WORKING TITLE: STEM CELLS AND THEIR USES

# FORWARD: AIM OF THE BOOK

The aim of this book is to provide information on stem cell biology, the use of stem cells and the ethical issues surrounding them in a format that allows the reader to determine the depth of their engagement with the subject. By writing a book that caters to all levels of scientific knowledge there is a danger that it becomes too generalised, however, the hope is that this book will provide a reference text applicable to all.

Unfortunately there is limited information that is publicly available and accessible to readers with no scientific background. How then can one be expected to generate an informed opinion of the topic

**basic**

**NO SCIENTIFIC TRAINING?**

In writing this book it was important to provide access to all readers irrespective of scientific training. The wide ranging and far reaching implications of stem cell research mean that everyone should have a basic understanding of the issues surrounding stem cells. Experimentation with, and utilisation of stem cells is already starting to shape the future of medicine and will start to directly affect routine medical treatments in the near future. The very fact that you are reading this book suggests that stem cell research has somehow touched your life.

If you are looking for a brief overview of stem cells, their uses and ongoing research the first section of each chapter will give a clear overview of the field free from scientific jargon. It is likely that many readers are primarily interested in the therapeutic implications of stem cells as a route to treating a family member affected by degenerative disease. For this reason later chapters on stem cell therapies are grouped by disease states rather than by stem cell type. Links are also provided to the latest research and current clinical trials.

Whilst much of this information is widely available on the Internet this book aims to bring together the key findings and provide sources of further information in a clear, unbiased and user friendly manner. It is also important that the book dispels myths and false promises of stem cell therapies in order to give the reader realistic expectations of current and future therapies and in turn allow them to make critical judgements on commercial therapies that are offered.

**advanced**

**Studying biology at university?**

More advanced readers will appreciate the option to continue through the chapter and access more in depth analysis of the latest research and take advantage of the fully referenced text and hyperlinked articles.

**expert**

**Currently researching stem cells?**

With the specialisation that occurs at the front-line of scientific research it is not always easy to keep abreast of the wider implications of stem cells or developments. The hope is that this book will provide you with enough information to give you an insight in to other stem cell fields whilst also providing links to seminal research papers allowing you to rapidly broaden your knowledge.

### Each chapter will have:

**Title**

**Learning outcomes -** learning outcomes may be wrong name you want the lay person to learn about the same things as the more advanced people just in less depth

**Required knowledge -** sources to find this i.e. cell biology and link to websites or books

**Overview -** lay person text

**Intermediate text -** Undergrad text - requires a knowledge of american and british syllabus??

**Detailed text -** Post doc text

**References -** AT END OF CHAPTER RATHER THAN BOOK?

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[INTRODUCTION TO STEM CELLS - basic](#h.zi36kus8qu9k)

[THE CELL CYCLE AND STEM CELLS - advanced/expert](#h.k827kn57zz2g)

[WHY ARE THEY IMPORTANT? - Overview - basic /advanced](#h.mc40y6kuz3al)

[The properties of stem cells allow the development of therapies to regenerate damaged tissue - Regenerative medicine](#h.wdu0bfayonou)

[May allow treatment of previously untreatable diseases/conditions](#h.whtya0fb0tfs)

[Study of development](#h.whtya0fb0tfs)

[Study of tissue](#h.w683evssn2md)

[Study of rare diseases and developmental disorders](#h.slslg1kbfm1a)

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[TYPES OF STEM CELLS - DEVELOPMENT - basic](#h.e5uk7ayxw347)

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[Embryonic:](#h.n5c4bb4vvxwc)

[Mouse](#h.rzjp1ip07tot)

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[THE MOVE TO XENO FREE CULTURE](#h.9rbxn9aqo2t0)

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[Tra-1-60 and Tra-1-80](#h.whpwzm80letj)

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[Somatic Cell Nuclear Transfer (SCNT)](#h.4qbn2nfhlkqt)

[Somatic stem cells - adult - HSC](#h.7y2m3olc15ud)

[Somatic stem cells - adult - MSC](#h.5n6ypwqzorng)

[Sources](#h.a4ohs3yks7y0)

[Multipotent](#h.hdg4xf5jppj)

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[MSC from ESC](#h.8s28ktc6890d)

[Current MSC usage](#h.wmm0nbyvyhcd)

[IPS](#h.l1279fhu63kd)

[human IPSC](#h.hb3m5337urmc)

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[IPS characteristics](#h.euebn46tazak)

[benifits of IPSC](#h.braucxhl0iam)

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[Summary of cell types? table?](#h.krtktj75zytm)

[2. The uses of stem cells -](#h.sfnd0ri1c8sk)

[Drug testing and assay development](#h.3o3lzp5n0l08)

[EACH STEM CELL TYPE TO BE NEW CHAPTER WITH BASIC INTRO THROUGH TO MORE DETAILED CULTURE ETC. EACH DIFFERENT AREA SUCH AS ETHICS OR USES TO HAVE BASIC INTRO](#h.nax6y5stqycm)

[Regenerative medicine](#h.3aovw1n73uw8)

[Tissue engineering](#h.b94r7lcqbyhc)

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[ECM matrix](#h.mcr8qck2i859)

[Tissue regeneration examples - NAME BY DISEASE RATHER THAN TISSUE](#h.gkfjehqqhkuj)

[Cartilage - FOCAL CARTILAGE DEFECTS, OA AND RA](#h.n8h5t2on643l)

[vascular -](#h.vcvma4kwemc7)

[blood - TREATMENT OF LEUKAEMIA AND BLOOD CANCERS, MYLOMA](#h.n7k0e7j6t1ch)

