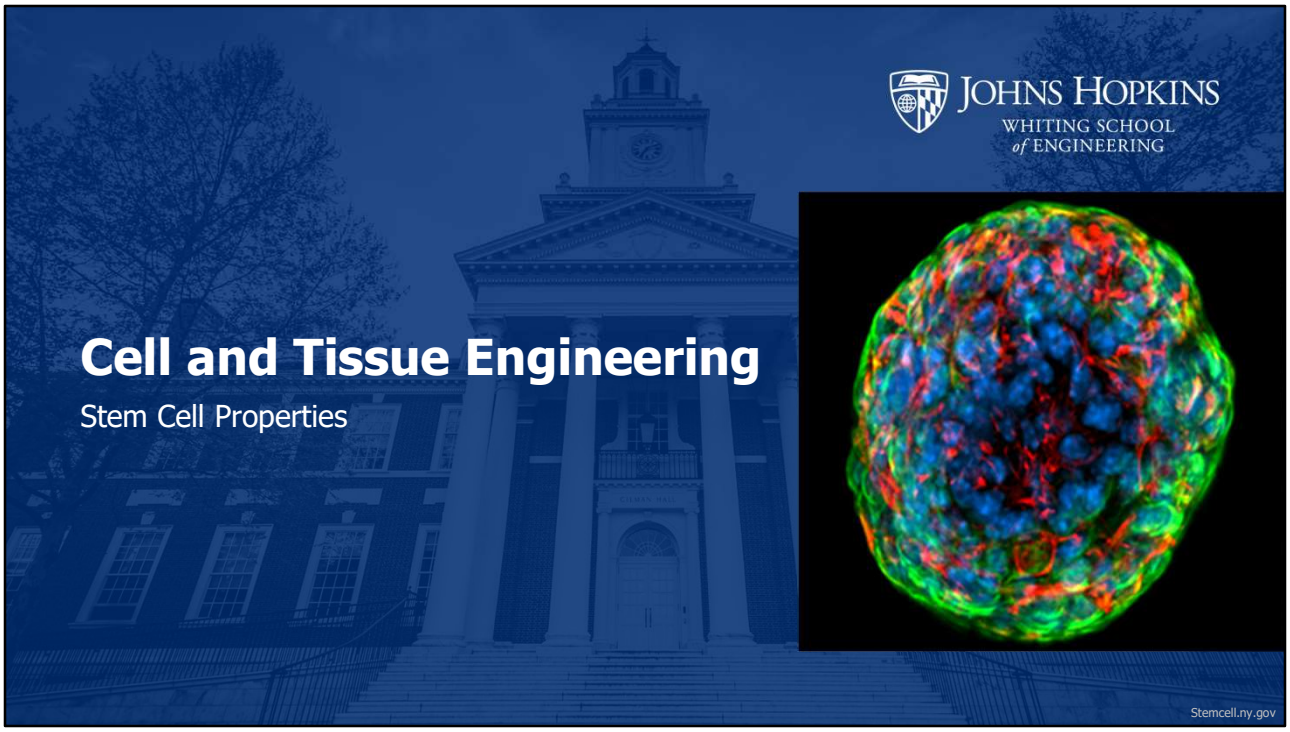
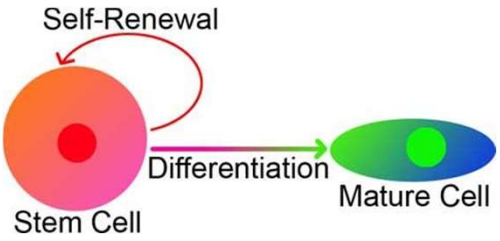


Welcome to cell and tissue engineering. This is Ethan Nyberg and this module we will be discussing stem cells.

What Are Stem Cells?

1. Self-renew



2. Potential to differentiate into 2+ lineages

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Nursingcrib.com; Independent.com

Every mature cell in your body came from a stem cells.

With that point of view, it is no surprise how excited the medical and scientific communities are about the potential use of stem cells for healing the human body.

SO, to begin, let's talk about what a stem cell is exactly

IN order to be a stem cell it must have 2 properties – the first is the ability to **self renew** – to create more stem cells.

The second ability is to **differentiate** into **2** or more different lineages, to have some degree **of plasticity** in cell fate.

