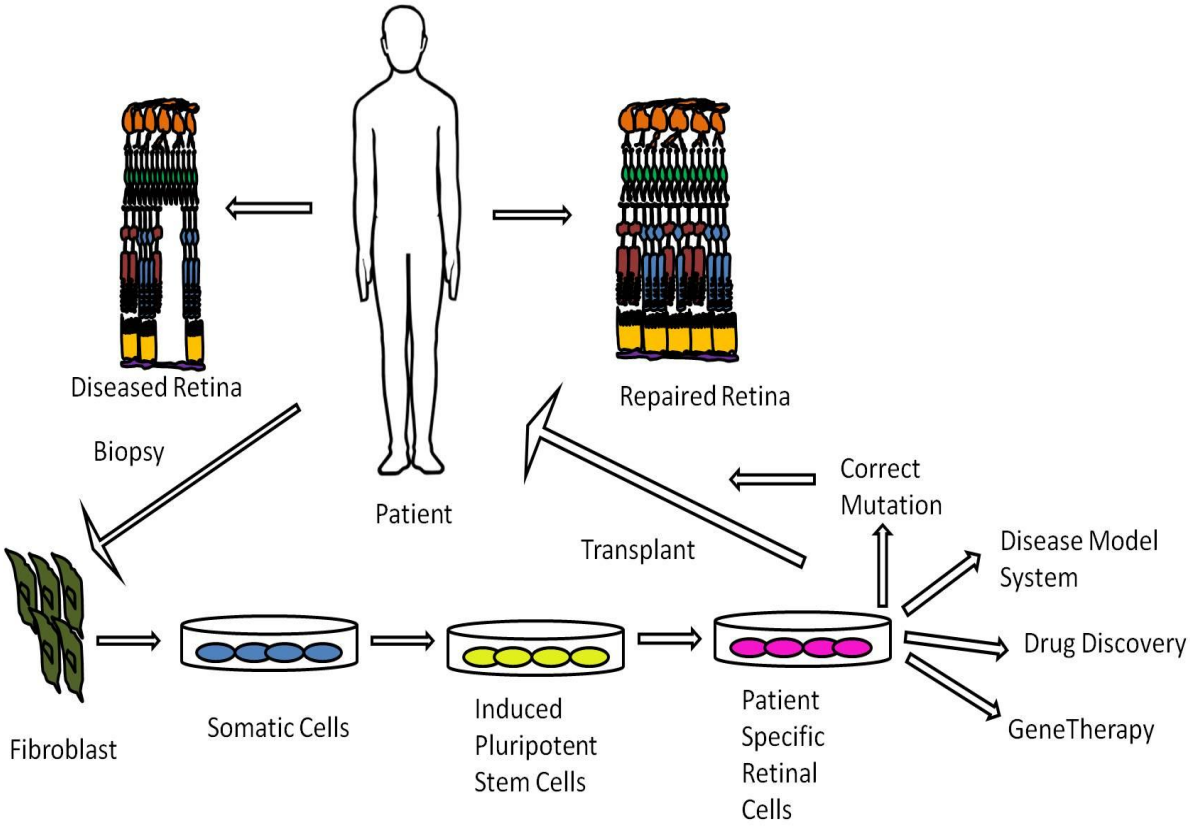


Name different types of the cell used in tissue engineering?

Homework Assignment 4

Give an example of tissue engineered structure. Describing which cells were used along with different materials. Was this construct implanted in an animal or human?

Tutorial 5 –Cell Therapy



What is the Purpose of Tutorial 5?

Objective 1 To consider usage of patient specific retinal cells

Objective 2 To think about clinical trails

Objective 3 To prepare for the final assignment

Activity 5.1 Summarise main idea from each paragraph.

In recent years, stem cells have often been portrayed by the news & media as a new wonder therapy that could regenerate virtually any damaged organs and tissues in the human body. But are stem cells really a new discovery and what is their impact in medicine?