[liver -](#h.uj3tag8hcqev)

[bone - NON UNION FRACTURES, BONE CANCERS](#h.510vk36ujvyp)

[eye - GLAUCOMA,](#h.eckf3ijgbo4q)

[skin - TRAUMA, SKIN GRAFTS, MYSKIN](#h.kcxkf0qo3exb)

[heart - HEART DEFECTS DUE TO ISCHAEMIA FROM CORONARY EVENT](#h.s86xu5vs2e03)

[neurons - STROKE, PARKINSONS,](#h.fjv7ruu5g89z)

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[Importance of scaffolds and vascular](#h.tlbrmdfhbzb5)

[3. Regulations (and ethics?)](#h.gbpcnix5i54v)

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[What do the regulations say](#h.e2a1e8fb7ujj)

[Who regulates](#h.2eonmo8xmj36)

[Patents](#h.gjaug7bsll58)

[4. Future of stem cells](#h.yr3dzqitgpgr)

[What does the failure of Geron mean?](#h.tz536c56q6x3)

[Can we afford stem cell therapies?](#h.tz536c56q6x3)

[Is there a place for hESC with IPS](#h.tz536c56q6x3)

[5. Glossary](#h.po1lln78sx6p)

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# 1. WHAT IS A STEM CELL AND WHY ARE THEY IMPORTANT?

Scientific American.(2005). "The Future of Stem Cells." Financial Times and

Scientific American. New York. June 20. 35. <http://news.ft.com/reports/stemcells2005> in docs

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**Suppliment issues of nature:**

Invitrogen/Nature. (2006). "Insight: Stem Cells." Invitrogen and Nature Publishing.

June 29. 1059-102. <http://www.nature.com/nature/supplements/insights/stem_cells/index.html> includes useful glossary

Insight stem cells tech features

<http://www.nature.com/nature/supplements/tech/7180/index.html>

Nature insight: epigenetics

<http://www.nature.com/nature/supplements/insights/epigenetics/index.html>

For more information on genomic imprinting please see the reviews by Li and Pray (103, 104).

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## INTRODUCTION TO STEM CELLS - basic

REQUIRED KNOWLEDGE:

1. Basic knowledge of the cell - <http://en.wikipedia.org/wiki/Cell_(biology)>

Your body is made up of around 50 trillion microscopic cells, the vast majority of which perform specialised tasks such as producing stomach acid to break down food, or contracting as part of a beating heart. These specialised cells do not divide and instead will continue to perform their specialised function for the rest of their lifespan, the length of which can range from years for bone and neural cells to hours in the case of some blood cells. Once cells die or are damaged they need to be replaced in order for the body to continue to function as normal. On average your body replaces a trillion worn out blood cells every day <http://www.ncbi.nlm.nih.gov/pubmed/8499622> . That's 1,000,000,000,000 or over 11 million cells every second. If the majority of cells in the body do not divide then where do these new cells come from? The answer is stem cells. Only a small number of stem cells are needed to produce the vast numbers of cells that are lost every day due to normal wear and tear (FIG1). It is this fantastic ability to reproduce and repopulate the body that has lead researchers to ask whether stem cells can be harnessed as a therapy.

FIGURE 1 - COMPACT COLOUR

FIGURE 1

A single stem cell is able to divide to produce both a replicate of itself and produce a more specialised progenitor cell. Through successive rounds of division these progenitor cells gain specialised features and loose proliferative potential such that they ultimately differentiate in to a highly specialised non-dividing cellular population (tissue).

The fundamental definition of a stem cell is a cell that can divide and give rise to either new identical stem cells or daughter cells that are further specialised (differentiated) in some way. Depending on the timing and external factors the ratio of each type of cell generated may change. The retention of stem cell properties (sometimes referred to loosely as 'stemness') by the parent cell ensures that stem cell pool is replenished and allows it to divide almost without limit. Daughter cells may have a variety of properties depending on the parent stem cell but in this generalise model the daughter cells have the characteristics of progenitor cells i.e. cells that are more specialised (differentiated) than the parent cell but still retain the ability to divide.

At each division the cell number doubles from 2 to 4 to 8 to 16 to 32 to 64 etc such that the growth of the cell population is exponential. Thus a small number of stem cells can give rise to large numbers of more specialised cells. The cells created during division are determined by the internal and external signals acting on the cell inducing them to become one particular cell type. Depending on the origin of the stem cell it may divide rapidly and regularly such as those that replace the blood, skin and the lining of the gut or may divide only when given specific signals such as in the pancreas and liver (marrow migrates to liver?) - bone.

A number of reports have been written to engage the general public and provide easily accessible information to the lay person such as (FT, NIH). The NIH website is also an excellent source of up to date information.

BRIEF HISTORY OF STEM CELLS

**UNIQUE PROPERTIES OF STEM CELLS**

Stem cells are referred to as unspecialised as they have no tissue specific features and thus cannot perform any specialised functions within the body. However, they give rise to cells that are able to specialise (differentiate) in to one of the many different cell types of the body. this process of differentiation will be cover later in the book but for now it is important to note that it is a multi stage process, determined by the parent stem cell and external factors. understanding these factors is critical for scientists to be able to control differentiation of stem cells to produce the cells of interest.

stem cell fate was originally thought to be determined through the location and origin of a cell such that a stem cell in the ?? would only give rise to ?? cells but recently there have been cases where transdifferentiation has been reported.

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**SUMMARY**

This introduction has introduced the common elements stem cells it is important that the reader understands that in order to have an informed opinion of any stem cell research they must first understand the type of stem cell that is being refereeing to. A stem cell derived from waste liposuction fatty tissue will have very different properties and ethical issues than stem cells derived from human embryos. Later chapters are devoted to each of the types of stem cells but for now we will focus on some common features.