Types of Stem Cells

Embryonic stem cells

Somatic or adult stem cells

- Tissue resident (bone marrow, muscle, brain, skin teeth, heart, liver…)

Induced pluripotent stem cells

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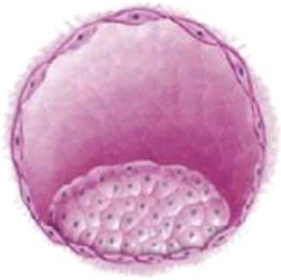
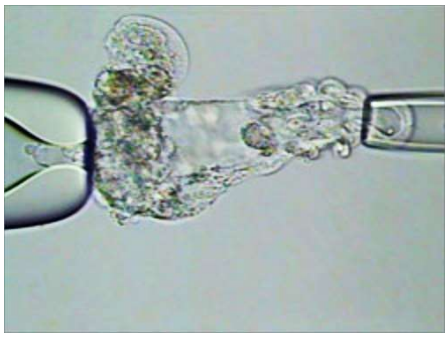
First, let's cover the three categories of stem cells – **embryonic**, **somatic** and **induced** stem cells

Embryonic Stem Cells

Nanog and Oct4





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Pfcla.com; Obsidianwings.blogs.com; http://stanmed.stanford.edu/2012spring/backstory.html

Embryonic stem cells – So as the name described, this type of stem cell is derived from embryos. Most are from embryos created using IVF or *in vitro* fertilization, and then donated for research purposes.

These cells are derived from the inner cell mass of a blastocyte – recall our module on morphogenesis! - In this image on the right you can see a micropipette holding an embryo.

Using precise laser techniques the embryo is opened on the side and the blastocyst – the dense collection of cells at the bottom of the two left images-- is removed.

After removal, these cells from the blastocyst are typically plated on a **support** or **feeder cell layer** which provides adhesive contacts and need growth factors.

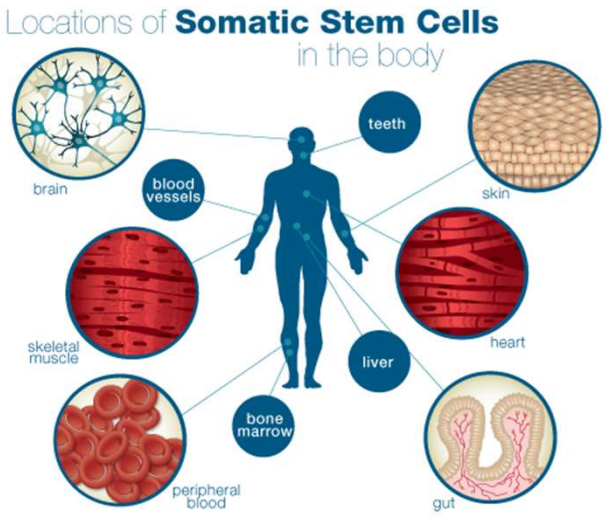
In order to work with **sufficient numbers** researcher expand these cells through **passaging** and creation of **cell lines** which we discussed earlier this semester.

Identification of embryonic stem cells and **checks** to see that the culture conditions have maintained the embryonic phenotype include measuring the expression of two important transcription factors – **Nanog** and **Oct 4**.

Use and **regulation** of these cells is one of the most controversial topics in medicine today. We'll get into that more in later videos in this module.

Somatic (adult) Stem Cells

Tissue resident (bone marrow, muscle, brain, skin teeth, heart, liver…)



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Learn.genetics.utah.edu

Somatic or adult stem cells are found in the adult body.

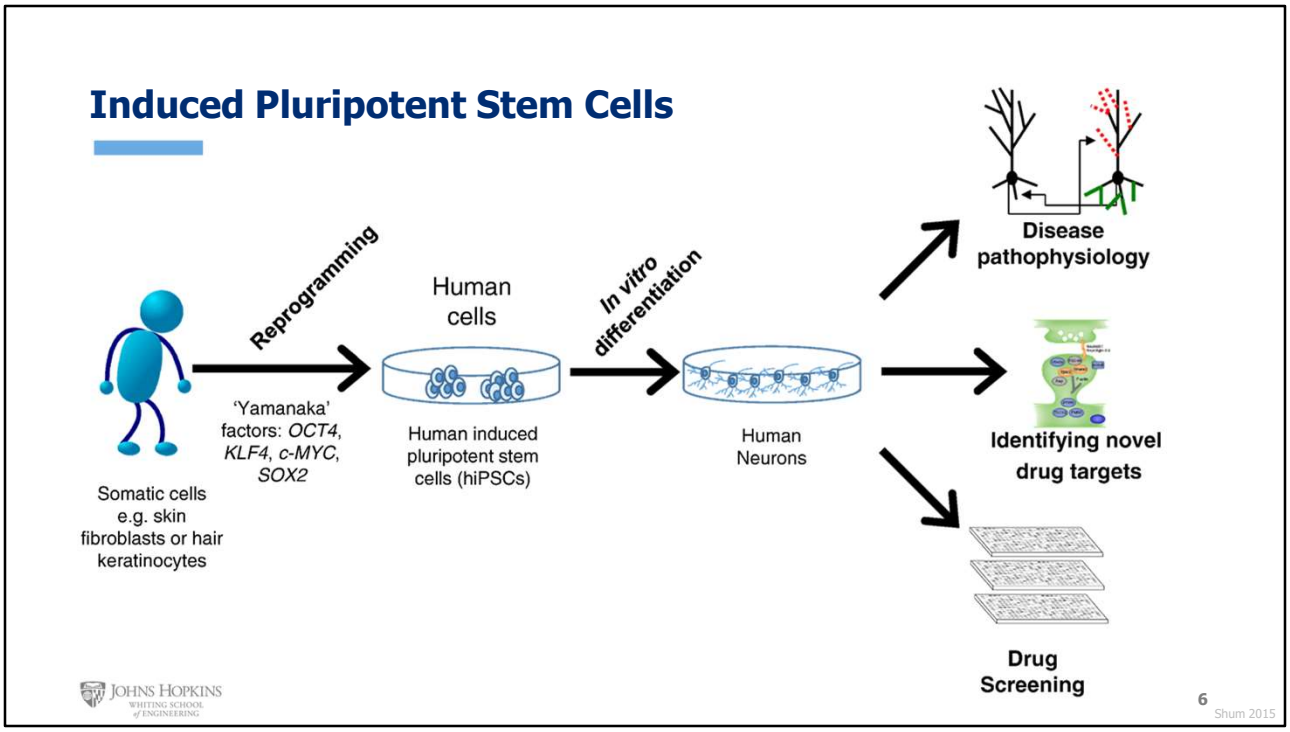
These cells can be lost or depleted though age and injury repair.

