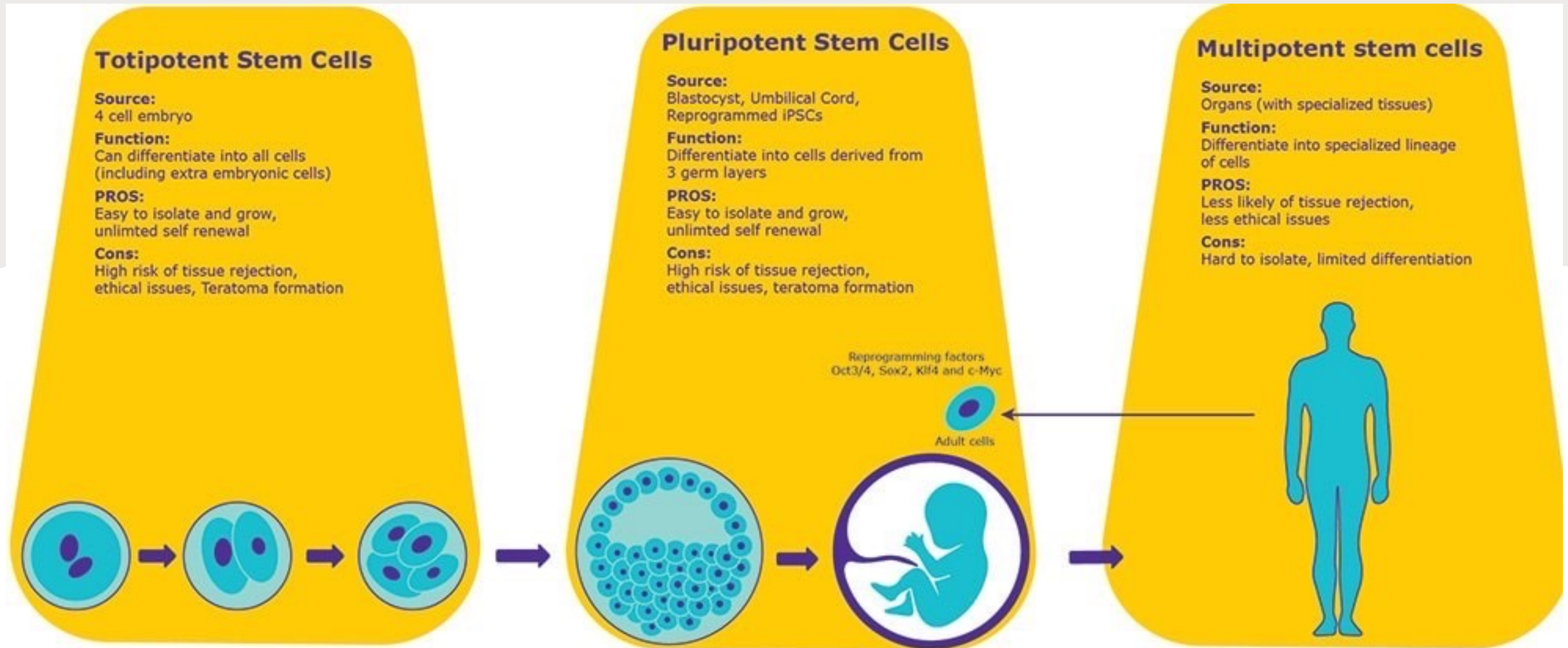
iPSC as powerful models for translational medicine

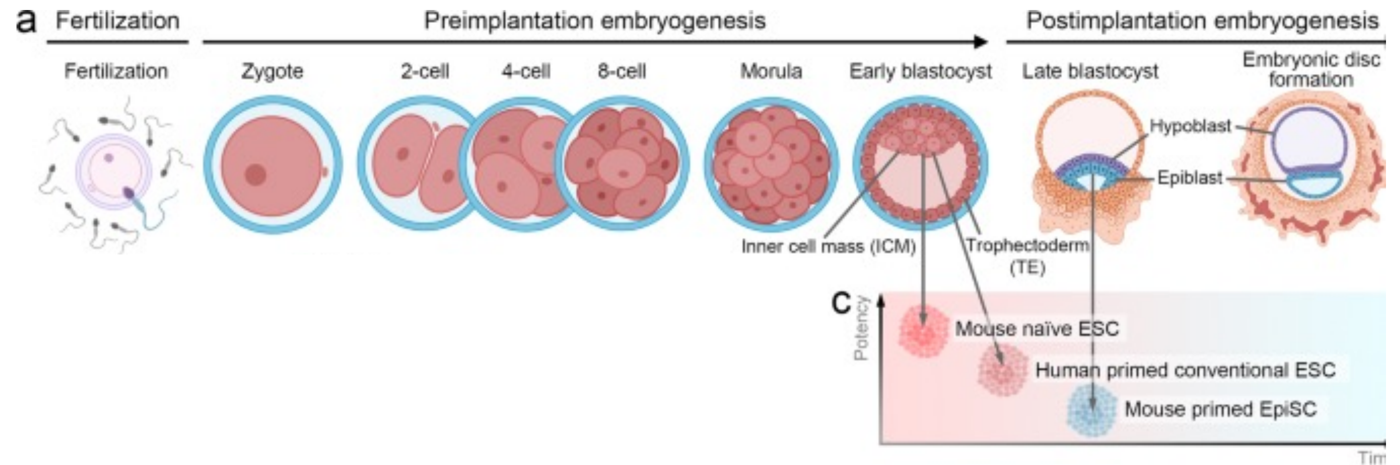
Types of stem cells



https://www.sigmaaldrich.com/IT/en/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/stem-cell-culture/what-are-stem-cells

Dynamics of stem cells development

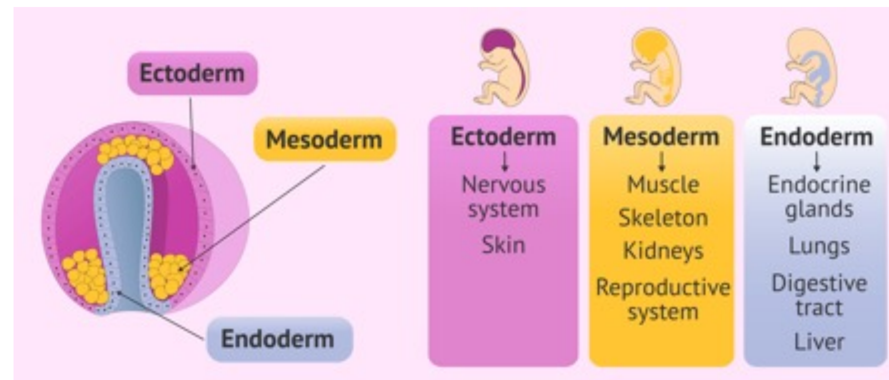
- Differences in environmental conditions applied to each cell in the mass result in the generation of the first heterogeneous population: the ICM and trophectoderm (TE)
- Trophoblasts organize surrounding structures such as the chorion, which supports embryogenesis, whereas the ICM is critical for the formation of the embryo
- After implantation of a blastocyst in the maternal endometrial epithelium, the ICM undergoes subsequent morphogenetic changes.
- The ICM of a postimplantation blastocyst contains epiblasts and hypoblasts
- The morphogenetic events include the polarization of the epiblast, which forms the central lumen that develops into the amniotic cavity; creation of the amniotic epithelium, which forms the amniotic sac membrane; and differentiation of primordial germ cells, which are precursors of eggs or sperm.
- Moreover, extraembryonic mesenchyme cells derived from the hypoblast surround the generated structure to isolate it from the outer cell membrane (OCM) formed by the trophoblast
- Thereafter, epiblasts in the ICM form a primitive streak, gastrulate and differentiate into three germ layers: The ectoderm, mesoderm, and endoderm[47].