IMAGE?

**- advanced**

Using their ability to continually divide and replicate themselves stem cells are able to multiply and provide all the cell types within any multi-cellular organism. However, this definition is somewhat simplistic. there are many different types of stem cell and so using this term generically is not always wise, instead we should also refer to the potency of the cell to help us define it. the potency or plasticity of the cell is its ability to produce daughter cells of differing cell types **(see table 1)**. stem cells range from totipotent (able to form every cell type of a blastocyst), through to unipotent stem cells that can only give rise to one self and one specialised cell type per division.

Totipotent - the capacity to develop into every type of cell needed for full development, including extra­embryonic tissues such as the placenta and umbilical cord.

Pluripotent - the ability to give rise to types of cells that develop from the three germ layers (mesoderm, endoderm and ectoderm) from which all the cells in the body arise. Pluripotent cells thus have the potential to develop into every cell type in the human body, but cannot develop into an embryo on their own.

Multipotent - the potential to differentiate into a limited number of specific cell types in order to regenerate the tissue in which they normally reside.

**expert**

INSERT TABLE OF:

POTENCY / DEFINITION / EXAMPLE DAUGHTER CELL TYPE / reference?

Thus in order to fully understand stem cells and their uses we should consider each of the stem cell classes individually, this is covered in chapter **1.1?**

In order to understand what a stem cell is we need to look at where the various types of stem cell come from and how their origin affects their functional properties.

## THE CELL CYCLE AND STEM CELLS - advanced/expert

introduce the cell cycle, checkpoints and how stem cells are similar to cancer cells.

## 

## WHY ARE THEY IMPORTANT? - Overview - basic /advanced

The importance of stem cells is a result not only of their potential to allow researchers try and rebuild damaged or diseased organs but also in the tremendous potential they hold for allowing researchers to study developmental pathways as well as providing cells for clinical testing that may be more accurate, more cost effective and ethically more favourable than animal models.

### The properties of stem cells allow the development of therapies to regenerate damaged tissue - Regenerative medicine

### May allow treatment of previously untreatable diseases/conditions

### Study of development

### Study of tissue

### Study of rare diseases and developmental disorders

### Drug screening

## 

## TYPES OF STEM CELLS - DEVELOPMENT - basic

The majority of cells within your body are known as somatic cells and are differentiated in to highly specialised roles. These cells have little to no proliferative potential, **although some cells such as ….... have a mesenchymal cells and .... have the ability to repair local tissue to some extent.**

By observing that a single cell can give rise to an entire organism in development it is clear that during this period cells have the potential to form many different cell types and have a huge proliferative potential. It is also evident that as cells specialise (differentiate) they must loose this proliferative potential and ability to give rise to diverse cell types. Using this simple observation researchers could start looking for stem cells early in development.

### INTRODUCTION TO DEVELOPMENT **- advanced/expert**

In order to understand the origin of stem cells we first need to understand a little about mammalian development. What follows is a rather brief account of development as this subject is beyond the scope of this book.

At conception a single sperm penetrates an egg (oocyte) to fertilise it and bring about the process of cellular proliferation. The newly formed cell initially divides ...........

morula blastoma etc

epiblast

trophoblast

cleavage

meso endo and ecto derm layers

## THE STEM CELL NICHE

MENTION THAT THIS CONTROLS IF A STEM CELL STAYS A STEM CELLS AND WHAT IT WILL DIFFERENTIATE IN TO.

# 

# Fetal Stem Cells

EACH STEM CELL TYPE TO BE NEW CHAPTER WITH BASIC INTRO THROUGH TO MORE DETAILED CULTURE ETC. EACH DIFFERENT AREA SUCH AS ETHICS OR USES TO HAVE BASIC INTRO

INTRO

ORIGIN/SOURCE

* cord blood

INITIAL DERIVATION TECHNIQUES

MARKERS/IDENTIFICATION

USES

PROBLEMS

# 

# 

# Epiblast

EACH STEM CELL TYPE TO BE NEW CHAPTER WITH BASIC INTRO THROUGH TO MORE DETAILED CULTURE ETC. EACH DIFFERENT AREA SUCH AS ETHICS OR USES TO HAVE BASIC INTRO

Ebiblast stem cells are derived from the .....

### 

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# Embryonic:

EACH STEM CELL TYPE TO BE NEW CHAPTER WITH BASIC INTRO THROUGH TO MORE DETAILED CULTURE ETC. EACH DIFFERENT AREA SUCH AS ETHICS OR USES TO HAVE BASIC INTRO

ESC ARE NOT A REAL CELL TYPE IN THE NORMAL ORGANISM, INSTEAD THEY ARE A TRANSIENT STAGE. THEY ARE DO NOT EXIST FOR MORE THAN A FEW DAYS. this was part of the reason it tool so long to grow them in a lab as scientists tried to understand and then alter the factors trying to induce spontaneous differentiation.