Typically, somatic stem cells generate the **type of tissue in which they reside.**

We've already heard about one type of somatic stem cell this semester – the **hematopoietic stem cells** which reside in the bone marrow and form the blood cells of your body.

Several years after the discovery of hematopoietic stem cells, researchers discovered the second stem cell population in bone marrow - **bone marrow stromal cells or mesenchymal stem cells**. These cells have been shown to differentiate into bone, cartilage and fate among other things. You'll read more about these cells in the reading this week.

As you can see from this list there are <u>many</u> tissues that contain a **progenitor** population – including the brain! Two locations in the brain have been identified as containing cells which can regenerate **neurons**, as well as **glial** cells.



Induced pluripotent stem cells have only been around for the last decade -- These are adult cells that are removed from the body and reprogrammed into a stem-cell like state

What researchers found what that you can start with a **fibroblast** – a differentiated phenotype that can easily be harvested from the skin – and genetically **reprogram** that cell by turning on the transcription factors **cMyc** and **Klf4** (Krupple like factor 4), **Oct 3/4,** and **Sox2**.

This process results in a cell population that is now technically **<u>un</u>differentiated** – no longer making fibroblast-specific proteins – and is self-renewing.

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https://www.sciencedirect.com/science/article/pii/S0018506X1530009X

Extending the Pluripotency of Stem Cells

ESCs, Adult Stem Cells, iPSCs traditionally only give rise to embryonically derived tissues (Pluripotent)

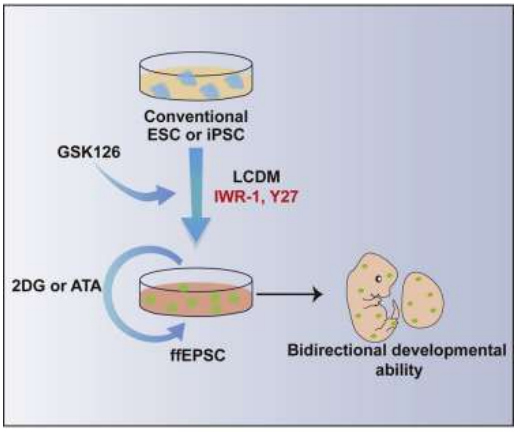
Extra-embryonic tissues are needed for complete development (yolk sack, placenta).

Cells that can give rise to both extra- and embryonic tissues are closer to totipotency.

2017 Yang – Extended Pluripotency

Extended Pluripotent Stem Cells (EPSCs)

Bidirectional developmental ability



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Zheng, Ran, et al. "Derivation of feeder-free human extended pluripotent stem cells." *Stem Cell Reports* (2021).

In recent years, we have begun to move past some of the limits of iPSCs – which are only pluripotent to embryonically-derived tissues.

You might recall that during development, there are extra-embryonic organs, such as the **placenta**.

Cells with **increased** potential for **extra-embryonic** tissues are closer to proper **totipotency**.

These cells can be developed in the lab using additional factors and are called **extended pluripotent stem cells**, and demonstrate **bidirectional** developmental ability.

https://www-sciencedirect-com.proxy1.library.jhu.edu/science/article/pii/S2213671121003076

Differences Between Types of Stem Cells

- Embryonic stem cells
- Somatic or adult stem cells
  - Tissue resident (bone marrow, muscle, brain, skin teeth, heart, liver…)
- Induced pluripotent stem cells

- **Totipotent** – any cell in the body, ability to become a complete mammal
- **Pluripotent** – many cell types in the body, are three germ layers, cannot become a complete mammal
- **Multipotent** – more than one cell type

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Some of the major differences between these stem cell types is their **potency,** which we touched on briefly in the morphogenesis module.

