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School of Chemical Technology

CHEM-E3225, Cell- and Tissue Engineering, 5 cr

TOPIC 2 (Stem) Cells for tissue engineering

See Birla: Chapter 2) Cells for Tissue Engineering – note that we will cover Culturing of Stem Cells in Topic 4

We will look at:

1. Cells in Tissue Engineering and Exctracellular Matrix (ECM)
2. Cell Sourcing
3. The Cell Transplantation process
4. Stem cell – basics
5. Recreating stem cells (from differentiated to undifferentiated)

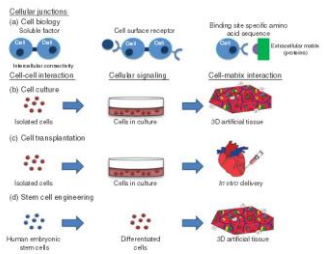
Why do we need to understand these ?

- Because the singalling, cell junctions and cell behaviour are essential for the ECM (extracellular matrxi), which we will look at in the next topic
- Because these are the functions in the ECM that the cells (as implants) will need to acquire in order to perform normal functions
- Because different stem cells behave differently, and some are more easy to work with than others. Also, if they behave incorrectly, there may be malfunction of the tissue and serious complications/death of cells

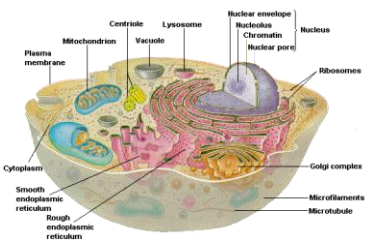
1. Cells in tissue engineering - tissue formation, signalling, ECM, cell junctions and cell behaviour

ECM is covered in Birla in Chapters 2 and 3 (2.3, p. 47 and 3.12. p. 114) read both of these; Today we cover 2.3. and in next topic, Topic 3 we will go into more detail on the ECM (refers t0 3.12)

Cells in tissue engineering



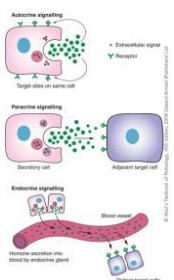
Cell structure



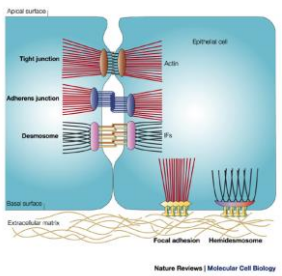
Types of cell signalling

- Endocrine
- Paracrine
- Autocrine
- Contact-dependent signalling*
  (= Direct cell to cell signalling)

* Not shown in this illustration



Cellular junctions



Nature Reviews Molecular Cell Biology 5, 542-553 (2004)

Main proteins (1-3):
1. Tight junction: Claudins, occculins, JAMS (=junctional adhesion proteins)
2.Adeherens junction: cadherin-catenin complexes, nectin-afadin – complexes
3. Desmosome junction: cadherins, armadillo proteins, desmoplakin
(IFS=Intermediate filaments)

Gap junctions – specialized intercellular connection between animal cells – ions, molecules and electrical impulses pass

Cellular junctions (previous power point 1/2)

1.Tight junctions
- between adjacent cells
- prevalent in epithelial tissue
- provide adhesion and barrier functions, hold cells together and provide a semipermeable barrier.
- Proteins are typical for tight junctions eg. Claudins, occulins and junctional adhesion proteins (JAMS) - provide a link with intracellular actin (**Actin** is a globular multi-functional protein that forms microfilaments.)

2. Adherens junctions
- connect adjacent cells together
- connect the intracellular actin bundles of adjacent cells
- provide increased structural stability.
- two types of major protein complexes at adeherens junctions: cadherin- catenin complexes and nectin- afadin complexes.