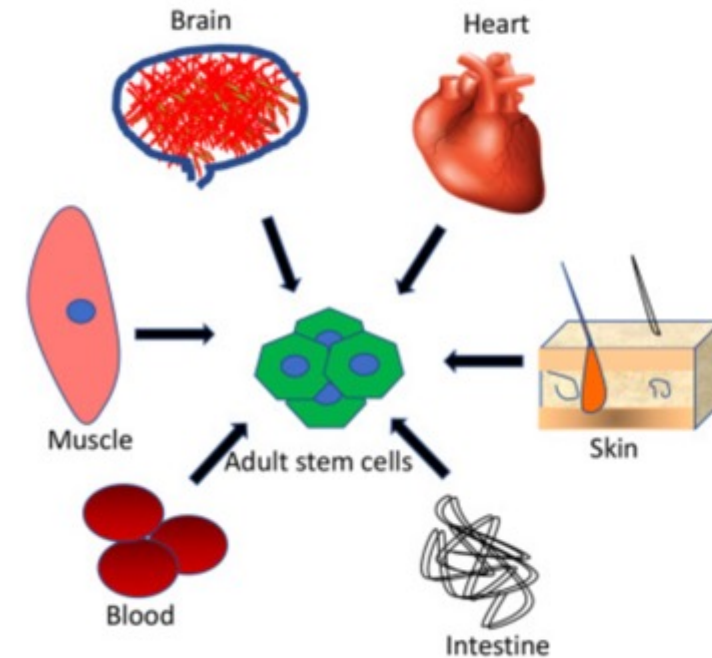
Adapted from Che, 2022

totipotent

pluripotent



Adult stem cells



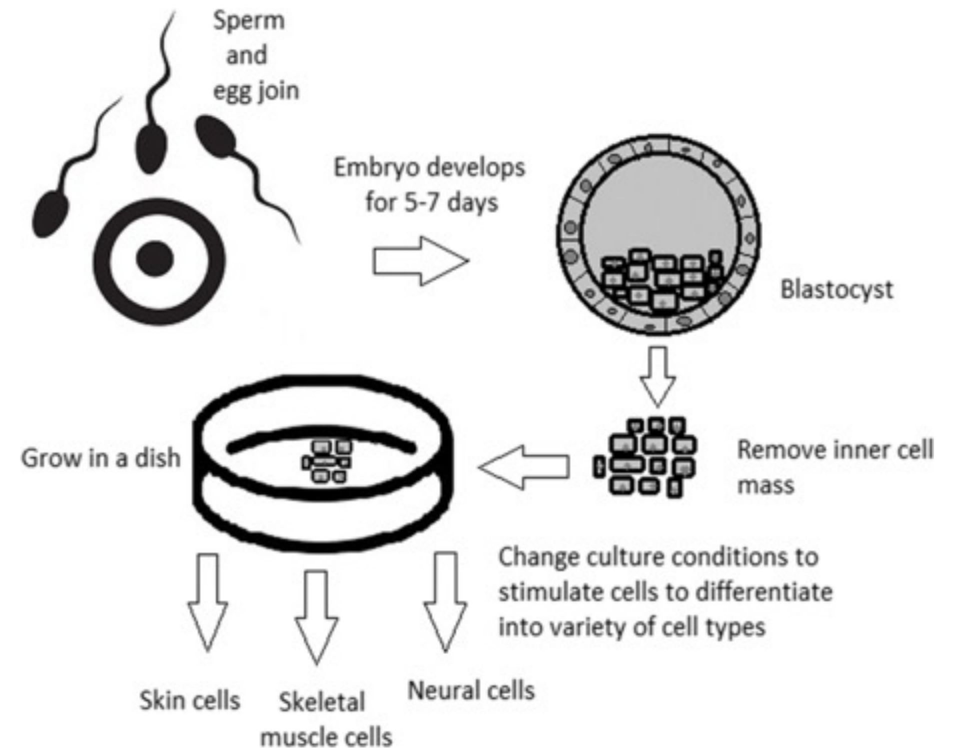
- Adult stem cells, also known as somatic stem cells or resident stem cells, are a rare population of undifferentiated cells, located within a differentiated organ, in a specialized structure, called a niche, which maintains the microenvironments that regulate the growth and development of adult stem cells.
- The adult stem cells are self-renewing, clonogenic, and multipotent in nature, and their main role is to maintain the tissue homeostasis. They can be activated to proliferate and differentiate into the required type of cells, upon the loss of cells or injury to the tissue.
- Adult stem cells have been identified in many tissues including blood, intestine, skin, muscle, brain, and heart. Extensive preclinical and clinical studies have demonstrated the structural and functional regeneration capabilities of these adult stem cells, such as bone marrow-derived mononuclear cells, hematopoietic stem cells, mesenchymal stromal/stem cells, resident adult stem cells, induced pluripotent stem cells, and umbilical cord stem cells.

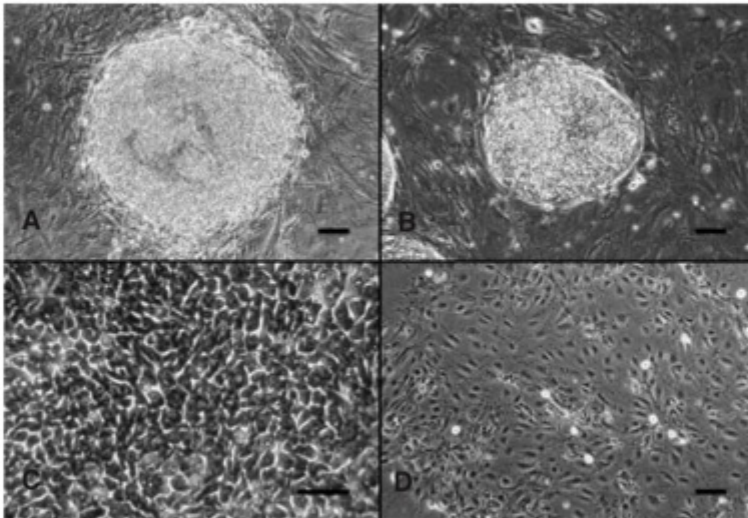
Mohamed Al-Rubeai • Mariam Naciri, Stem Cells and Cell Therapy, Volume 8, Springer Verlag

ESC

Development:

- initially isolated from mouse (Evans and Kaufman 1981, Martin et al. 1981)
- then primate ESCs (Thomson et al 1995)
- Human ESCs (Thomson et al 1998)





Generation:

•the Inner Cell Mass (ICM) is removed from its normal embryonic environment and cultured under appropriate conditions