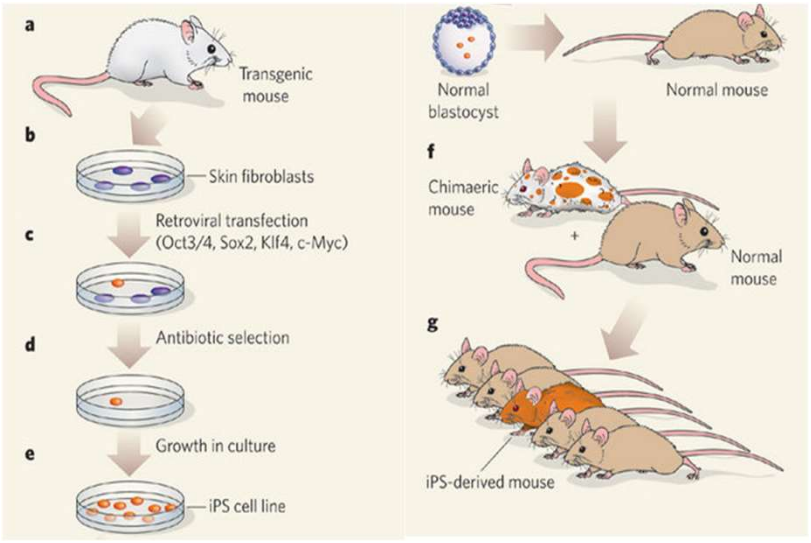
An embryonic stem cells that are produced in the first couple cell divisions have the potential to not only become all **embryonically** derived cells in the human body **but also to become a complete human being,** for this reason they are considered them **totipotent**

The embryonic stem cells used in research are derived at later stages of morphogenesis and are considered **pluripotent** however – that is they only able to form all of the **tissues of the human body**

Somatic stem cells however are more limited and have been shown to generally only differentiate into the **cells within their host tissue** – these cells are typically considered **multipotent**.

The potential of induced and expanded pluripotent stem cell is not fully understood – but recent studies in mice (not humans!) have demonstrate the ability **to create a fertile** organism from these cells which would put them in the totipotent category.

Testing for Pluripotency



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Let's look at how researchers are testing for pluripotency with mouse IPS cells…

This procedure should be familiar to you from our module on genetic engineering.

First, the skin fibroblasts are harvested, they are modified using retroviral techniques to express the 4 transcription factors we discussed.

Cells that successfully incorporated the reprogramming also express an antibiotic resistance gene that is used in selection. Application of the antibiotic to the culture will kill all of the cells that did not reprogram. Any cells without the gene are killed during the selection process.

Once selected, the IPS cells are expanded, and then injected into a mouse blastocyst, which is put into a mouse.

One of the resultant offspring will be chimeric – that is expressing both IPS genes and genes from the parent mice. This chimeric mouse is mated **again** with a normal mouse resulting in a mixture of offspring – some normal and -- in this case you can see 1 that expresses only the IPS code. This mouse was **derived from germ cells expressing** the IPS code.

Stem Cell Culturing and Availability

- Embryonic stem cells
- Somatic or adult stem cells
  - Tissue resident (bone marrow, muscle, brain, skin teeth, heart, liver…)
- Induced pluripotent stem cells

Easy to harvest and expand

Limited quantities and experience with expansion

Control not fully understood

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Mcb.berkeley.edu

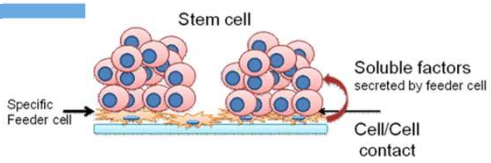
Another difference is ease of culture and availability.

The methods for obtaining Embryonic Stem Cells is complex, but it is fully **worked out** and **reproducible**. These cells readily expand in culture either on feeder layers or in embryoid bodies. This gives researchers **large** numbers to work with, but is not easily translated to human use.

Adult stem cells must be **isolated** from their host tissue – this typically means **total** or partial **destruction** of that tissue and **extensive** isolation protocols. These protocols differ from lab to lab, but what remains constant is a **low yield** and a **weak** understanding of how to expand these cells while **maintaining their stemness**.

Induced pluripotent cells are **easy** at the start – you can simple collect skin cells. However the genetic manipulation required makes this cell type **unavailable for mass research**. There is a **low efficiency** in their creation and **variation** found in their genetic profiles.

