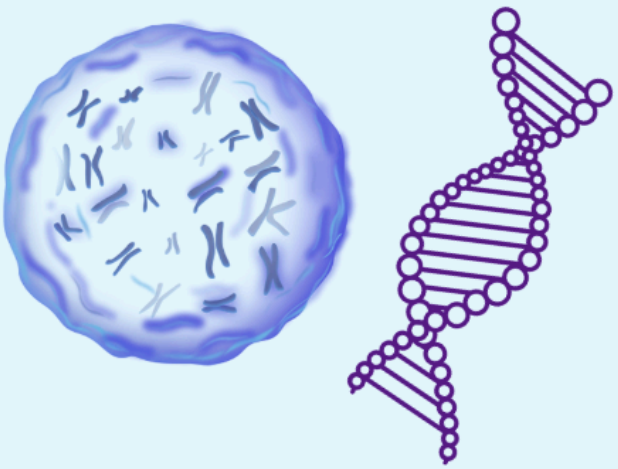
CEDB30004

Stem Cells in Development & Regeneration



H1 Complete Subject Notes

L1: Intro to Stem Cells

LOS

- Defining different types of stem cells
- Examples of sources of different stem cell types
- Intro to molecular and functional properties of stem cells

Stem cells

- **A stem cell can be defined by two essential functional properties**

1. **Self renewal**: ability to divide and maintain the stem cell pool
2. **Differentiation**: ability to produce specialised cell types

- **Characteristics:**
  - **Functional characteristics:** ability to self renew & differentiate into specialised cells
  - **Molecular characteristics:** 'stemness' which are the molecular signals that govern the functional properties
- Potency refers to *what* and *how many* different specialised cells that stem cells can give rise to (the potential of differentiation).

Potency	Capability	Example
Totipotent	ALL cell types & extra embryonic tissue (whole organism)	Zygote, blastomere
Pluripotent	All body cell lineages (not placenta)	Embryonic stem cells, iPSCs
Multipotent	Multiple related cell types; more than one mature cell type of the body	HSC -> all blood cells
Unipotent	One cell type lineage only	Spermatogonia -> sperm

- Gold standard potency test
- **Functional engraftment in vivo** (e.g., inject putative pluripotent cells into a blastocyst -> chimeric animal).
  - The stem cells must successfully attach, survive and integrate into the host tissue and function normally -> **proves true potency**.
  - *Functional engraftment means cells integrate into a tissue in vivo and carry out the normal function of that tissue, proving true potency.*

L2: Early Development and Cell Potency

Development

- The process of generating a complex body from a single cell (zygote)
- Early development establishes:
    - totipotency ⟶ pluripotency transitions
    - first lineage decisions
    - patterning mechanisms (Hippo/YAP, FGF/MAPK)
    - emergence of epiblast (embryo proper)

Preimplantation stages

- Zygote ⟶ cleavage ⟶ morula ⟶ compaction ⟶ blastocyst
- **Zygote:** totipotent, maternal ⟶ embryonic transition
- **Cleavage:** symmetric divisions, no growth
- **Morula:** 16-cell
- **Compaction:** polarity established
- **Blastocyst:** ICM + trophectoderm