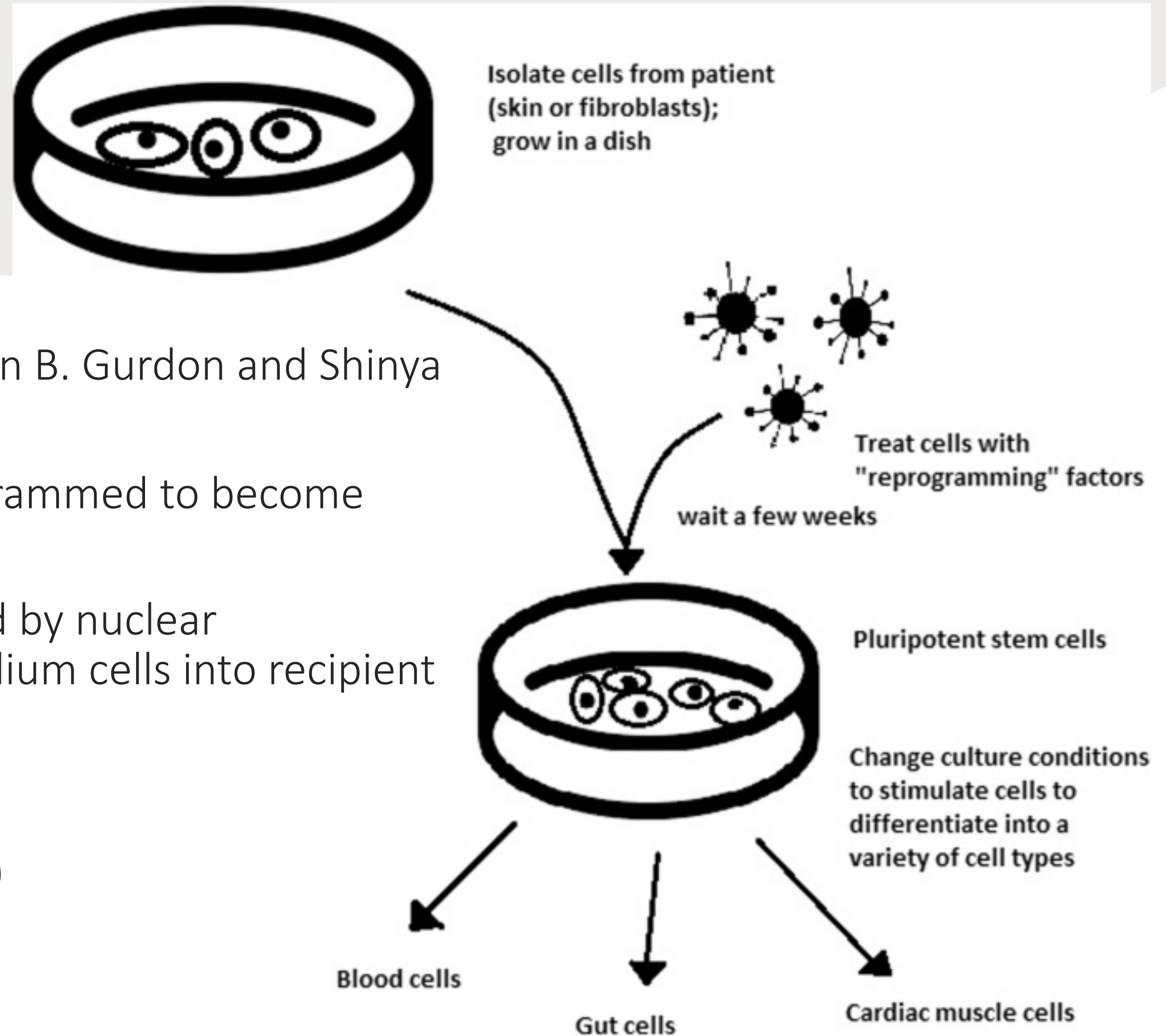
•the ICM-derived cells continue to proliferate and replicate indefinitely

•they still maintain the developmental potential to form any cell type of the body
→ pluripotency

- Strengths and Limitations:
  + pluripotent
  + self renewal = unlimited growth
  + resemble human stem cell state the closest
- →allogeneic, thus immune rejection
  →availability and ethics
  →teratoma formation

IPSC

- Nobel Prize in Physiology or Medicine 2012 Sir John B. Gurdon and Shinya Yamanaka
- "for the discovery that mature cells can be reprogrammed to become pluripotent“
- the fate of fully differentiated cells can be changed by nuclear transplantation of Xenopus laevis intestinal epithelium cells into recipient eggs (Gurdon 1962)
- mouse iPSCs (Takahashi and Yamanaka 2006)
- human iPSCs (Takahashi et al. 2007, Yu et al. 2007)



Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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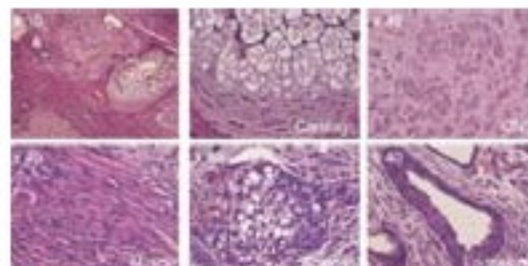
DOI 10.1016/j.cell.2006.07.024
Cell 126, 663-676, August 25, 2006 ©2006 Elsevier Inc.

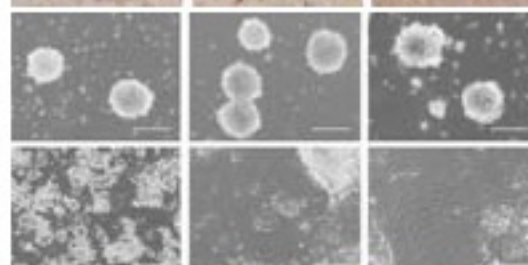
Induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, **Oct3/4, Sox2, c-Myc**, and **Klf4** in the FBX15 locus, under ES cell culture conditions.

Various tissues present in teratomas derived from iPS

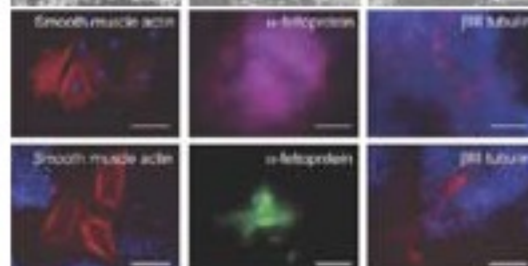


Neural tissues and muscles in teratomas

In vitro embryoid body formation and differentiation



In vitro differentiation into all three germ layers.



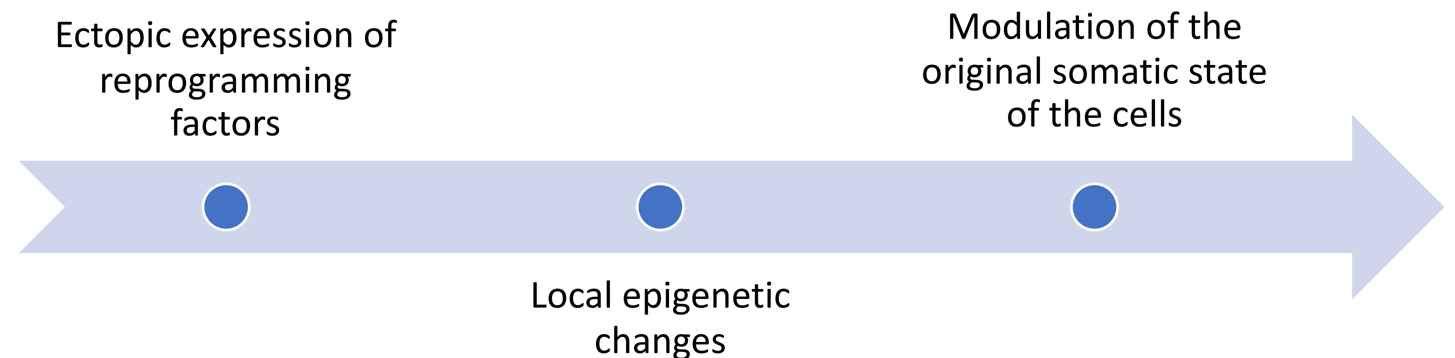
These cells, which were designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes.

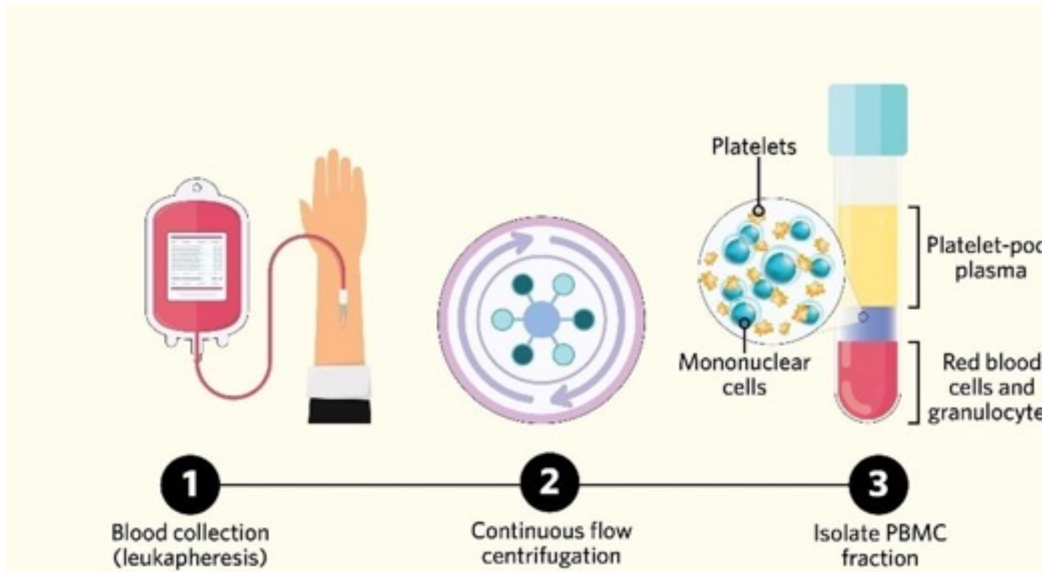
1- Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers.