3. Desmosome junctions
- anchor adherent cells together and perform a function similar to tight junctions and adeherens junctions
- three protein families: cadherins, armadillo proteins, desmoplakin

Cellular junctions (previous power point 2/2)

4. **Focal adhesion**
- large macromolecular assemblies through which mechanical force and regulatory signals are transmitted between the extracellular matrix and an interacting cell

5. Hemidesmosome Junctions
- anchor cells to the underlying basement membrane (we will come back to basement membrane and the ECM)

6. Gap junctions: (not in the previous power point illustration)
- a specialized intercellular connection between a multitude of animal cell types
- directly connect the cytoplasm of two cells, which allows various molecules, ions and electrical impulses to directly pass through a regulated gate between cells = typical for eg.nerve cells

A few words about ECM and BM (to be covered also in later lectures)

- **Extracellular matrix** (**ECM**)
  - collection of extracellular molecules secreted by cells
  - provides structural and biochemical support to the surrounding cells.
  - the composition of ECM varies between multicellular structures; however, cell adhesion, cell-to-cell communication and differentiation are common functions of the ECM
- The animal extracellular matrix includes the interstitial matrix and the basement membrane (BM)
  - The **(BM) basement membrane** is a thin matrix of tissue
  - separates the epithelium (skin, respiratory tract, gastrointestinal tract, etc), mesotehlium(pleural cavity, peritoneal cavity, pericardial cavity, etc) and endothelium (blood vessels, lymph vessels, etc) from underlying connective tissue

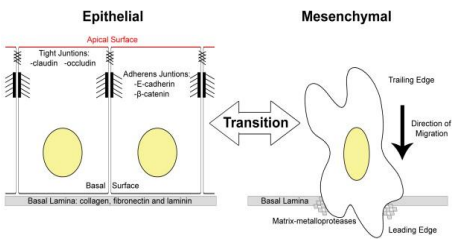
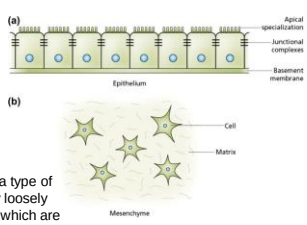


FIGURE 8.1. Epithelial vs. Mesenchymal.Epithelial cells adhere together by tight junctions and adherens junctions localized near the apical surface. Epithelial cells also have a basal surface that rests on a basal lamina (ECM). Mesenchymal cells in contrast do. Not have well-defined cell-cell adhesion complexes, have front-end/back-end polarity instead of apical/basal polarity, and mesenchymal cells are characterized by their ability to invade the basal lamina.

Chapter 8 – Molecular Organization of Cells Jon D. Ahlstrom

http://dx.doi.org/10.1016/B978-0-12-398358-9.00009-2
Principles of Tissue Engineering (Fourth Edition), 2014, 147–160



Mesenchyme is a type of tissue formed by loosely associated cells which are srrounded by the ECM

FIGURE 7.9. Most tissues are composed of epithelial (a) and mesenchymal (b) components.

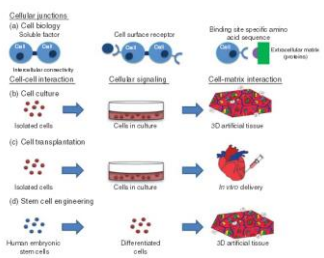
Chapter 7 – Molecular Biology of the Cell J.M.W. Slack http://dx.doi.org/10.1016/B978-0-12-398358-9.00007-0
Principles of Tissue Engineering (Fourth Edition), 2014, 127–145

Cell behavior

What do cell do?

- Attach: generate the contact site between the cell and substrate
- Spread: increase the contact area between the cell and substrate
- Proliferate: increase in cell number by division
- Differentiate: become specialized for particular functions.
- Apoptosis: programmed cell death, an active process requiring metabolic activity by the dying cell
- Migrate: move from one place to another
- Function: carry out certain tasks, e.g. molecule transport, molecule metabolism, and energy conversion

Cells in tissue engineering



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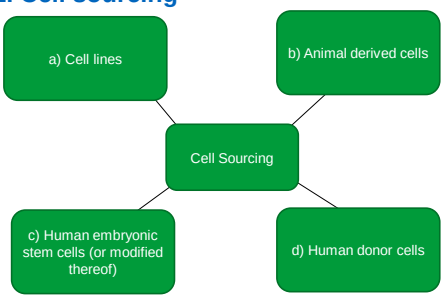
2. Cell Sourcing