Contrary to popular belief, it's been understood for millennia that different organs have regenerative potential. The Greek poet, Hesiod, recognised this ability in the liver over 2800 years ago when he wrote about the legend of Prometheus. Such observations prove true in our modern era when the liver can regenerate even after 70% of it is has been surgically removed. This process is now attributed to the presence of stem cells in this organ. However, it wasn't until 1868 that the term 'stem cell' was first used when the German scientist, Ernst Haechel, was studying evolution in multiple species including crustaceans. In his native language, he called it the 'stammzelle' (stem cell in English) and he recognised the unique capability of these cells: namely, to replicate and make more stem cells whilst also being able to produce differentiated cell types, such as heart, liver and blood cells.

Such stem cells found in organs of the adult body are known as 'adult stem cells'. Perhaps the most well studied of the adult stem cells are blood (or haematopoietic) stem cells. In the 1960s it was recognised that a blood transfusion from a healthy donor could restore the wellbeing of someone exposed to harmful levels of radiation. Many of these findings were precipitated following the horrific radiation injuries in civilian populations after atomic bombs were dropped on in Hiroshima and Nagasaki. Now blood transfusion is commonly-used to help the recovery of patients undergoing treatment for cancer. This is because the chemo- and radio-therapy used damages their own blood stem cells, which are replaced by an infusion of healthy stem cells from a suitable donor. Indeed, pioneering work in the field of blood transfusion led to Peter Medwar being awarded the Nobel Prize in Medicine in 1960. Other adult stem cells being investigated as therapies include those in skin (for treatment of burns), cornea (for repairing damage to the eye) and brain (for injuries such as stroke).

In parallel to the discoveries being made in adult stem cell research, work was starting with a different class of stem cells, known as the embryonic stem cells. During the 1960s and 70s, scientists found that testicular cancers formed bizarre tumour masses that didn't just contain testicular material but also had hair, bone, neurons and so on. The cause of these strange tumours was identified as embryonal carcinoma cells, which are stem cells that have become mutated. A few years later in 1981, better behaved embryonic stem cells were isolated from mouse embryos. These remarkable cells could be grown in the lab for long

periods of time and yet be coaxed into become virtually every cell type in the mouse (about 200 different cell types). Indeed, when implanted into early stage mouse embryos, these embryonic stem cells can contribute to every tissue of the adult mouse. The embryonic stem cells could also be genetically modified and used to make new strains of mice with specifically-engineered changes in their genome. This finding revolutionised our understanding of genetics and disease, which ultimately led to the Novel Prize in Medicine being jointly awarded to Mario Capecchi, Martin Evans and Oliver Smithies in 2007.

It took another 17 years before human embryonic stem cells (hESC) were isolated in 1998, largely because different conditions were needed to grow these cells relative to their mouse counterparts and the ethics of working with human embryos. Close regulation by government groups such as the Human Fertilisation & Embryology Authority (HFEA) make sure all work is carried out in an ethical manner. Thus, hESCs can only be produced from spare embryos donated by consenting couples undergoing in vitro fertilisation (IVF, or test-tube baby) treatment. The hESCs are often considered as master or 'pluripotent' stem cells because they make many if not all of the cell types in the human body. Unlike in the mouse, the intention with hESCs is not to make new humans but rather to use their power to make replacement cells for sick patients. This is slow and painstaking work but the first clinical trials have begun to repair macular degeneration (eye disease) by the US company Advance Cell Technologies (ACT) with initial results expected from just a few patients in 2014.

A major breakthrough came with cloning of Dolly the Sheep in 1997 by Keith Campbell, Bill Ritchie and Ian Wilmut at the Roslin Institute in Scotland. This work disproved the central dogma that stated development was unidirectional i.e. the embryo becomes the fetus which becomes the adult. In these 'cloning' experiments, the nucleus of a fertilised sheep egg was replaced with the nucleus from an udder cell. This 'reconstructed nuclear transfer embryo' was transplanted into a surrogate sheep mother, which gave birth to Dolly. Since this landmark discovery, many scientists worked tirelessly to see if different somatic cells (e.g. skin cells, blood cells etc) could be converted into stem cells in the lab but without the need for nuclear transfer and fertilised eggs, which is ethically-sensitive in many countries.