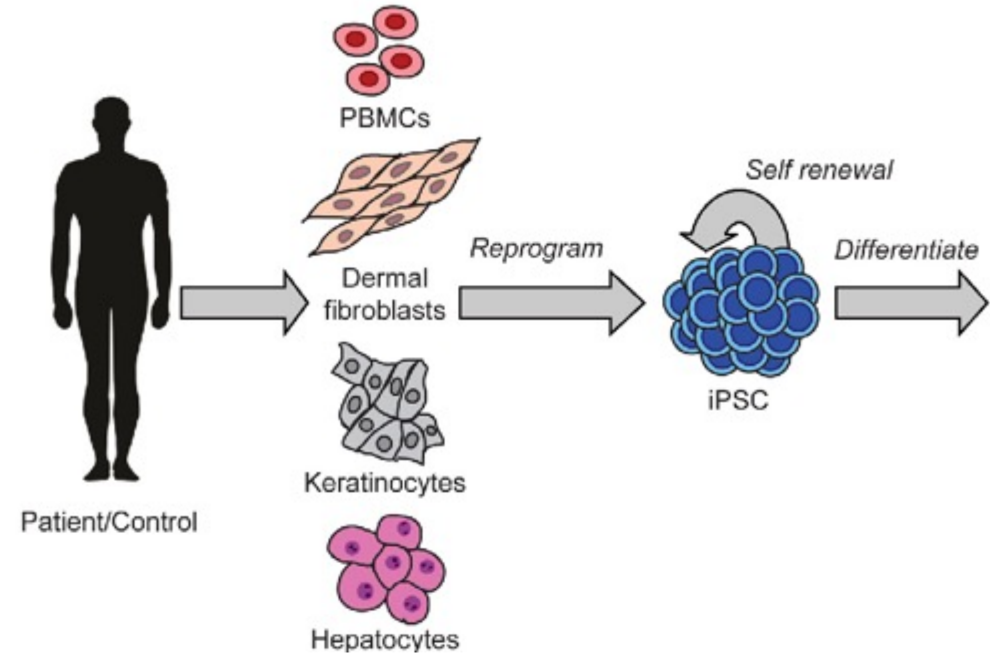
2- Following injection into blastocysts, iPS cells contributed to mouse embryonic development, but embryos failed to develop beyond mid-gestation stage.

REPROGRAMMING iPSC

- Reprogramming is achieved over several weeks by **forced expression of genes that are known to be master regulators of pluripotency.**
- At the end of this process, these master regulators will remodel the expression of an entire network of genes.
- Features of differentiated cells will be replaced by those associated with the pluripotent state, essentially reversing the developmental process.
- mesenchymal genes → initially repressed by OCT4 and SOX2
- Proliferation → enhanced by MYC
- epithelial genes → induced by KLF4
- self-sustaining regulatory network providing cells with pluripotency factors is reactivated
- ectopic expression of pluripotency factors then no longer needed
- key role of chromatin remodeling & epigenetic makeup (origin) of the cells







SOURCE OF IPS CELLS

REPROGRAMMING METHODS

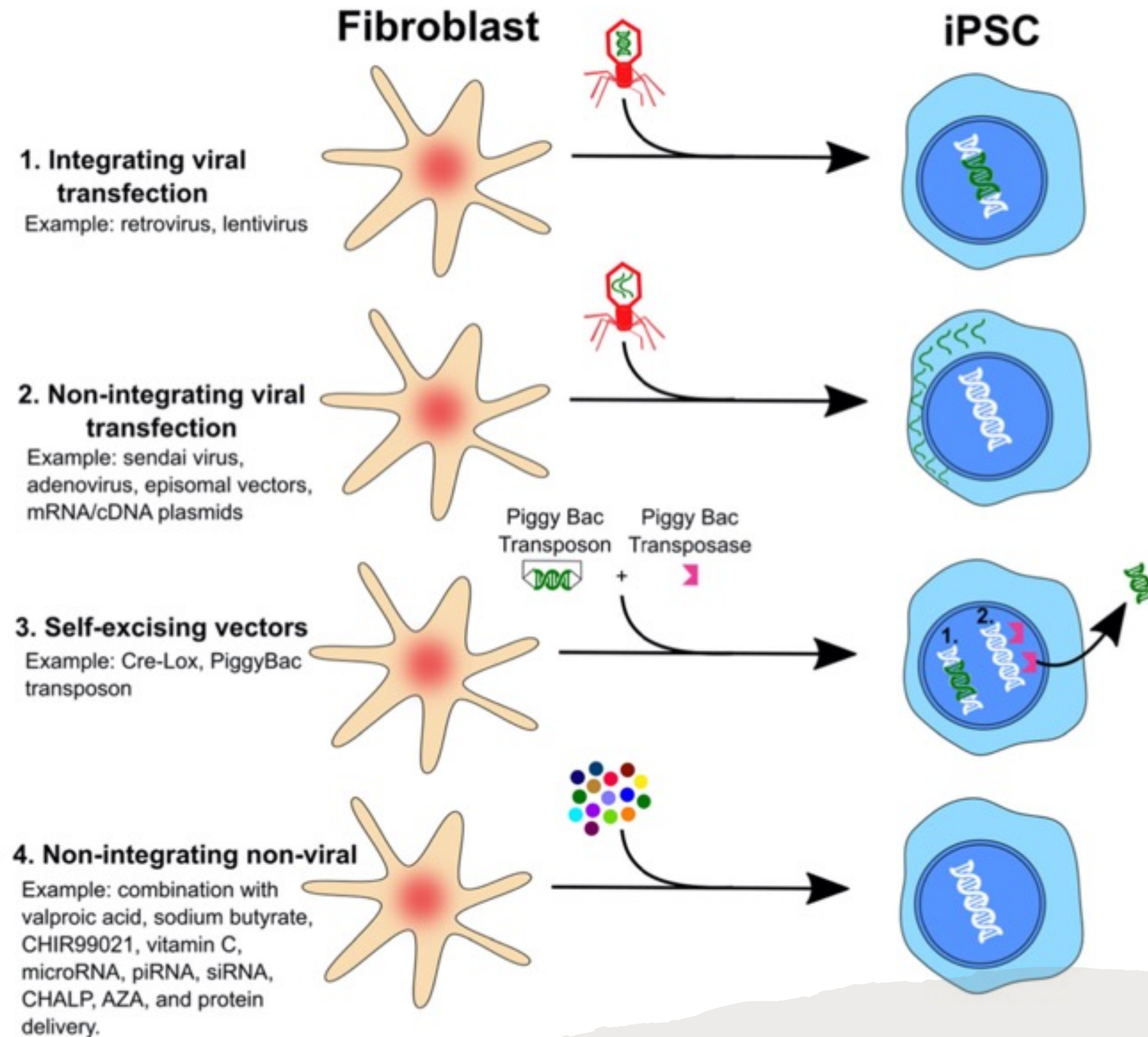


Fig. 2. The four key methods for delivering reprogramming factors. Integrating viral systems were the first to be used to deliver transcription factors to generate stem cells, but they have the disadvantage of incorporating their genetic material and contributing to teratoma formation. By avoiding integration, novel methods (non-integrating vectors, self-excising vectors, and non-integrating non-viral vectors) represent iterative improvements upon this initial methodology. Such approaches provide significant advances in the safety and efficacy of iPSCs, which may then be applied for downstream scientific and clinical applications.