2. Cell sourcing



Birla 2014, figure 2.2. modified

Considerations for cell sourcing

- Autologous vs. allogeneic
- Animal-derived vs. human-derived cells
- Cell lines
- Stem cells
- Stem cell engineering

Cell sourcing

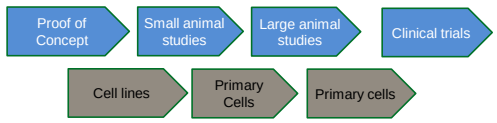
Requirements of ideal cell source:
Cells need to

1. Be safe and not to trigger tumor creation
2. Improve fuctional performance of the host tissue
3. Functionally integrate with host tissue
4. Be applicable to noninvasive delivery methods
5. Not to trigger the host immune responce
6. Tolerate the processing conditions required to develop clinical therapies

Stem cells should:

7. Be sensitive to social end ethical issues
8. Have a demonstrated potential to differentiate with high efficacy to the cell type of interest

Cell sourcing during the progression of Tissue Engineering Studies (Birla 2014. figure 2.3.



Different cell sources are suited for different parts of the technology development process for fabrication of 3D artificial tissue

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3. The cell transplantation process

3. The cell transplantatoin process (Birla 2014) figure 2.4.) an example !

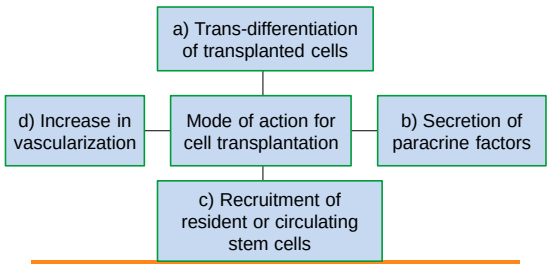
Human embryonic stem cells isolated from inner cell mass of blastocyst /Alternative today is IPSc and similar technology !

The cells are cultured in controlled in *in virto* conditions

Cells are differentiated to a specific cell lineage depending on the application

During transplantation process the diffrentiated cells are delivered to the site of injury through a delivery vehicle

Expected modes of action of cell transplantation (Birla 2014, figure 2.5)

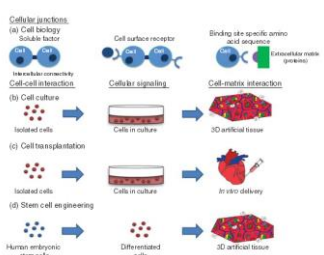


4. Stem cells – basics

A stem cell is an unspecialized cell that can both self-renew (reproduce itself) and differentiate into functional genotypes

- origin, types (embryo/adult), differentiation, potency, niche

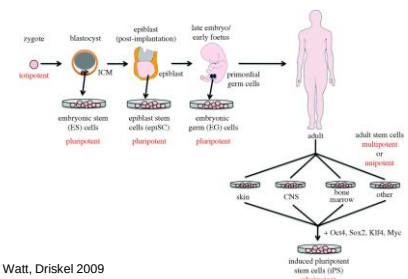
Cells in tissue engineering



Stem cells in tissue engineering

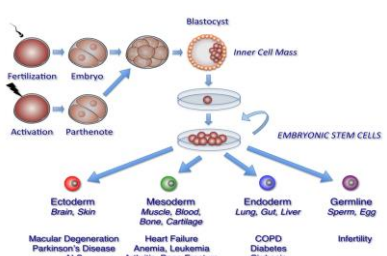
- 1. Stem cells can be used for generation of the right type of somatic cells
- 2. Stem cells can be programmed with physico-chemical cues or recombinant DNA techiques, and used as building blocks for the development of a variety of tissues