Culturing ESCs



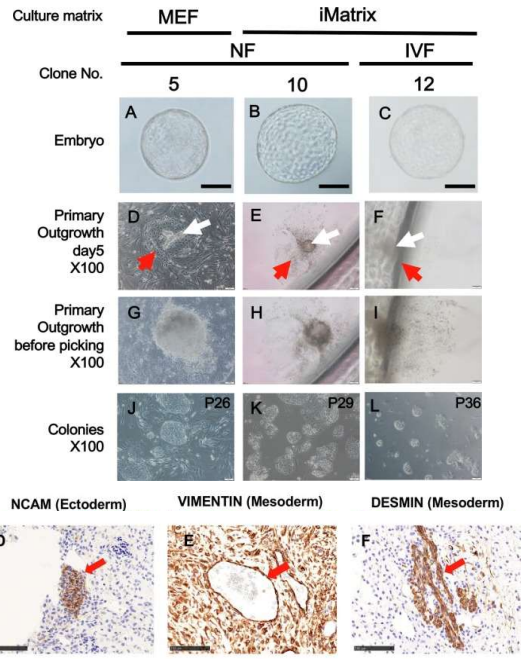
ESCs on Feeder cells vs xeno-free media

Impact of xenogenic processing on future product development ($$$, Regulation)

Marmoset example

Testing for pluripotency with teratoma formation *in vitro*

- NCAM – Neuronal Cells – Ectoderm
- VIMENTIM – Vascular Cells – Mesoderm
- DESMIN – Muscle Cells – Mesoderm



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Kishimoto, Keiko, et al. "Establishment of novel common marmoset embryonic stem cell lines under various conditions." *Stem Cell Research* 53 (2021): 102252.

We just mentioned that ESCs are not easily translated to human use, and here's why: the use of feeder cells and media factors shift the resulting cell line into the **xenogenic category**.

There have been recent efforts to shift ESC culturing to a fully **synthetic** process, using specialized **gels** and synthesized recombinant media components, but this process has been slow and expensive.

On the right, you can see an example of the ESC process with marmoset embryos, comparing the traditional feeder cell layer approach with the synthetic approach.

Finally, the gold standard of pluripotency has been the formation of **teratomas** – here at the bottom you can see staining for different embryo-layer derivatives → **Neurons from ectoderm** and vascular and muscle cells from **mesoderm**.

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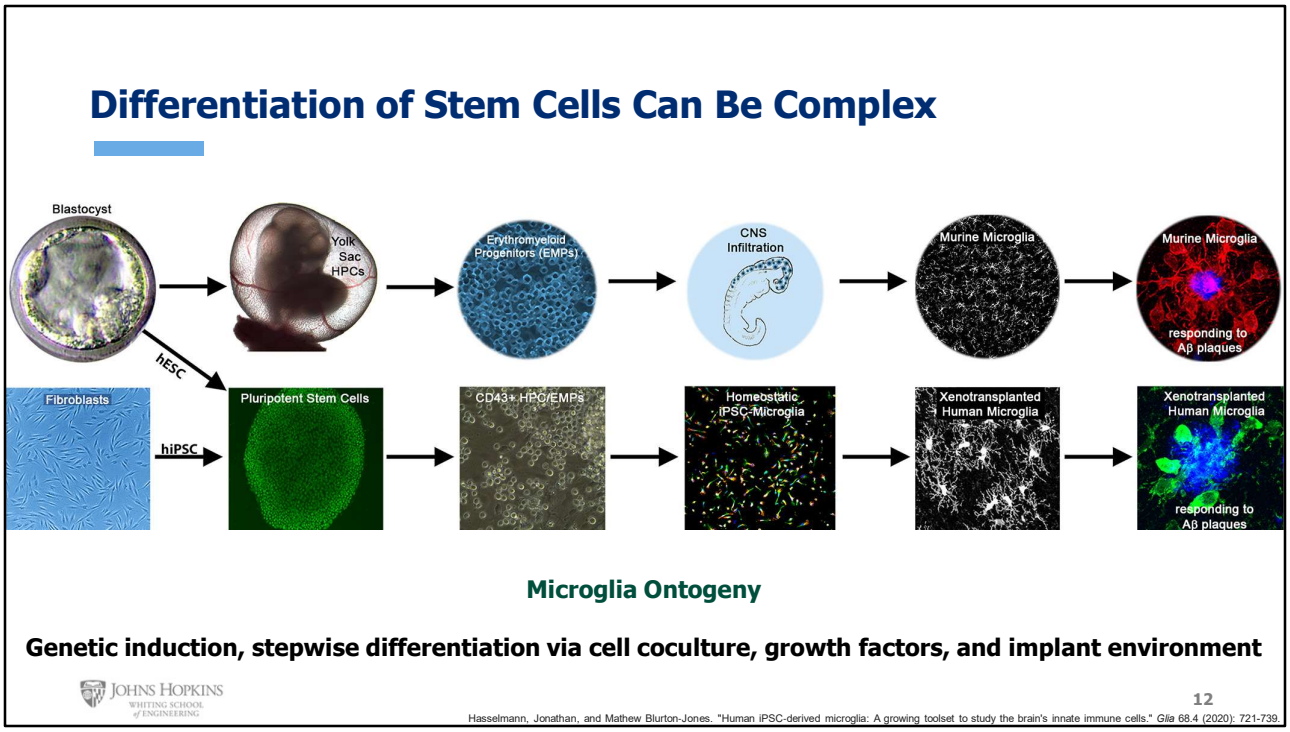
https://www-sciencedirect-com.proxy1.library.jhu.edu/science/article/pii/S1873506121000982



Differentiation of stem cells into target tissues is complex because our native blue-print to follow from natural development is *also* complex.