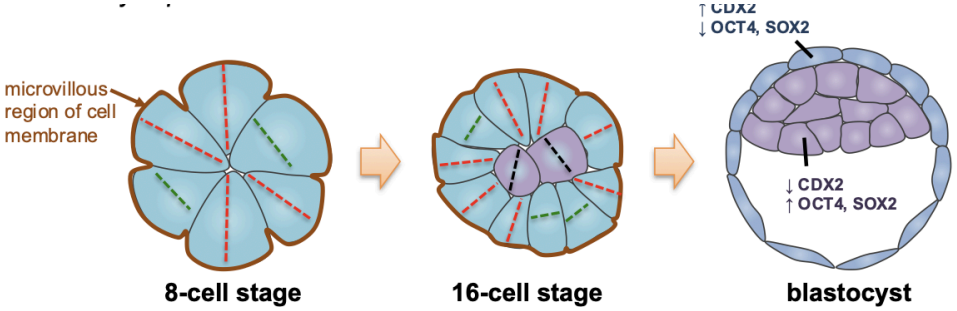
Stages of mammalian embryogenesis

- Fertilisation -> zygote
    - Sperm and egg fuse to form a single totipotent cell (**zygote**); this contains all the DNA needed for the embryo and extraembryonic tissues
- Cleavage -> morula
    - The zygote divides rapidly without growing (**cleavage**), and each division produces smaller cells called ***blastomeres***.
    - Early blastomeres are totipotent.
    - After several division (16 in humans), the embryo is called a **morula.**
- Blastocyte formation
    - Fluid accumulates inside the morula forming a **blastocyst**
    - Cells differentiate into
        - Trophectoderm (becomes placenta)
            - *Outside cells become committed to trophoblast fate by 32-cell stage*
            - *Expresses* **CDX2**
        - Inner cell mass -> embryo proper
            - *Inside cells become committed to ICM fate by 64-cell stage*
            - Expresses **OCT4 + SOX2 + NANOG**
    - ICM cells are pluripotent
    - ***Zona pellucida*** surrounds the blastocyst serving as protection (does not contribute to the ICM, or any part of embryo or placenta)

- **Compaction (8-cell stage)**
    - **Marks the beginning of epithelisation**
    - Calls flatten and adhere via **E-cadherin**

- **Outer cells polarise -> apico-basal polarity**
- **Division types:**
    - **Conservative** -> 2 outer cells
    - **Differentiative** -> 1 inner + 1 outer cells

- **Blastocyst Cavitation (32-cell stage)**
    - **Outer cells** -> **trophoblast**
        - epithelium with tight junctions and active transport of Na+/water
        - CDX2+
        - Forms placenta
    - **Inner cells** -> **ICM** -> pluripotent embryonic tissue
    - OCT4/SOX2/NANOG
    - Will split into epiblast + hypoblast



Lineage Specification

Transcription factors

Factor	Function	Fate
OCT4	Maintain pluripotency *Essential for identity of the ICM; expressed In all totipotent, pluripotent and germ cell lineages*	ICM, epiblast
SOX2	Supports pluripotency *Key pluripotent factor; binds with OCT4*	ICM, epiblast
***NANOG**	Epiblast identity *Pluripotency; becomes future embryo*	Epiblast
***GATA6**	Hypoblast identity *Extra-embryonic, primitive endoderm*	Hypoblast
CDX2	Trophoblast identity	Trophoblast

*stochastic expression at ICM stage

L3: Pluripotency and Stem Cells

Derivation of Embryonic Stem Cells (ESCs)

- First derived in 1981 where mouse ICMs were isolated via immunosurgery
- **Culture conditions**
    - Cultured on MEF (mouse embryonic fibroblast) in a teratocarcinoma
    - Medium with 10% foetal bovine serum
    - Blastocysts in diapause (arrested development)
- **Key concept:** Early ESC derivation was informed by teratocarcinoma cell lines which are pluripotent but karyotypically abnormal!
- ESC are immortal, self renewing, pluripotent

3 classic stem cell lines that can be isolated from a blastocyst

- **Embryonic stem cells (ESC)**
    - **Origin:** Epiblast (ICM)
    - **Potency:** Pluripotent
    - **Features**: Immortal, self-renewing, pluripotent -> gives rise to embryo
- **Trophoblast stem cell (TS)**
    - **Origin:** trophectoderm
    - **Potency**: Multipotent
    - **Gives rise to:** placenta lineage
- **Extraembryonic endoderm cell (XEN)**
    - **Origin:** primitive endoderm/hypoblast
    - **Potency**: Multipotent
    - **Gives rise to:** yolk sac lineage

ESC characterisation

ESC must show:

- **Self-renewal**: ability to divide while maintaining pluripotency
    - Proliferate indefinitely
    - Telomerase activity (long telomeres)
    - Normal karyotype maintained
    - Marker expression profiles
    - Glycolytic metabolism
- **Pluripotency**

Functional tests for pluripotency

1. **In vitro differentiation (easiest)**

- Change culture ⟶ ESCs spontaneously differentiate into ectoderm/endo/mesoderm.
    - **Significance:** Shows that the cells can differentiate spontaneously into derivatives of the three germ layers: ectoderm, mesoderm, endoderm

L11: Epithelial Stem Cells

What is an Epithelium?