Origin of stem cells



Watt, Driskel 2009

Embryo & germ layers

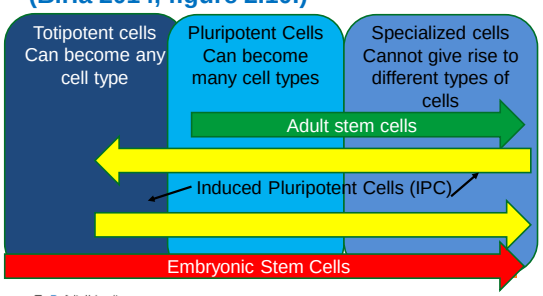


AGING (2011) 3: 5, 494-508

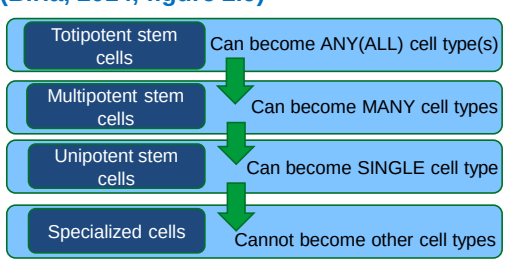
Different types of stem cells

- Totipotent stem cells can give rise to any cell type and even an entire organism.
- Pluripotent stem cells, e.g. embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSs), can differentiate into cell types of all three germ layers, but cannot give rise to an entire organism.
- Multipotent stem cells, e.g. adult stem cells, can differentiate into several cell types that belong to particular germ layers, not all three.
- Unipotent stem cells can generate a single differentiated cell type.

Stem cell sources in tissue engineering (Birla 2014, figure 2.10.)



Differentitation potential of Stem cells (Birla, 2014, figure 2.9)



Multipotent stem cells (adult)

- Multipotent stem cells that can differentiate into several cell types that belong to particular germ layers, not all three germ layers
- Adult stem cells have been isolated from human bone marrow, blood, muscle, pancreas, umbilical cord blood, fat, liver, brain, and et al.
  - Mesenchymal stem cells
  - Hematopoetic stem cells
  - Endothelial stem cells
  - Intestinal stem cells etc.

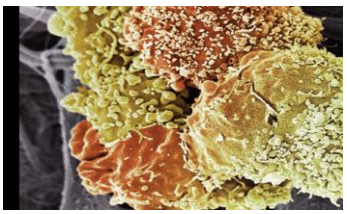
Embryonic stem cells (ESC)

- Derived from the inner cell mass of a blastocyst (early-stage preimplantation embryo)
- ESC are pluripotent stem cells as they have the ability to differentiate into any cell type and to propagate
  - A normal karyotype
  - Maintain high telomerase activity
  - Exhibit a long-term proliferative potential

Applications of ESC

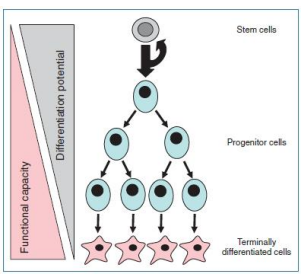
- There are several potential applications to use ESC inregenerative medicine:
  - Blood and immune-system related genetic diseases, cancer
  - Type 1 diabetes, Parkinson's disease, blindness, spinal cord injuries
  - Research of early human development and genetic diseases, *in vitro* systems of toxicology testing
- Ethical issues: Should the embryos at a pre-implantation stage be considered to have the moral or legal status as more developed human beings?
- Technical issues: Graft-versus-host disease

ESCs are probably the most complex human therapeutic imaginable "



Vigilance for chromosomal abnormalities will be high as [1] human embryonic stem cells move into the clinic. FOX, L (2008) FDA scrutinizes human stem cell therapies, *Nature Biotechnol.* 26 (6) 598.

Differentation vs. functionality

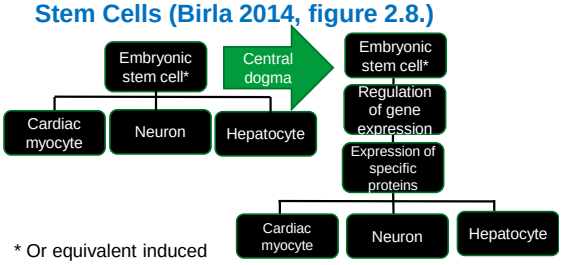


Principles of regenerative medicine (2008), Atala, Lanza, Thompson, Nerem

Stem cell differentation

- Stem cell differentation is dependent both on genetics and environmental factors
- Stem cells receive exogenous signals, which can cause an activation of intracellular signalling mechanisms
- Genetic manipulation of stem cells is also one possibility to control differentation in high efficacy
  - Recombinant DNA techniques