The major breakthrough came in 2006 by Shinya Yamanaka in Japan. To the astonishment of the world's scientific community, his group showed skin

cells from a mouse could be 'reprogrammed' into stem cells by adding just 4 genetic factors (Oct4, Sox2, Klf4 and cMyc), now known as the Yamanaka Factors. In 2007, Yamanaka went on to show this process also worked in human cells in a process called 'induced pluripotency' giving rise to human induced pluripotent stem cells (hiPSC). Already, hiPSC have been used to produce retinal cells, which are being transplanted into patients with eye disease in clinical trials in Japan.

It is noteworthy that transplantation is not the only use for hiPSCs and hESCs. For example, new drugs are currently tested in animals to determine whether unwanted side effects occur in organs such as the heart, liver and brain. These cell types can be made from hESC and hiPSC, so there is growing interest by the pharmaceutical industry in reducing or replacing animals with drug testing platforms that use human stem cells instead. Furthermore, because hiPSC can be made from patients who harbour genetic disorders, an exploding area of research is to recreate these disorders in the lab in what is becoming known as 'disease in a dish' technology. This gives new opportunities to better understand these disorders and develop novel drugs or genetic therapies. It is for these reasons that the Nobel Prize for Medicine in 2012 was awarded jointly to John Gurdon in the UK for his early work on reprogramming and to Shinya Yamanaka for his work on hiPSC.

Unequivocally, the long history of stem cells shows their potential in biomedicine and there is every indication that this utility will expand in the future. However, progressing stem cells from bench to bedside takes decades of hard, slow work, which is not so exciting for the media to present. Nevertheless, the coming years should prove to be an exciting time for stem cell research and medicine.

Induced Pluripotent Stem Cells Applications

Patient-specific iPSC can be generated using skin biopsies (or other tissue biopsies) to obtain fibroblasts (or other somatic cells) that can be reprogrammed using specific ''reprogramming transcription factors''. Following iPSC expansion and differentiation into retinal cells (photoreceptors or RPE or laminated retinal tissue), these cells could be transplanted back into the patient to repair retinal structures and restore vision. This technology can be further used for various applications such as disease modeling, drug screening, and gene therapy.

Transplantation of iPSC-derived RPE sheet into first AMD patient (2013)

Using iPSC-derived RPE cells started in 2013 at the RIKEN Center for Developmental Biology in Japan. iPSC lines were generated from AMD patients' skin cells and differentiated into RPE cells that were then seeded onto collagen gel forming a monolayer without synthetic scaffold or matrices. Once confluence was achieved, the constructs were treated with collagenase, resulting in a sheet of RPE cells ready for transplantation (1.3mm by 3.0 mm) into the damaged patient retina (figure 5.1).

Figure 5.1 RPE sheet generated from patient's iPS cells

A 70-year-old female patient suffering from exudative age-related macular degeneration (AMD) became the world's first recipient of an experimental transplant of a cell sheet derived from induced pluripotent stem cell (iPSCs).

Final Assignment

Write a review article on Can/Should we grow own body parts? You are given an opportunity to select which question you want to write essay on.

You are encouraged to use tables and figures in your report. Below I have listed some of question that will help you to get started with the article. You have to answer these questions using the information gained from reading the scientific articles.

However you still have to structure your report and the writing should be coherent. Referencing is very important so make sure you use information from the scientific articles.

Can we grow own body parts?