An epithelium is a sheet of cells that:

- Lines **surfaces**, **cavities**, and **tubes** of the body (skin, gut, lungs, ducts, cornea, reproductive tracts, blood vessels).
- Functions as **barrier + interface** between **environment vs internal tissues**.
- Has **rapid turnover** → requires **resident stem cells**.
- Has **polarity** → 2 functional surfaces:
    - **Apical side:** faces lumen/external environment → special transporters, absorptive or secretory functions
    - **Basolateral side:** faces basal lamina and tissue → signalling, anchoring, blood supply

Key concept: polarity allows selective barrier + directional transport (example: gut glucose uptake requires apical SGLT1 + basolateral GLUT2).

Cell Junctions

Epithelia behave like **sealed, coordinated tissue**, not loose cells. Junctions enforce:

- Integrity
- Selective transport
- Tissue mechanics + morphogenesis
- Stem cell positional signalling

Junction hierarchy (apical → basal):

Tight Junctions (TJ)

- Seal spaces between cells -> prevent **paracellular leak**
- Most apical; Maintain **polarity** by preventing membrane protein diffusion
    - Example: CFTR must **stay apical**, GLUT2 must **stay basolateral**
- Proteins: Claudins, Occludin, ZO-1

Adherens Junctions (AJ)

- Form a **circumferential actin belt** for **shape + movement**
- Located just below tight junctions
- Drive **tissue remodelling** (e.g., neural tube closure, crypt budding)
- Proteins: **E-cadherin**, β-catenin (links to actin)

Desmosomes

- Provide mechanical resilience; spot weld cells
- Link **intermediate filaments (**keratin**)**
- Crucial for tissues under mechanical stress (skin, oesophagus)

Hemidesmosomes

- Anchor **epithelium** to **basal lamina**
- Protein: α6β4 integrin, plectin, binds laminin & collagen IV

Clinical correlation: Defects → blistering (pemphigus, epidermolysis bullosa)

L23 - Diabetes

Diabetes

- Diabetes = chronic disorders where **glucose cannot be regulated**, resulting in **persistent hyperglycaemia**
- Symptoms: **polydipsia, lethargy, weight loss, polyuria**
- **Pancreas compartments**:
    - **Exocrine (98–99%)**: acinar + duct cells → digestive enzymes to gut
    - **Endocrine (1%) — Islets of Langerhans**: hormone-secreting clusters, highly vascularised for rapid systemic response
- **Islet endocrine cell types**:
    - β-cells (insulin, ~75%)
    - α-cells (glucagon, ~15%)
    - δ-cells (somatostatin, ~5%)
    - PP-cells (pancreatic polypeptide, ~3%)
    - ε-cells (ghrelin, <1%)

Types of Diabetes

- **Type 1** = **autoimmune**, T-cell mediated destruction of β-cells → no insulin production
- Evidence of **genetic susceptibility (HLA)** and affects ~145,000 Australians (~0.6%)
- Consequences: ↓life expectancy by **~12.2 years** + severe long-term complications (blindness, CVD, neuropathy, amputations)

Current Therapy & Limitations

- **Insulin replacement** (injections or pump + CGM) controls glucose but **cannot fully replicate physiological homeostasis**
- **Hypoglycaemia risk** remains - major morbidity + mortality concern

Why Replace β-Cells?

- T1D pathogenesis = **β-cell loss** → replacement is rational & **curative-potential**

Transplantation Options & Limitations

- **Whole pancreas transplant**
    - Effective, ~85% achieve insulin-independence at 1 yr but requires **major surgery** & **donor shortage**
- **Islet transplantation**
    - Delivered via **portal vein**, minimally invasive, but **requires multiple transplants** + long-term **immunosuppression** + **limited donors** (<15 per yr)
- **Conclusion**: Not scalable → **PSC-derived cell therapy needed**

Pluripotent Stem Cell Approach

- Human **ESCs** or **iPSCs** can be differentiated into **pancreatic lineages** following **embryonic developmental signalling cues** (Activin A, WNT, FGF, TGFβ, RA, etc.)
- **Final target cell number per patient ~10^9 cells** (≈1M islets x 1000 cells)