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Start with history of ES culture and development. I.e. start with EC then mouse then human

* EC was first step to developing ES cells. Solter, D. (2006). "From teratocarcinomas to embryonic stem cells and beyond: A history of embyronic stem cell research." Nature Reviews Genetics 7(4): 319-27. <http://www.nature.com/nrg/journal/v7/n4/full/nrg1827.html>
* Mintz, B., et al. (1975). "Normal genetically mosaic mice produced from malignant teratocarcinoma cells." Proceedings of the National Academy of Sciences of the United States of America 72(9): 3585-89. **demonstration that meso ecto and endo formed from EC cells**
* because of their properties they were soon used as model of mammalian development Martin GR (1980). "Teratocarcinomas and mammalian embryogenesis". *Science* **209** (4458): 768–76. [doi](http://en.wikipedia.org/wiki/Digital_object_identifier):[10.1126/science.6250214](http://dx.doi.org/10.1126%2Fscience.6250214). [PMID](http://en.wikipedia.org/wiki/PubMed_Identifier) [6250214](http://www.ncbi.nlm.nih.gov/pubmed/6250214).
* ES cells first derived in 1981 by 2 groups
  + Evans, M.J., et al. (1981). "Establishment in culture of pluripotential cells from mouse embryos." Nature 292(5819): 154-6.
    - revealing a new technique for culturing the mouse embryos in the uterus to allow for an increase in cell number, allowing for the derivation of ES cells from these embryos
  + Martin, G.R. (1981). "Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells." Proceedings of the National Academy of Sciences of the United States of America 78(12): 7634-8.
    - published her paper in December and **coined the term “Embryonic Stem Cell”**.She showed that embryos could be cultured *in vitro* and that ES cells could be derived from these embryos

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### Mouse

Extraction and culture of ESCs was first attempted in the mouse model.

1. how
2. hanging drop
3. different lines
4. references
5. lif, BMP and single cell culture
6. basis of most of our knowledge of pluripotency genes etc
7. relevance to hESC

Mouse ES cells have been qualified by their ability to form chimeric mice. The ESC are implanted in to the blastocyst and allowed to develop undisturbed. The resulting mouse is then analysed for chimerism and the ability of the implanted ESC to contribute to all body tissues. This is the gold standard experiment. Although technically possible this experiment cannot be performed with human-mouse or human-human chimeras due to the ethical concerns surrounding this.

### Human

full definition including chimera and how technically no hESC is pluripotent.

Embryonic stem cells or ESCs are cells taken from a developing embryo immediately post gastrulation at around day 8 in humans. It is at this time that the so called inner cell mass of cells is formed.

* Embryonic stem cells are derived from the inner cell mass of the early embryo at the blastocyst stage.
* Initially demonstrated using mouse embryos in the 1960’s
* First demonstrated in human cells by Thomson et.al. in 1998 (science 288: 1145) <http://www.sciencemag.org/cgi/content/full/282/5391/1145>

#### Source

* hESC’s are derived from ‘waste’ fertilised embryos produced during IVF.
* The process is not perfect and many embryos are used for relatively few successful hESC cultures.
  + http://stemcells.nih.gov/info/ethics.asp
* However, once established cell lines can be maintained indefinitely (in theory).
* initially cells isolated using enzymatic methods (xeno) now have mechanical only methods

### THE MOVE TO XENO FREE CULTURE

* Remove mef
* remove matrigel
* cell isolation - problems with old non gmp lines
* route to fully defined culture system

#### initial culture methods - MEF

* Embryonic stem cells only exist in the developing embryo for a number of days. The prolonged culture of these cells is completely artificial.
* In order to maintain a pluripotent cell population special culture conditions are required.
* Originally hESC were derived on a mouse embryonic fibroblast (MEF) feeder layer.
* Feeder layers
* Labour intensive
* Difficult to scale up
* Lot to lot variability
* Introduction of pathogens
* MEF are irradiated. This operates to inhibit cell proliferation long before general metabolism is appreciably affected.
* More recently human cells have been utilised as feeder cells
  + Human fetal/epithelial cells – Richards et al (2002)
  + Derived from human ES – Stojkovic et al (2004)
* MEF thought to keep bFGF around longer - bFGF induces gremlin expression which in turn is known to inhibit the induction of differentiation by BMPs
  + MEF conditioning of media shown to reduce BMP activity - possibly because of FGF production and stabilisation.
* Fibroblasts secrete FGF as well as other growth factors
* thought that MEF can stabilise FGF2 possibly through protease inhibitors or binding proteins that modulate stability - mechanism unconfirmed
* degrades rapidly to levels below those need to maintain pluripotency unless very high levels are used - mTeSR
* MEF are irradiated to prevent proliferation - high energy irradiation operates to stop cell cell proliferation long before cell metabolism is appreciably affected
* some labs using human derived feeders
  + - fetal = Richard et al 2002
  + and even ES-derived feeders
    - stojkovic et al 2004
* In 1950’s discovered that MEF condition the media
  + Puck developed MEF system in 1955 to help with difficult to culture cell lines - adapted for mouse ES cells (Evans 1972) and then hESC

#### MEF FREE CULTURE

* feeder layers are very labour intensive, difficult to scale up and produce a lot of variability
* Feeder cells introduce potential contamination and complication of xeno/allogenic pathogens or cell byproducts

Ludwig-Thomson\_2006\_**Feeder-Independent\_Culture\_of\_Human\_Embryonic\_Stem\_Cells**.pdf

* Human cells can incorporate non-human slacilic acid (Martin 2005) = antigen and therefore creates immune response
* Subsequent developments allowed culture in conditioned media

**Basic Fibroblast Growth Factor Support of Human Embryonic Stem Cell Self-Renewal**

**Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells**

**Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells**

* Recently fully defined medias have been developed that can be made to clinical grade.
* Amit et al 2000 found you can replace serum in hESC cultures with MEF+bFGF+KOSR
* KOSR contains albumax - poorly defined lipid rich bovine albumin = problamatic for clinical use
* Xu et al 2001 developed feeder free system using same media
  + Nature biotech 24,185:187 **Derivation of human embryonic stem cells in defined conditions**
  + note called **Defined culture media for hESC**
* TeSR1 ludwig et al 2006
  + cDNA microanalysis formed basis of genes influenced and selected factorsfor texting