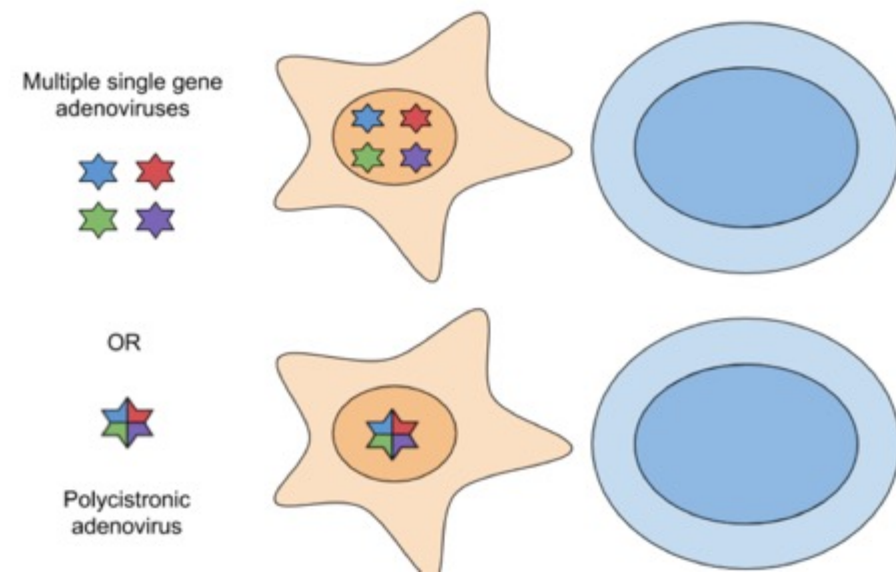
Adapted from Liu, 2020

Non-integrating iPSC delivery methods

Viral Vectors

- **Adenovirus**: Adenoviral vectors infect dividing and nondividing cells and have an ~8kb packaging capacity. With this packaging capacity, reprogramming factors can be delivered either as a single polycistronic transgene or with four different adenoviruses, each expressing one factor. These vectors don't integrate into the genome and are instead lost by dilution via cell division. A drawback to this approach is it has lower levels of efficiency at generating iPSCs, usually several orders of magnitude lower than retroviruses; however, because they are less likely to cause insertional mutagenesis, adenoviral vectors are considered a safer way to express reprogramming factors for therapeutic applications.

- **Sendai viral vectors**: Sendai virus is a single stranded, negative sense RNA virus. It's a member of the *Paramyxoviridae* family of viruses, which also includes measles and mumps. Sendai transduces a wide range of cell types and replicates in the cytoplasm independent of the cell cycle. A challenge of using Sendai is that since it's replication competent, it's difficult to eliminate the virus from all cells, even after many passages. Ban et al developed a temperature sensitive Sendai virus



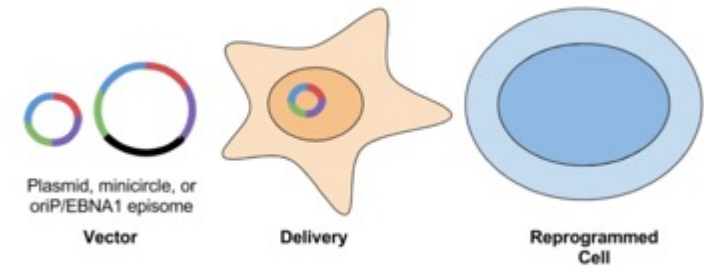
https://blog.addgene.org/delivery-methods-for-generating-ipscs

Transient episomal delivery

Non-integrating methods have a **smaller genetic footprint** compared to integrating approaches. These methods **eliminate the risk of insertional mutagenesis, the presence of a genetic scar, and incomplete silencing of transgenes.** Overall, non-integrating approaches are safer than integrating methods, with RNA and protein delivery considered the safest since there's minimal risk of lingering expression of reprogramming factors.

- **Non-replicating**
  - **Plasmids:** Generating iPSCs with plasmid-based expression requires serial transfection of 1 or 2 plasmids that express the reprogramming factors of interest. The advantages of this method are that it's relatively simple to implement and doesn't require time-consuming production of virus. In theory this is a non-integrating approach; however, in practice integration can occur. Okita et al describes a protocol for generating iPSCs by plasmid transfection where 2 of 11 clones tested had plasmid integration. Another challenge is that multiple transfection makes it difficult to control the dose of plasmid the cells receive over the whole reprogramming period and the plasmid will get diluted faster when cells are actively dividing. The typical drawbacks of using transfection will still exist: transfection efficiency is cell-type dependent and larger plasmids have lower rates of transfection.

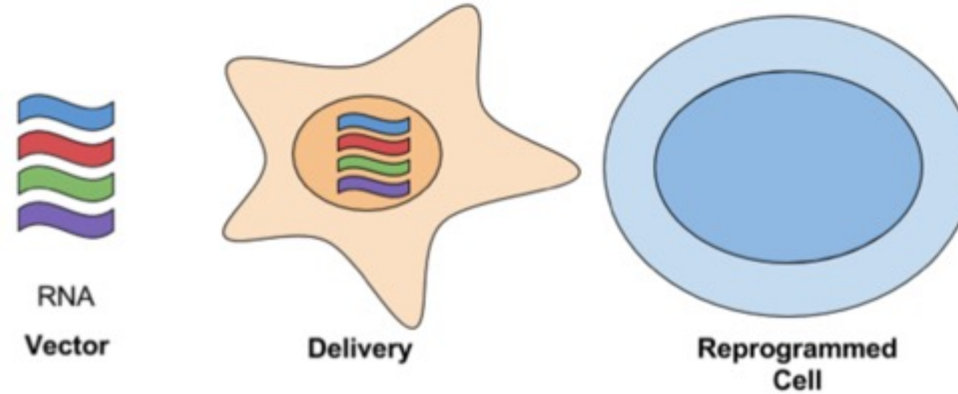
  - **Minicircles**: Minicircles are like mini-plasmids. They contain only a eukaryotic promoter and the cDNA(s) to be expressed and they don't integrate or replicate. Their small size leads to higher transfection efficiencies and they tend to express for longer periods of time than traditional plasmids due to lower activation of DNA-silencing mechanisms. While minicircles are typically smaller than traditional plasmids, Jia et al generated iPSCs using a ~14.5kb minicircle expressing a 2A peptide polycistronic cassette comprised of OCT4, SOX2, LIN28, NANOG, and a GFP reporter. Minicircles are removed from cells by dilution with each cell division, but it can still take several passages for the minicircle to be completely removed.



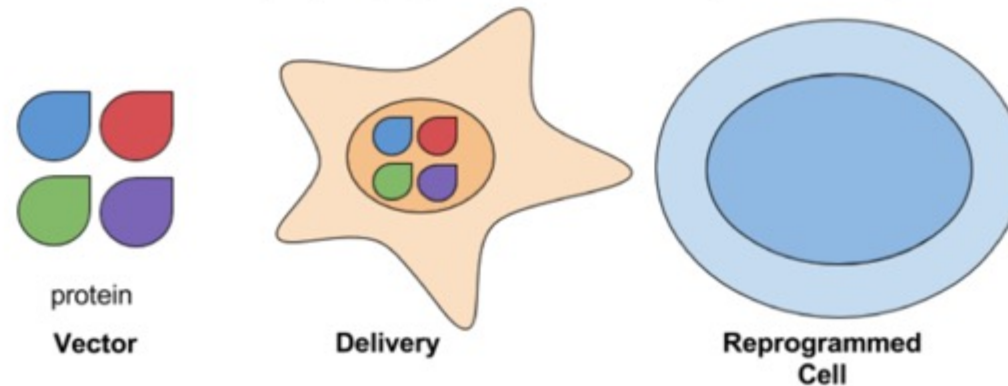
https://blog.addgene.org/delivery-methods-for-generating-ipscs

DNA-free methods

- **RNA Delivery**: For this approach, *in vitro* transcribed RNAs are serially transfected using a cationic vehicle. The following synthetic modifications are used to protect the RNAs from cellular defenses against ssRNA: phosphatase treatment, substituting cytidine with 5-methylcytidine, and/or substituting uridine with pseudouridine. The key advantage of using RNA to reprogram cells is that it's simple and efficient. It has a high efficiency of reprogramming when compared to other methods, even when compared to integrating viral approaches. It's also has high marks for safety.