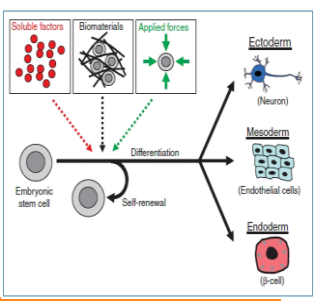
Differentiation of Human Embryonic Stem Cells (Birla 2014, figure 2.8.)



* Or equivalent induced pluripotent (totipotent) cell ??

Stem cell modulation

Stem cell fate decision is regulated by their microenvironmental cues, such as biological, chemical, and physical cues.



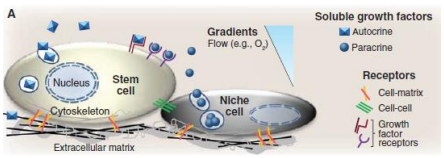
Atala et al. 2008 Principles of regenerative medicine

Stem cell niche

- The tissue-specific micronvironment where the stem cells reside
- Stem cell niche can consist of any combinations of chemical, physical and cellular elements, for example:
  - Chemical: Growth factors, hormones, ions, ROS
  - Physical: Matrix elasticity, topography, cell shape, fluid shear stress
  - Cellular: stem cell progeny, cell-cell communication

Stem cell niche

- The niche protects stem cells and regulates their function whether the cell will remain quiescent, self-renew, differentiate, migrate or undergo apoptosis

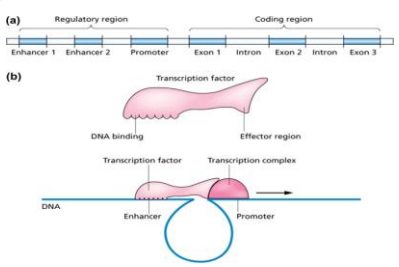


2009 Science 324:1673 34

5 . Recreating stem cells

- *Induced pluripotent stem cells (iPS)*
- *Challenges of reprogramming*

How do signals regulate cell function & What are transcription factors ?



J.M.W. Slack **Chapter 7 – Molecular Biology of the Cell** FIGURE 7.2. a) Structure of a typical gene. b) Operation of a transcription factor. Principles of Tissue Engineering (Fourth Edition), 2014, 127–145 http://dx.doi.org/10.1016/B978-0-12-398358-9.00007-0

Signal transduction in the cell

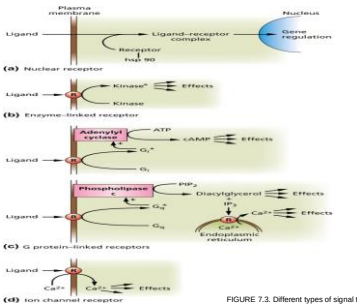


FIGURE 7.3. Different types of signal transduction.

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Principles of Tissue Engineering (Fourth Edition), 2014, 127–145
J.M.W. Slack http://dx.doi.org/10.1016/B978-0-12-398358-9.00007-0
Chapter 7 – Molecular Biology of the Cell

Reprogramming of stem cells

- Mature cells can also be turned into pluripotent, ESC-like cells
- Theoretically, if harnessed correctly reprogramming could be used as an additional strategy for repair damaged tissue using patients' own mature cells
  - No need for isolation and expansion of rare adult stem cells
- Removes the ethical problem of using ESC

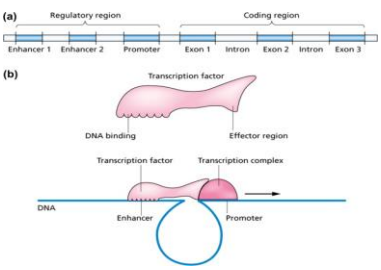
Induced pluripotent stem cells (iPSC)