1. What are the stem cells?
2. State types of stem cells
3. What are induced pluripotent stem cells?
4. How are they generated etc?
5. Explain the difference between embryonic and iPS cells.
6. Advantages of iPS cells over embryonic.
7. Any example of application of iPS cells in treating any diseases.
8. Challenges for making transition from bench to bed side.

Should we grow own body parts?

1. What are the stem cells?
2. State types of stem cells
3. What are induced pluripotent stem cells?
4. How are they generated etc?
5. Explain the difference between embryonic and iPS cells.
6. Advantages of iPS cells over embryonic.
7. Ethical issues concerning usage of stem cells giving examples.

Report Structure

- Introduction
- Main Body
- Conclusion
- References

Tutorial 6 – Feedback tutorial

What is the Purpose of Tutorial 6?

- To receive feedback on final assignments.
- To share examples of best practice with the other pupils in your group.
- To write targets for improvement in school lessons.
- To reflect on the programme including what was enjoyed and what was challenging.

Final assignment feedback

What I did well…	What I could have improved on…
•	•
•	•
•	•

My target for future work is…

Tutorial 7 – Feedback tutorial

What is the Purpose of Tutorial 7?

- To reflect on what went well in the program
- To identify what could have been better
- Overall achievement from the program

Reflecting on Uni Pathways

What did you most enjoy about Uni Pathways?
•   •   •

What did you find challenging about the programme?	How did you overcome these challenges?
• • •	• • •

Appendix 1 – Referencing correctly

When you get to university, you will need to include references in the assignments that you write, so we would like you to start getting into the habit of referencing in your Brilliant Club assignment. This is really important, because it will help you to avoid plagiarism. Plagiarism is when you take someone else's work or ideas and pass them off as your own. Whether plagiarism is deliberate or accidental, the consequences can be severe. In order to avoid losing marks in your final assignment, or even failing, you must be careful to reference your sources correctly.

What is a reference?

A reference is just a note in your assignment which says if you have referred to or been influenced by another source such as book, website or article. For example, if you use the internet to research a particular subject, and you want to include a specific piece of information from this website, you will need to reference it.

Why should I reference?

Referencing is important in your work for the following reasons:

- It gives credit to the authors of any sources you have referred to or been influenced by.
- It supports the arguments you make in your assignments.
- It demonstrates the variety of sources you have used.
- It helps to prevent you losing marks, or failing, due to plagiarism.

When should I use a reference?

You should use a reference when you:

- Quote directly from another source.
- Summarise or rephrase another piece of work.
- Include a specific statistic or fact from a source.

How do I reference?

There are a number of different ways of referencing, and these often vary depending on what subject you are studying. The most important to

thing is to be consistent. This means that you need to stick to the same system throughout your whole assignment. Here is a basic system of referencing that you can use, which consists of the following two parts:

1. **A marker in your assignment**: After you have used a reference in your assignment (you have read something and included it in your work as a quote, or re-written it your own words) you should mark this is in your text with a number, e.g. [1]. The next time you use a reference you should use the next number, e.g. [2].
2. **Bibliography**: This is just a list of the references you have used in your assignment. In the bibliography, you list your references by the numbers you have used, and include as much information as you have about the reference. The list below gives what should be included for different sources.

   a. **Websites** – Author (if possible), title of the web page, website address, [date you accessed it, in square brackets].

   E.g. Dan Snow, 'How did so many soldiers survive the trenches?', http://www.bbc.co.uk/guides/z3kgjxs#zg2dtfr [11 July 2014].

   b. **Books** – Author, date published, title of book (in italics), pages where the information came from.

   E.g. S. Dubner and S. Levitt, (2006) *Freakonomics*, 7-9.

   c. **Articles** – Author, 'title of the article' (with quotation marks), *where the article comes from* (newspaper, journal etc.), date of the article.

   E.g. Maev Kennedy, 'The lights to go out across the UK to mark First World War's centenary', Guardian, 10 July 2014.

Notes

Can/Should We Grow Our Body Parts?

Key Stage 4 Programme

researchersinschools.org