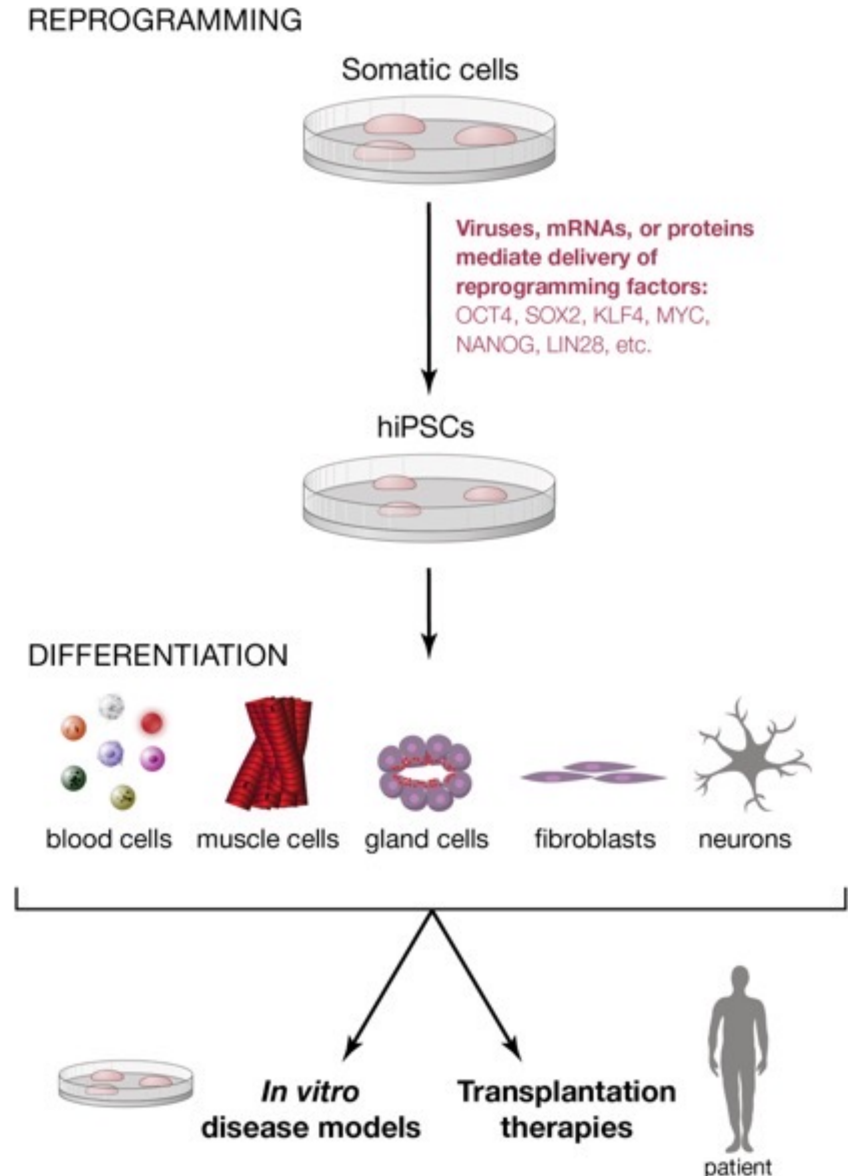


- **Protein Delivery:** Delivering reprogramming factors as protein is another way to avoid exposing cells to exogenous genetic material. Proteins are delivered to cells by fusing them with peptides that help mediate their transduction, such as the polyarginine peptide described in Zhou et al and Kim et al. This is considered a safer approach for developing therapeutic applications, but it can take a long time to reprogram cells. In Kim et al, cells were exposed to reprogramming proteins for 8 hours a week for 6 weeks, and still required further culturing to generate iPSC colonies. Reprogramming efficiencies are also low and it's difficult to purify the reprogramming proteins needed to perform these experiments.



Applications of iPSCs

- Regenerative Medicine: iPSCs can be differentiated into specialized cells for transplantation
- Drug Discovery and Screening: iPSCs can be used to test the effectiveness and safety of new drugs
- Disease Modeling: iPSCs can be used to understand disease mechanisms and develop targeted therapies



Induced Pluripotent Stem Cells Meet Genome Editing

Organoids: complex tissue in a dish

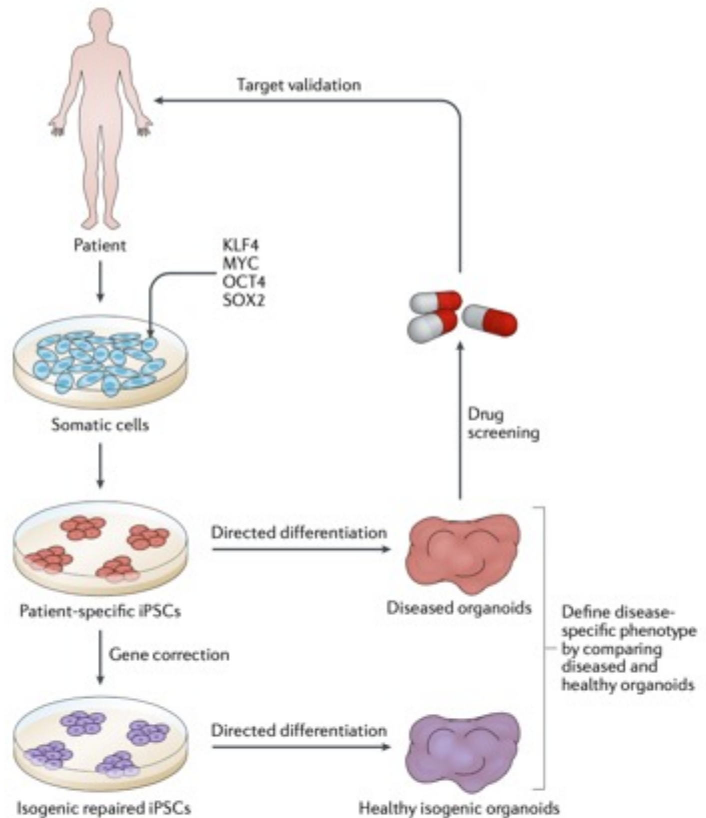


Fig. 2 | **Application of organoids derived from iPSCs to disease modelling and drug discovery.** Remarkable progress has been made in the differentiation of increasingly complex multicellular and diverse organoid systems across many tissues. We propose that parallel differentiation of organoids from patient-derived induced pluripotent stem cells (iPSCs) as well as genetically corrected, isogenic control iPSCs will allow attribution of an organoid-level disease phenotype to a specific molecular lesion. Once a clear organoid-level readout is established, diseased organoids can be used in drug screening and validation studies.

Table 1 | **Phenotypes modelled in 2D and 3D systems based on iPSCs**

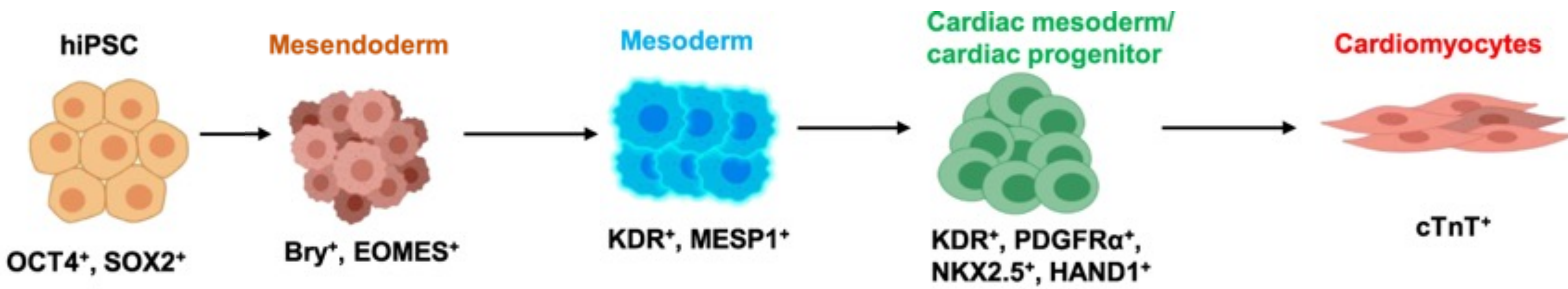
Tissue	2D phenotype	3D phenotype	Refs
Blood	Oligopotent differentiation	Multipotent differentiation and engraftment	10,11,108
Neural	Neural differentiation, gene expression and neurite formation	Cortical organization, regional specification, cell–cell interactions and neuronal migration	15,19,20,31,32,40,47
Cardiac	Action potential and contractility	Self-organization and integration of biophysical cues	16,84
Gastrointestinal	Differentiation	Bile secretion, motility and cell–cell interactions	46–49