#### SUBSTRATE

* Matrigel
  + An extracellular matrix produced from mouse fibroblast cell line.
  + Xenogenic components
  + Batch to batch variablilty
  + Undefined
* Lamanin and defined substrates such as Synthemax

Synthetic Peptide-Acrylate Surfaces For Long-Term Self-Renewal and Cardiomyocyte Differentiation of Human Embryonic Stem Cells in Defined Medium. <http://www.nature.com/nbt/journal/v28/n6/full/nbt.1629.html>

* Generally hESC cannot be cultured as single cells - although there are many papers where people have done this so need good argument why its no good
  + No clonal amplification
  + More labour intensive
* Difficult to count
* Difficult to manipulate

#### hESC MARKERS

* Generally hESC cannot be cultured as single cells
  + No clonal amplification
  + More labour intensive
* Difficult to count
* Difficult to manipulate

#### 

#### PLURIPOTENCY

If hESC are pluripotent then cells should theoretically form all of the body tissues.

* How can we test this?
* Mouse embryonic stem cells can be used to form chimera
* hESC can only be analysed using a teratoma assay in humans.

#### EMBRYOID BODIES

* In routine culture hESC are maintained in a pluripotent state.
* If removed from the culture surface and allowed to differentiate cells will self assemble into embryoid bodies
* aggregates of cells
* originally imposed using hanging drop method but now use non-attachment plastic ware
* to a limited extent cells recapitulate embryonic development
  + Disorganized differentiation - unlike embryo
  + will form a hollow ball (cystic embryoid body)
  + cardiac myocytes form
  + AFP = fetal liver, beta 3 tubulin found only in neurons

Embryoid bodies analysed for 3 germ layers

* Mesoderm (smooth muscle)
* Endoderm (AFP)
* Ectoderm (beta 3 tubulin)

#### GENETIC CONTROL OF PLURIPOTENCY

##### Oct 4

* Octamer binding protein 4 (POU5F1) POU class 5 transcription factor 1.
* Homeodomain transcription factor.
* binds ATTTGCAT
* has homeodomain (a region coding for a protein domain that can bind DNA) ~60aa
* homeobox genes encode transcription factors that switch on cascades of other genes
  + specificity of homeobox is increased with interaction with other transcription factors
* Hox genes - determine embryonic regions along the anterio-posterior axis
  + typically found in organised cluster. Linear order of the genes is directly correlated to order of regions they affect as well as the timing in which they are affected.
* Oct4 -/- embryos only capable of forming trophectoderm
* Oct4 downregulated in the trophectoderm but readily detected in the ICM.
* Oct 4 expression starts in early embryo - lost during differentiation.
* Necessary but not sufficient to maintain pluripotency.
* Oct 4 overexpression can also cause differentiation - it is thought that a cofactor in a limiting amount acts with Oct4 and thus Oct4 overexpression may dilute the active complex
* Sox2 necessary as cofactor for Oct4 transcription of some genes

##### Sox2

* Transcription factor that plays an essential role in transcription of several Oct4 target genes.

##### Nanog

* Tir Nan Og ‘land of forever young’ in celtic mythology.
* Also homeodomain
* expressed in a restricted number of cell types and only a subset of Oct4 positive cells
* not present in unfertilised egg
* Helps to regulate pluripotency but is dependant upon Oct4
* not present in unfertilised egg
* expressed during ICM/blastocyst stage but decreases prior to implantation - presumably to decrease expression of pluripotent cells
* Nanog function requires presence of Oct4. Oct4 knockout cannot be rescued by overexpression of Nanog

#### hESC markers - not genetic control

##### 

##### Alkaline phosphatase

* not specific to ES cells
* function not fully understood
* Hydrolase enzyme responsible for dephosphorylating molecules such as nucleotides, protein and alkaloids
* under alkaline conditions enzyme present eveywhere but elevated in liver, kidney, bone, placenta and embryo

##### SSEA 3+4

* Stage specific embryonic antigen
* SSEA3 and 4 are synthesised during oogeniesis and are present on the membranes of primate and human oocytes, zygotes and early cleavage-stage embryos
* Human ESC do not express SSEA1

##### Tra-1-60 and Tra-1-80

* name of antibodies against unidentified proteins on hESC
* Tra stands for battle of Tragalgar!

**COULD USE THIS TO LEAD IN TO IPS CELLS?**

#### IMMORTALITY

Telomerase

* Allows hESC to escape senescence in culture.
* Prevents cellular ageing - immortal - similar to cancer cells
* Theoretically allows infinite expansion of an ESC culture.
* allows cells to maintain telomere length and not age between generations

#### RISKS OF hESC USAGE

* Uncontrolled teratoma formation.
  + hESC will need to be differentiated before use
* Allogenic immune response.
  + Immune suppression is associated with its own complications/risks
* Xenogenic components from MEF and culture media.