- Considered as surrogates to ESC cells but contain number of genetic and epigenetic differences
- Can provide an unlimited supply of autologous cells for generation of transplants without the risk of immune rejection
- iPSC cells have been succesfully generated from several somatic cell types, such as skin cells, neural cells, adipose tissue derived cells and blood cells.
  - The specific donor cell type affets the kinetics and the efficiency of the programming
  - Cells that are in their early stage of development are more amiable to reprogramming compared to terminally differentiated cells

iPSC derivation methods

- Pioneering methods:
  - Mature cells can be reprogrammed to become pluripotent by nuclear transfer (John B. Gurdon, 1962)
  - Induced pluripotent stem cells by the introduction of multiple transcription factors (Shinya Yamanaka, 2006)
  - Together they received a Nobel Prize in Physiology and Medicine 2012

- Breaktrough of creating iPSC from adult human cells: James Thomson, 2007 and Shinya Yamanaka, 2007
  - Thomson factors: *OCT4, SOX2, NANOG* and *LIN28*
  - Yamanaka factors: *OCT4, SOX2, c-MYC* and *KLF4*



http://dx.doi.org/10.1016/B978-0-12-398358-9.00007-0

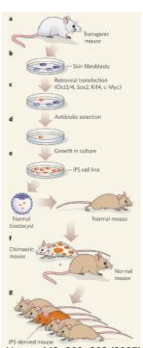
FIGURE 7.2. a) Structure of a typical gene. b) Operation of a transcription factor.

J.M.W. Slack **Chapter 7 – Molecular Biology of the Cell**

Principles of Tissue Engineering (Fourth Edition), 2014, 127–145

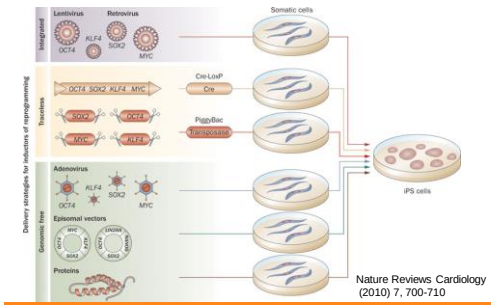
Creation of iPS cells (this is the original approach)

(A) Creation of a transgenic mouse that was resistant to selected antibiotics. The resistance was linked to the expression of pluripotency markers (Pou5f1 or Nanog).
(B) Isolation of the fibroblasts of genetically modified mice.
(C) The isolated fibroblasts were introduced genes for four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) by retroviral transfection. (Takahashi & Yamanaka 2006)
(D) Antibiotic selection. Only the cells that expressed to puripotency factors survived.
(E) The cells that resembled ESC were isolated, and expanded into stable iPS-cell lines.
(F) Creation of chimaeric mise by injecting iPS cells into blastocysts of normal mice
(G) The chimaeric animals were crossed with normal mice, which led to the birth of offspring carrying the genetic content of an iPS cell.



Nature **448,** 260–262 (2007)

Other approaches: Modification of stem cell genome



Nature Reviews Cardiology (2010) 7, 700-710

Challenges in older technologies for reprogramming cells to pluripotency

- Low efficiency: The rate at which somatic cells were reprogrammed into iPS cells in Yamanaka's original mouse study was 0.01–0.1%.
- Genomic insertion: The use of transcription factors includes a high risk mutations being inserted into the target cell's genome. Genomic insertion can be avoided by using a different vector for input, such as plasmids, adenovisuses and transposon vectors, but often lead to lower throughout.
- Tumors: Some of the reprogramming factors (i.e. Myc-family proteins) are oncogenes that bring on a potential tumor risk.
- Incomplete reprogramming: Reprogramming has the challenge of completeness because the genome-wide epigenetic code must be reformatted to that of the target cell in order to fully reprogram a cell.

Reprogramming with CRISPR/Cas system

- CRISPR/Cas systems are used by various bacteria and archaea to mediate defense against viruses and other foreign nucleic acid.
- These systems form the basis for a large variety of more efficient tools for reprogramming
- http://www.crisprtx.com/programs/regenerative-medicine
- http://gizmodo.com/everything-you-need-to-know-about-crispr-the-new-tool-1702114381