Here's an example showing *in vivo* and *in vitro* microglia ontogeny.

(Along the Top) Lineage-tracing studies in mice have shown that microglia follow a distinct developmental pathway in vivo. Pluripotent embryonic stem cells (ESCs) within the inner cell mass of the blastocyst give rise to hypoblasts which in turn produce the extraembryonic endoderm of the yolk-sac.

Hemogenic endothelium within the yolk-sac then gives rise to primitive hematopoietic progenitor cells (HPCs) that can further differentiate into EMPs. These cells then infiltrate into the developing central nervous system (CNS) where maturation under homeostatic conditions results in neatly tiled microglia that develop complex ramifications.

In contrast, pathological conditions, such as the development of amyloid plaques in a murine model of Alzheimer's disease (AD), induces robust migratory and morphological alterations.

(Along the Bottom) iPSC-derived microglia protocols seek to mimic in vivo

ontogeny in order to generate cells that **appropriately** recapitulate endogenous human microglia. This is accomplished by obtaining either human ESCs (hESCs) or reprogramming human fibroblasts (or other cells) into induced pluripotent stem cells (hiPSC).

These pluripotent stem cells can then be further differentiated into $CD43^{+}$ microglial progenitors that resemble in vivo HPCs/EMPs**. By providing additional key microglial growth factors and signaling molecules including CSF-1, IL34, and TGFβ, that mimic the homeostatic brain environment**, researchers can promote the further differentiation and maturation of large numbers of iPSC-derived microglia (iMGs).

Subsequent xenotransplantation of these microglia into the murine brain induces **similar** morphology and tiling patterns as endogenous murine microglia while retaining the ability to respond to Alzheimer's pathology

This example should remind you of the many impacts of mechanics, matrix, soluble factors, co-culturing cells that we have examined this semester.

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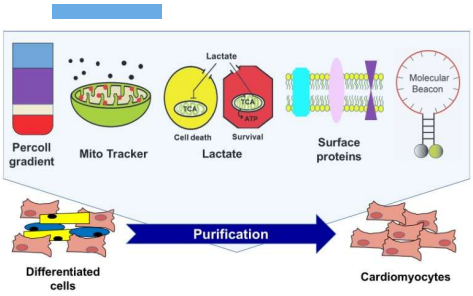
https://onlinelibrary.wiley.com/doi/full/10.1002/glia.23781?casa_token=hvdvXruBEa4AAAAA%3Azc9v_piOsh-A3lHquYTefTKzSXn756pJEONjdAcmmnOCq-Shfi1BSj2n0m1fRBQiY7GXQk4qrZ8p

From Stem Cells to Purified Cell Product



Heterogenous hit rate – how to achieve purity?

Massive number of cells needed for Tissue Engineering

Purification methods	Purity (%)	Yield or Viability (%)	Tumorigenicity	Scalability	Clinical applicability	Application to other type of cells
Manual enrichment	5 - 70%	> 70%	Not reported	Difficult	No	No
Physical enrichment by percoll gradient	40 - 70%	< 70%	No tumor detected	Difficult	Yes	No
Genetic modification-based method	> 95%	~ 70%	No tumor detected	Yes	No	Yes
Surface protein-based method	> 95%	~ 70%	No tumor detected	Yes	Yes	Yes
MITO tracker-based enrichment	Up to 99%	~ 70%	No tumor detected	Yes	Yes	No
Lactate-based enrichment	Up to 99%	> 80%	No tumor detected	Yes	Yes	No
mRNA-based method (Molecular Beacon)	Up to 97%	< 70%	No tumor detected	Yes	Yes	Yes
MicroRNA-based method	Up to 96%	~ 90%	No tumor detected	Yes	Yes	Yes
Microfluidic systems	< 80%	> 70%	Not tested	Difficult	Yes	Yes

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Ban, Kiwon, Seongho Bae, and Young-sup Yoon. "Current strategies and challenges for purification of cardiomyocytes derived from human pluripotent stem cells." *Theranostics* 7.7 (2017): 2067.

Even after you have an isolation and induction process established, there remains work before to arriving at a purified cell product.