#### CURRENT USES

* GRNOPC1 – oligodendrocyte progenitor cells
  + Phase 1 clinical trial
  + <http://www.geron.com/GRNOPC1Trial/>
  + Preclinical safety studies
* 12 month teratoma studies
* 5 % contamination with hESC showed teratoma
* Cyst formation – unwanted tissue
* Demonstrated low susceptibility to major humoral response – short term immune suppression.
  + Stem cells 2009; 27:2126 Long-Term Safety and Function of RPE from Human Embryonic Stem Cells in Preclinical Models of Macular Degeneration

Stargardt’s Macular Dystrophy

* causes progressive vision loss, usually starting in children between 10 to 20 years of age.
* eye is immune privilaged site
* Eventually, blindness results from photoreceptor loss associated with degeneration in the pigmented layer of the retina, called the retinal pigment epithelium (RPE)
* RPE - tissue consists of single cell layer adjacent to the rod cells and cone photoreceptors and blood vessels of choroid
  + basal surface of these cells rests on complex ECM known as Bruch’s membrane. This epithelium develops from multipotent cells in optic neuroepithelium = indespensible for eye angiogenesis and vision
  + adult human RPE can transdifferentiate into neurons
  + cell death is important process in several diseases - impairs visual function of retina photoreceptors
  + form blood-retina barrier
  + important role in phagocytosis of shredded photoreceptor membrane and movement of ions and metabolites between retina and blood
  + In retina detachment RPE act to heal the wound by undergoing EMT = form fibrous tissue
  + impede the generation of activated T-cells by interfering with induction of high affinity IL2 receptors, IL2 production and expression and expression of CD71 and cyclin A
* macular degeneration = impossible to read/recognise faces
  + dry - cellular debris accumulate between retina and choroid and can detach retina
  + wet - blood vessels grow up from choroid and detach retina
* choroid - contains bllod supply to macula
* macula is yellow spot near center of retina ~5mm
  + 2 or more layers of ganglion cells
  + in center is the fovea (small pit responsible for central vision)
* wet = treated with laser coagulation and medication
* dry = Currently no non-hESC treatment
* Cyclosporine A used as an immunosupressant

**Long-Term Safety and Function of RPE from Human Embryonic Stem Cells in Preclinical Models of Macular Degeneration**

* RCS rat model which looses vision over several months due to genetic trait.
* rat model evidence that implanting RPE cells can sustain photoreceptors and control deterioration of visual function
* immune deficient mouse
* show up-regulation od RPE genes and protein
* show down regulation of ES genes and protein
* GMP standard culture of cells
  + Pathogen testing - FDA regulations
  + tested for mycoplasma/TEM for viral particles/PCR for reverse transcriptase (virus)/HIV/HBV/HCV/CMV/HTLV-1/Parvovirus BP1/Karyotype
  + data outsourced

Demonstrated teratomas with hESC

* No teratomas in 79 immune deficient mice (NIH III) with differentiated cells
* No teratomas in 18 experimental rats
* Cells were produced in GMP conditions
* Differentiated cells should also have karyotype checked
* Differentiation protocol:
  + makes EBs
  + after 6-8 weeks RPE cultures are observed (pigmentation) - picked and isolate
  + RPE ‘lines’ could be expanded >5 passages
  + frozen
  + thawed and implanted (no culture) into subretinal space
* fig 4 - dose response
* fig 5 - high cell doses show long lasting effects
* fig 6 - histology shows benifit of implant at p90 and human cells interacting with mouse
* ACT – macular degeneration
  + <http://www.advancedcell.com/documents/0000/0244/RPE_Derived_from_ESCs_chapter49.pdf>
  + Phase 1 clinical trial
  + http://advancedcell.com/news-and-media/press-releases/advanced-cell-technology-files-ind-with-fda-for-first-clinical-trial-using-embryonic-stem-cells-to-tr/

### Drug screening

* 1. mouse vs human
  2. Zeno free and gmp quality lines
  3. undefined nature

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# Somatic Cell Nuclear Transfer (SCNT)

# 

# Somatic stem cells - adult - HSC

# 

# Somatic stem cells - adult - MSC

EACH STEM CELL TYPE TO BE NEW CHAPTER WITH BASIC INTRO THROUGH TO MORE DETAILED CULTURE ETC. EACH DIFFERENT AREA SUCH AS ETHICS OR USES TO HAVE BASIC INTRO

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20.

Bjornson, C.R., et al. (1999). "Turning brain into blood: A hematopoietic fate

adopted by adult neural stem cells in vivo." Science 283(5401): 534-37.

21.

Munsie, M.J., et al. (2000). "Isolation of pluripotent embryonic stem cells from

reprogrammed adult mouse somatic cell nuclei." Current Biology 10(16): 989-92.

22.

Verfaillie, C. (2002). "Adult stem cells: assessing the case for pluripotency." Trends

in Cell Biology 12(11): 502-08.

PNAS **Transplanted bone marrow generates new neurons in human brains.**

<http://www.ncbi.nlm.nih.gov/pubmed?term=Transplanted%20bone%20marrow%20generates%20new%20neurons%20in%20human%20brains>

**OTHER REFS THAT SHOW TRANSDIFFERENTIATION:**

Nature reviews **HOW CELLS CHANGE THEIR PHENOTYPE** <http://www.ncbi.nlm.nih.gov/pubmed/11994739>

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* Function in the body to maintain or repair damaged tissue
* Bone marrow transplants have been around for 40 years.
* Stem cell types
  + Hematopoietic
  + Mesenchymal
  + Neural
  + Epithelial
  + skin
* mesenchyme = embryonic connective tissue derived from mesoderm and differentiates into haematopoietic and connective tissue
* stromal cells = connective tissue cells which describes some aspects of MSCs

#### Sources

* Can be isolated from bone marrow, fat, blood (low numbers) periosteum and amniotic fluid - see REFERENCE **Multilineage Potential of Adult Human Mesenchymal Stem Cells**
* Isolation from adult tissues avoids the ethical issues of hESCs
* differences in source affect phenotype

REFERENCE: **Derivation of Clinically Compliant MSCs from CD105, CD24 Differentiated Human ESCs**