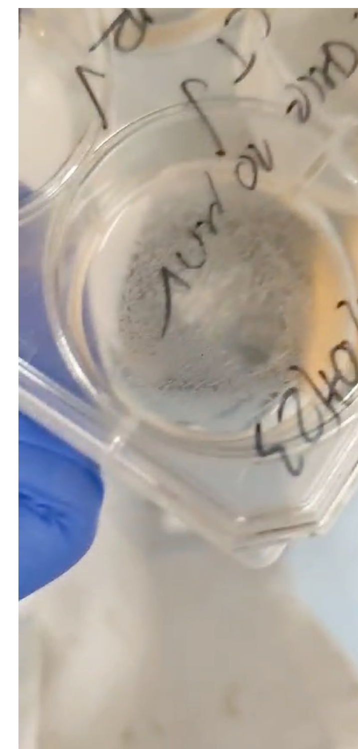
iPSCs, induced pluripotent stem cells.

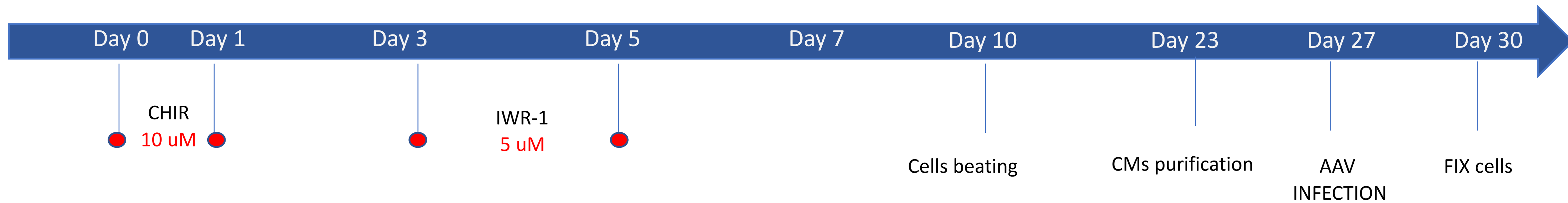
Rowe 2017

- An important advance in disease modelling with iPSCs occurred with the development of **iPSC-derived organoids**.
- Organoids are 3D multicellular **aggregates derived from stem cells that differentiate and self- organize to recapitulate the structural features and cell– cell interactions of mature tissues**.
- The soluble and biophysical cues used to guide organoid differentiation from PSCs have been incrementally refined to generate increasingly complex ‘tissues in a dish’.
- Although several limitations exist in current 3D technology, combining disease-specific iPSCs with 3D technology enables the **examination of spatiotemporal cellular interactions that could reveal the physiological disease status**, thus providing an **unprecedented drug-screening platform and offering a new option for tissue-replacement therapy.**
- Furthermore, investigators have achieved specification of particular regions of organs such as the brain and gastrointestinal tract.

Cardiac differentiation



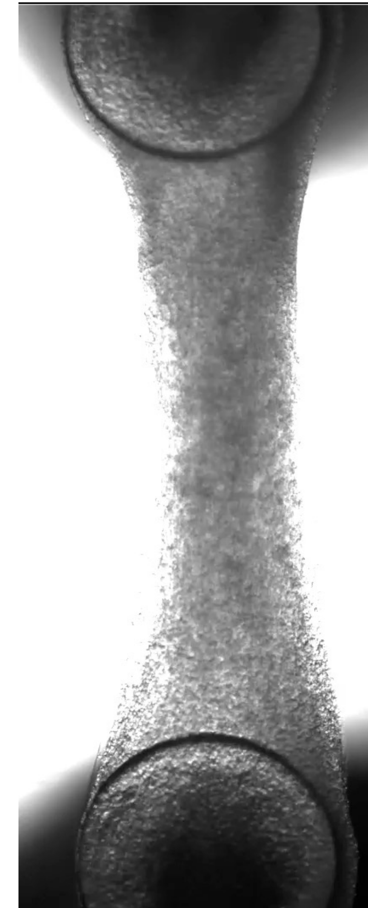




Engineered Heart Tissue

- Fibrin-based EHTs are anchored between two flexible silicone posts, which generate a preload

- EHTs perform auxotonic contractile work (the physiological form of cardiac contraction) against elastic posts.

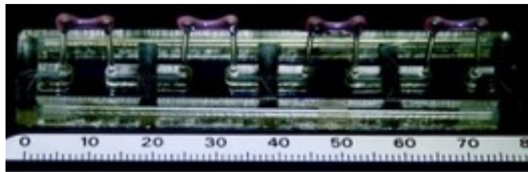


Fibrin-based Engineered Heart Tissue (EHT)

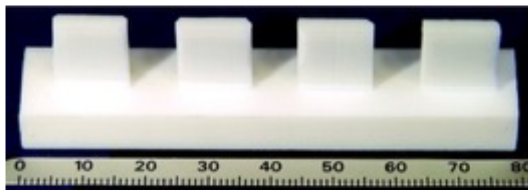
Circ Res. 2010 Jul 9;107(1):35-44. doi: 10.1161/CIRCRESAHA.109.211458. Epub 2010 May 6.

Development of a drug screening platform based on engineered heart tissue.

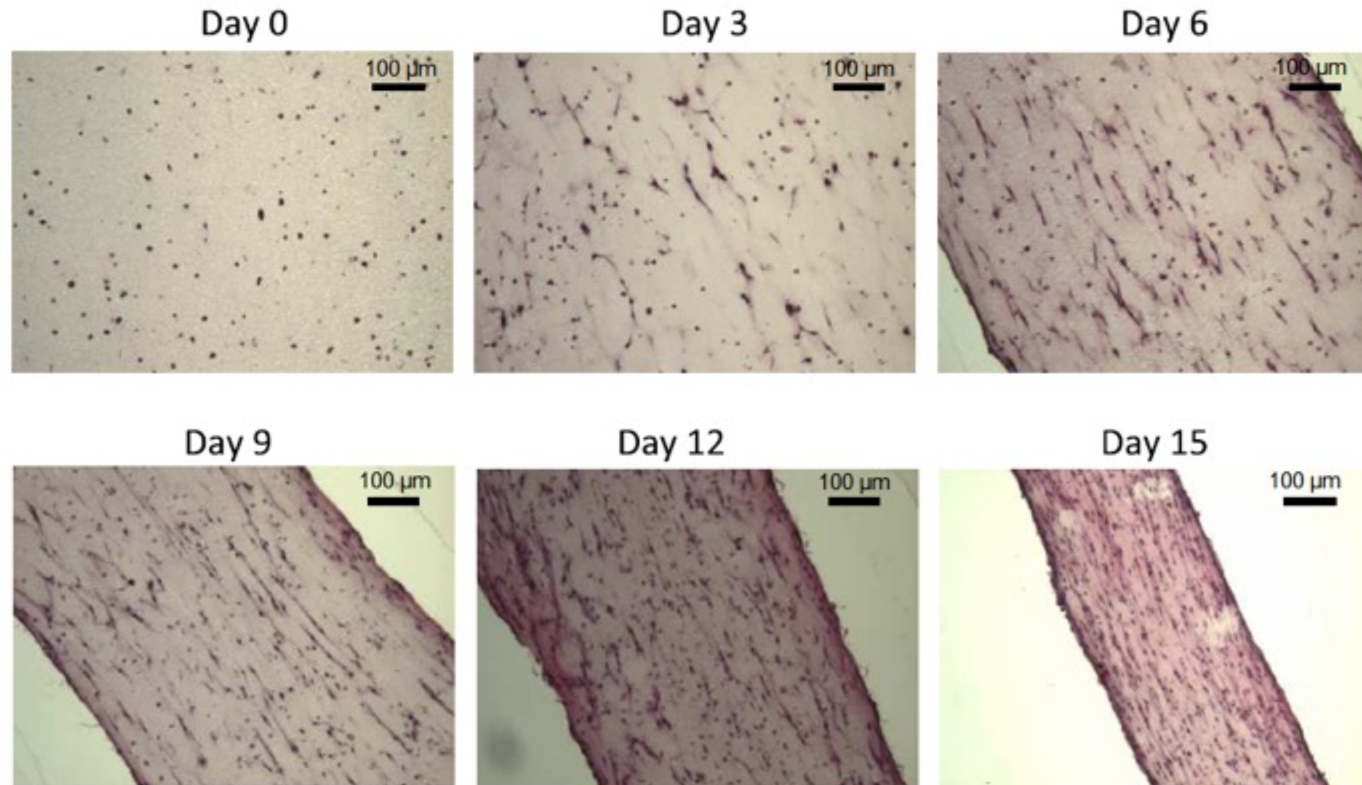
Hansen A[1], Eder A, Bönstrup M, Flato M, Mewe M, Schaaf S, Aksehirlioglu B, Schwoerer AP, Uebeler J, Eschenhagen T.