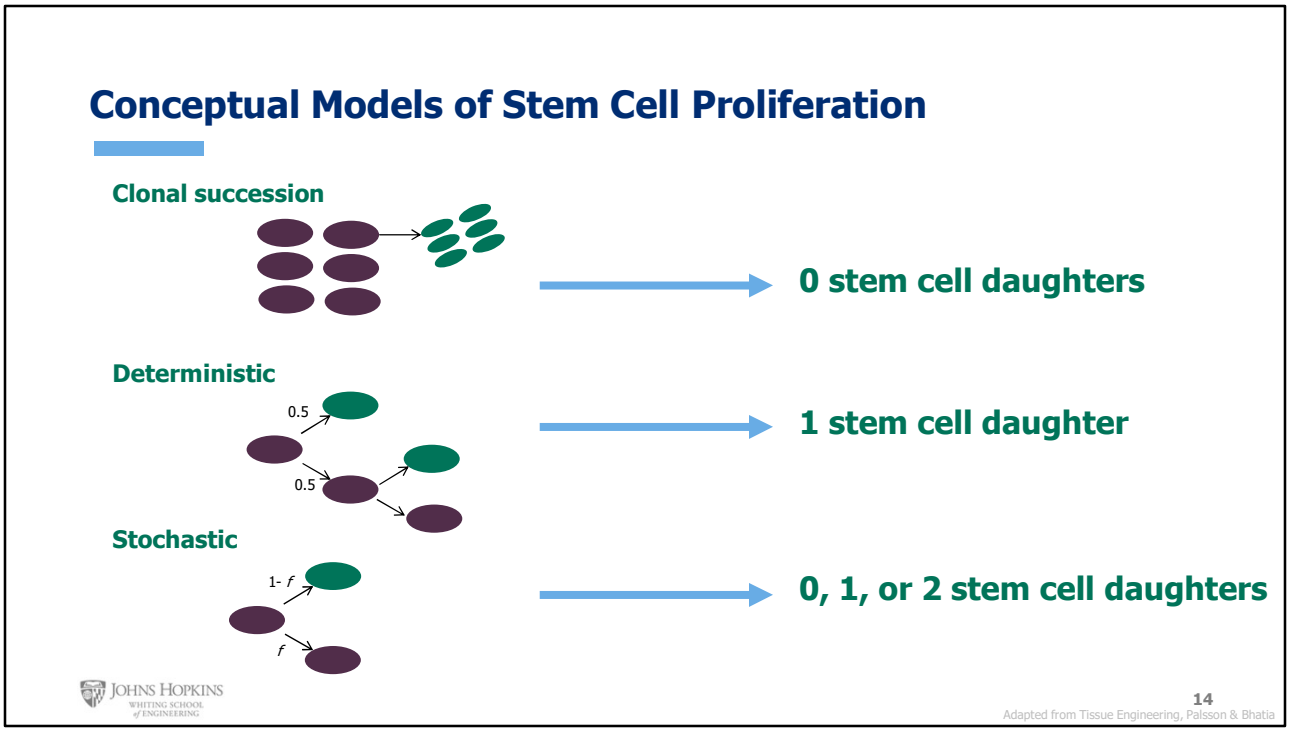
The isolation, **induction**, and **differentiation** processes each have heterogenous hit rates – the result is often variable batch to batch and contains mixed other cells.

There are a number of techniques in the table on the right to help achieve a purified cell product, including genetic based methods to select cells (similar to the antibody resistance gene we talked about earlier). You can also use selective cell attachment, flow cytometry, and MRNA methods.

Each of these techniques has different advantages in the **degree of purity** achieved and the ability to scale and apply the cells to the clinical environment.

Scalability is important because of the massive number of cells needed for Tissue Engineering – often we are considering trillions upon trillions of cells.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5479288/



Now, In order to achieve any use of stem cells, we need them to **divide** – to proliferate.

The last thing we are going to talk about in this lecture are **models** of stem cell proliferation.

There are 3 main models outlined in your reading this week. **Clonal** succession, **deterministic** and **stochastic** models.

The first model is **clonal** succession – in this model stem cells are believed to be existing in a **dormant** state waiting to be called to work. One at a time, each stem cell can be called upon to differentiate and proliferate into a colony of mature cells. The stem cells in this model are **available** for the **life time** of the organism, however once called into action they have a **limited life span**, therefore after some period of time a **mature clone may burn out** and need to be replaced by another.

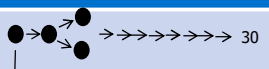
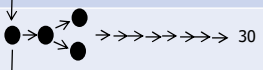
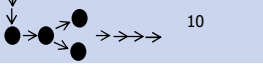
In the clonal model – each proliferation event results in **no stem cell** daughters. All daughter cells come from matured phenotypes.

The second method is **deterministic**. Here the stem cell can renew – with each division the stem cell will split into one **mature daughter cell** and one stem cell. Here I've written that the probability is 50% (or 0,5) but in reality this probability of self

renewal may depend on the tissue environment and be influenced by telomere length.

The last model is **stochastic** – Instead of predicting zero or 1 daughter cells that are stem in nature, this model predicts that it could be either of those or additionally 2 daughter cells. And that this happens in a stochastic way – meaning there is some degree of randomness in what the outcome is – it isn't just a set probability.