#### Multipotent

* Will form bone (osteocytes), cartilage (chondrocytes) and fat (adipocytes)
* They cannot reconstitute an entire organ.
* Unlike hESC, MSCs can be cultured as single cells.
* Do not require feeder cells

neural stem cells = neurons, astrocytes and oligodendrocytes

epithelial stem cells = lining of gut - absorptive cells, goblet cells, paneth cells and enteroendocrine cells

skin stem cells = basal layer of epidermis at base of hair follicle - keratinocyte, hair follicle and epidermis

#### Pluripotent? Transdifferentiation

REFERENCES:

Yu-et-al\_2006\_**Human ES cells reprogram myeloid precursors following cell-cell fusion**.pdf

Since fused cells would have more than the normal number of chromosomes, this phenomenon can be assayed via karyotyping analysis,

#### Markers

* There is no single test to detect an MSC - not fully defined. unlikely to be general MSC marker because:
  + no known mouse counterpart
  + stro-1 not exclusive to MSCs
  + expression lost throughout culture expansion so only useful in initial isolation
  + function of many markers is unknown
* colony forming unit fibroblasts (CFU-F)
  + simple assay involving the production of colonies from sparesly plated cells
* Normal to define a population rather than individual cells.
  + Characterised based on CD markers
* Functional test of differentiation

CD markers

* cluster of differentiation is naming system for surface markers present on WBC
* >350 CD markers for humans
* used in cell sorting methods and flow cytometery
* only a fraction of CD markers characterised but most have an important function

CD105

* Aka Endoglin
* Transmembrane protein
* Binds to TGF beta
* Not specific to MSCs
  + Also expressed on vascular endothelial cells.

stro-1

* Best known marker of MSCs
* Stro-1 negative populations are not able to form CFU-Fs
* Not a definitive marker
  + Stro-1 is not exclusive to MSCs
  + Stro-1 disappears during culture

#### Autologous therapy

* MSCs can be extracted, expanded, differentiated? and re-implanted into the same individual.
* Likely to avoid an immune response
* They will not help if there is a genetic component to the disease.

#### Potential issues

* Rare cells
  + large volumes of tissue in painful procedures.
  + Patient may not have enough cells for therapy
  + MSC numbers and proliferation decline with age
  + mobilisation of stem cells - side effects of treatments
* Limited expansion due to senescence – no telomerase
* Heterogeneious mixture of cells
  + Differing proliferation rates
  + Wide variety of morphologies

#### MSC from ESC

MSCs are downstream of hESCs and many attempts have been made to create MSCs from hESC.

* Limited differentiation
* Loss of pluripotency and thus teratoma formation
* Expansion of hESC but safety of MSC

#### Current MSC usage

* Bone marrow transplants
* Many clinical trials
  + http://www.clinicaltrials.gov/

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# IPS

EACH STEM CELL TYPE TO BE NEW CHAPTER WITH BASIC INTRO THROUGH TO MORE DETAILED CULTURE ETC. EACH DIFFERENT AREA SUCH AS ETHICS OR USES TO HAVE BASIC INTRO

* Pluripotent stem cell derived from non-pluripotent differentiated cell.
* Cloning demonstrated that differentiated cells contain all the information needed to form an embryo.
* Initial discovery of IPS technology was a methodically driven experiment involving a huge amount of work.
* Using their knowledge of genes important in embryogenesis 24 genes were selected and expressed in a somatic cell (MEF - Mouse)
* To find out which genes were important 24 – 1 genes were expressed in every combination
* 10 genes were found to be essential for colony formation
* 10 – 1 genes were then expressed in every combination and 4 genes were found to be important.
* Expression of these 4 genes was sufficient to induce pluripotency
* C-Myc, Oct4, Sox 2 and KLF4

#### human IPSC

* Developed by 2 labs concurrently,
  + Thomson et al Science 23, 318
  + Takahashi Cell 30, 313
* Takahasi used same genes as in mice
  + C-Myc, Oct4, Sox 2 and KLF4
* Thomson replaced c-myc with LIN28

#### 

#### induction

* Virus mediated induction of gene expression
  + Associated with cancer
  + Not clinically attractive
  + Multiple vectors create multiple integration sites
* K, Kaji developed single vector allowing non-viral transfection methods
  + (Nature 458,711)
* Recently direct protein penetration methods have been developed.

Current Opinion in Biotechnology 2009, 20:516–521

#### IPS characteristics

* Show the same molecular markers as ESC
* Express telomerase
* Form teratomas
* Mouse cells used to form chimeras

#### benifits of IPSC

* Allow generation of disease specific lines
* Allow patient specific lines – no immune rejection
* No ethical concerns
* No cell shortage
* However,
  + Young technology
  + Concerns of genetically modified cells in clinical setting
  + Incidence of cancer in animal models is higher than hESC
* http://stemcells.nih.gov/info/basics/basics1.asp
  + http://stemcells.nih.gov/info/ethics.asp
* Science 288: 1145
* Current Opinion in Biotechnology 2009, 20:516–521
* Lodish mol cell bio, chapter 23
* Regulations in the UK
  + http://www.ema.europa.eu/ema/index.jsp?curl=pages/special\_topics/general/general\_content\_000471.jsp&murl=menus/special\_topics/special\_topics.jsp&mid=WC0b01ac058022857d

ips

* 1. personalised medicine
  2. clinical grade
  3. risks of virus and cancer
  4. study of disease (put this here as specific to this line)

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# Cancer Stem Cells

EACH STEM CELL TYPE TO BE NEW CHAPTER WITH BASIC INTRO THROUGH TO MORE DETAILED CULTURE ETC. EACH DIFFERENT AREA SUCH AS ETHICS OR USES TO HAVE BASIC INTRO

similarity between stem cells and cancer

# Summary of cell types? table?

## 

# 2. The uses of stem cells -

THE LAY PERSON WILL BE INTERESTED BECAUSE THEY HAVE THE DISEASE. THEREFORE STRUCTURE THE BOOK SO THAT THEY CAN SEARCH BY DISEASE AND SO THAT THEY CAN FIND OUT WHERE TO FIND THE LATEST LITERATURE.