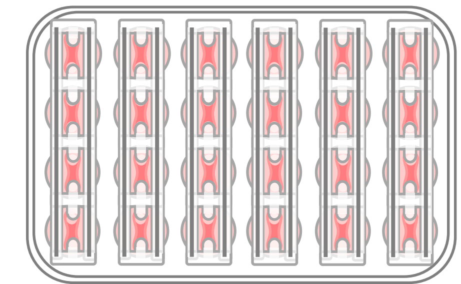


Silicon post rack with four EHTs, turned upside down

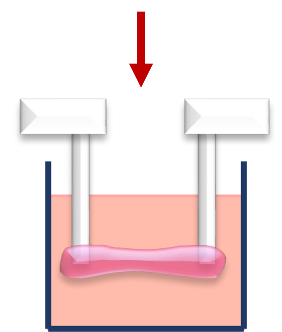


Teflon spacers for producing the casting molds





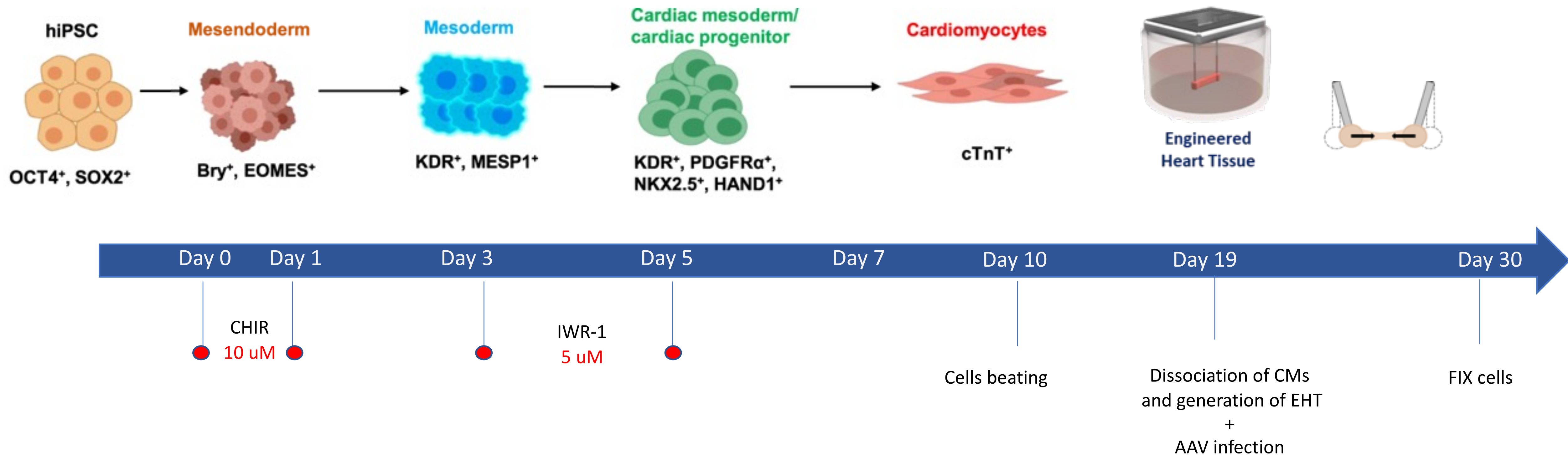
24-well plate



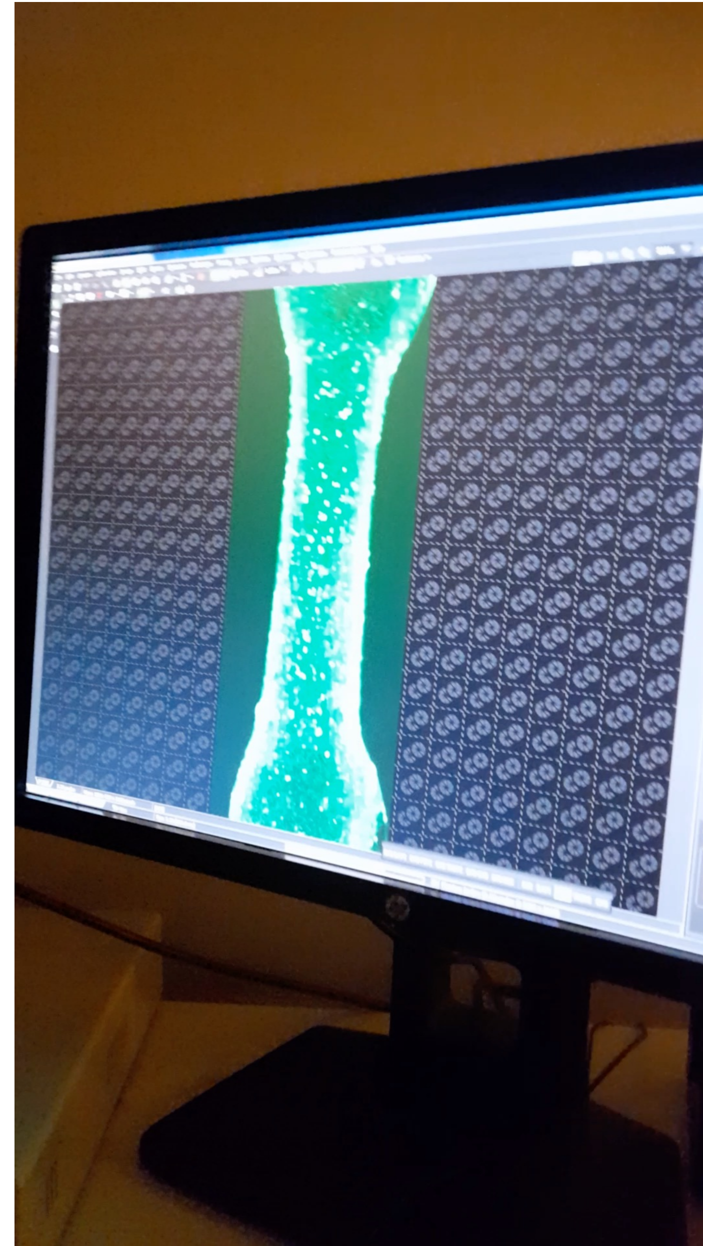
EHT anchored to silicon posts

Hirt et al., Circ Res. 2013

ENGINEERED HEART TISSUE



LIVE IMAGING



AAV6 CMV GFP

Crioinjury as a model of MI