Dynamic Models of Stem Cell Proliferation

Year	Telomere length (kbp)	Doublings	Cells	Committed Telomere length (kbp)
0	11.0		10^9	9.5
1			10^9	
2	10.9		10^9	9.4
3			10^9	
60	8.0		10^9	6.5
70	8.0		10^6	6.5
80	8.0		10^3	6.5

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Adapted from Tissue Engineering, Palsson & Bhatia

These conceptual models can be used to generate **dynamic computational models** to analyze rates of cell **division**, rates of **renewal,** and changes in the stem cell pool with organism **age**.

One simple model of an aging stem cell population is depicted in this table, Let's look at it quickly.

Starting at birth at year zero we have a telomere length of **11kpb** (no telomerase activity).

Cell Division results in a new -- but older -- stem cell (downward arrow) and a committed cell (rightward arrow) each with a telomere reduction of 50bp.

The committed cell will undergo 30 population doublings generating 10^9 cells total. These cells will have telomeres 1.5kbp shorter than the original stem cell – here you see 9.5 vs 11.

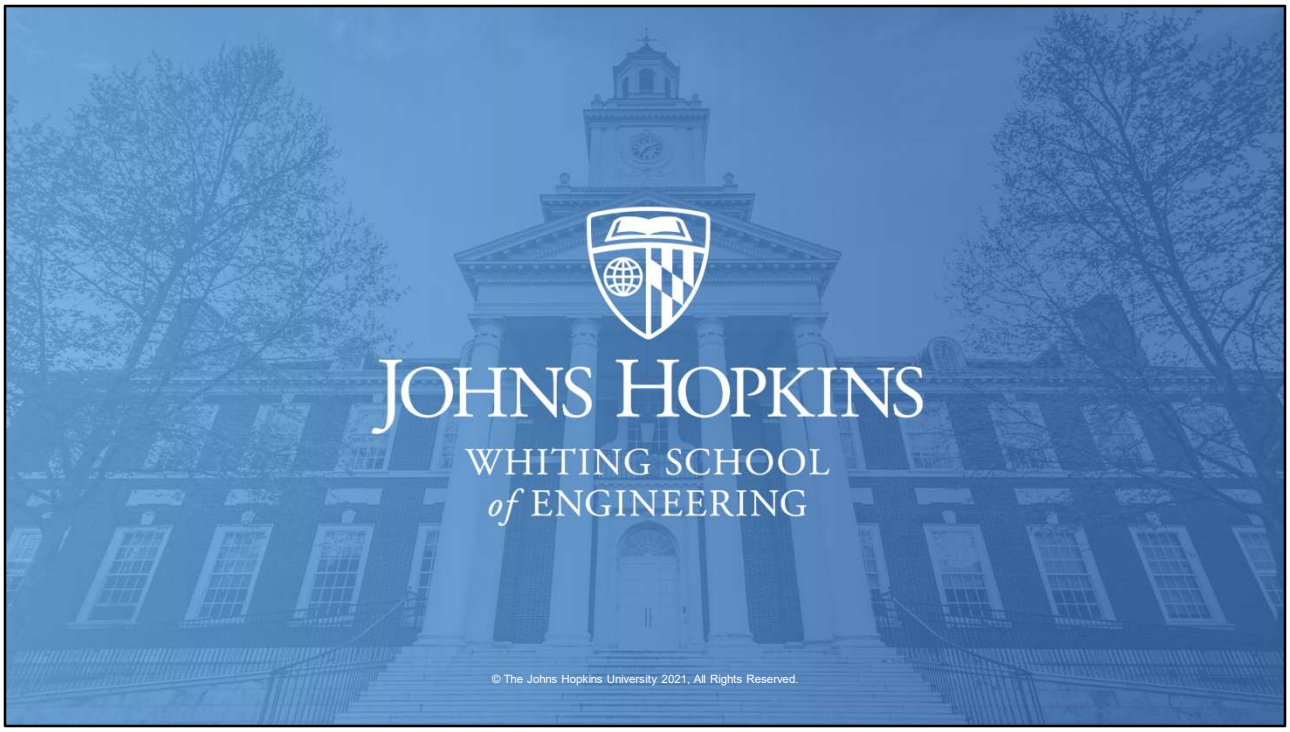
Now that stem cell daughter will sit in **G0** in the cell cycle for **1 year** and then the model has this cell once again enter into proliferation. This cell division is **asymmetric** – producing a new stem cell and a committed progenitor each time.

Around age 60 that stem cell population starts to go into **senescence**. You can see

here you only get 10^6. By age 80 we only get 1000 committed cells.

Although all of the parameters in this model are not fully researched, the model does demonstrate the power of using these quantitative techniques to understand stem cell dynamics.

In this case the potential of the stem cell pool may be correlated with the onset of diseases, and if this model were patient specific, it could also be useful for determining the abilities of autologous stem cell therapies.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