ALSO FOCUS ON PUBLICISED RESEARCH. THE LAY PERSON WILL SEE SOMETHING ON TV AND WANT TO KNOW IF IT IS VALID

WHERE CAN YOU GO TO FIND THE LASTEST RELIABLE RESEARCH AND WHERE CAN YOU GO TO GET THE TREATMENT - ENROLL ON TRIAL?

## Drug testing and assay development

#### EACH STEM CELL TYPE TO BE NEW CHAPTER WITH BASIC INTRO THROUGH TO MORE DETAILED CULTURE ETC. EACH DIFFERENT AREA SUCH AS ETHICS OR USES TO HAVE BASIC INTRO

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## Regenerative medicine

Nature insight regen med

<http://www.nature.com/nature/supplements/insights/regenerativemedicine/index.html>

The regeneration of a functional tissue in place of a lost or degenerated tissue.

* Implantation of stem cells or derived cells
* Tissue transplant
* Induced regeneration through biologically active molecules

### Tissue engineering

* The process of creating tissue from cells and scaffolding molecules
* Engineered tissue may be used in regen med

### 

### cell therapy

* The use of cells to treat a disease or tissue damage
* May or may not involve stem cells
* Stem cell plasticity – hESC are pluripotent, MSC are multipotent (although contention about transdifferentiation).
* hESC should **NOT** be implanted undifferentiated
* MSCs **CAN** be implanted undifferentiated
* Primary cells **MAY** be de-differentiated

#### ECM matrix

* Cell derived material between cells
* Tissue dependant
* Provides adhesion and insoluble signaling factors for cells
* Key to tissue engineering
  + Artificial/natrual scaffolds

nature med 14:213

* Lancet 2008; 372:2023
* Patient presented with severe reduction in bronchial tube
* Cadaver trachea decellularised to remove MHC proteins
* Chondrocytes derived from patient MSCs used to seed outside of trachea
* Epithelial cells from biopsy used to seed inside

#### Tissue regeneration examples - NAME BY DISEASE RATHER THAN TISSUE

##### Cartilage - FOCAL CARTILAGE DEFECTS, OA AND RA

* A thin layer of tissue covering the long bones allowing for frictionless motion
* Aneural, avascular and no lymph system
* Unique tissue containing only one type of cells
* Cells make up 2-5% of tissue by volume
* ECM mostly collagen and proteoglycans
* Adult cartilage does not repair after injury
* Damaged cartilage will not cause pain until it affects other tissues
* Cartilage defects are a major cause of disability and cost over $60 bn in the US alone
* Knee replacements often do not last as long as the patient and may not be possible to replace

mosaicplasty

ACI – Autologous chondrocyte implantation

clinical results

* Long term results show formation of inferior fibrocartilage
* Difficult to analyze due to lack of nerves innervation – no pain
* Lack of integration
* Donor site morbidity

neocartilage

* Chondrocytes can be made to form a cartilage like tissue in vitro
* Chondrocytes removed from cartilage after digesting ECM
* Chondrocytes cultured in high density with specific growth factors to mimic development

microfracture

[www.clinicaltrials.gov](http://www.clinicaltrials.gov)

* Review of cartilage therapy
  + Journal of Bone and Joint Surgery - British Volume, Vol 91-B, Issue 5, 565-576. **Articular cartilage tissue engineering** TODAY’S RESEARCH, TOMORROW’S PRACTICE?
  + *Osteoarthritis and Cartilage (2001)* ***10, 432–463***
* Good review of MSC therapies
  + Journal of the American Medical Association, February 27, 2008—Vol 299, No. 8925

##### vascular -

##### blood - TREATMENT OF LEUKAEMIA AND BLOOD CANCERS, MYLOMA

##### liver -

##### bone - NON UNION FRACTURES, BONE CANCERS

##### eye - GLAUCOMA,

##### skin - TRAUMA, SKIN GRAFTS, MYSKIN

##### heart - HEART DEFECTS DUE TO ISCHAEMIA FROM CORONARY EVENT

##### neurons - STROKE, PARKINSONS,

##### grnopc1

##### 

##### 

#### Importance of scaffolds and vascular

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# 3. Regulations (and ethics?)

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see stem cell primer for good background on history up to 2006

also see code of practise for use of stem cell lines 2010 <http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC003132>

“Of Clowns and Clones” published by cell biologist Robert A. Weinberg, in the magazine Atlantic Monthly. <http://www.theatlantic.com/doc/prem/200206/weinberg>

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## Why regulate

### Risks of stem cell therapies

Teratoma

### Risks of unlicensed use

cord blood banking - how some.people are making money off the public i.e. using adult stem cells in ungoverened way

### What do the regulations say

Code\_of\_Practice\_for\_the\_Use\_of\_Human\_Stem\_Cell\_Lines\_(2010) document in evernote

## Who regulates

FDA required a 200 book document from geron

## Patents

#### 

#### 

#### 

# 4. Future of stem cells

## What does the failure of Geron mean?

## 

## Can we afford stem cell therapies?

## 

## Is there a place for hESC with IPS

# 

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# 5. **Glossary**

see glossary from evernote