Stem Cells and Differentiation

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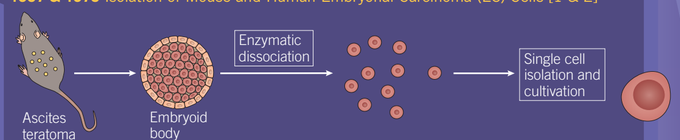
Stem cells are defined by their ability to self-renew and propensity to differentiate into functional cell types. Pluripotent Stem Cells (PSCs) differentiate into cells of all three germ layers (endoderm, ectoderm and mesoderm), whereas multipotent stem cells are more limited in their differentiation potential. The unique abilities of these cell types make them attractive tools for a wide range of applications, from regenerative medicine to drug toxicity screening. There are a large number of protocols for the maintenance of this pluripotent state, as well as for the subsequent directed differentiation of these cells *in vitro* to form specialized cell types.

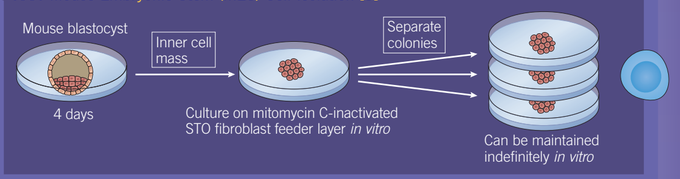
Pluripotent Stem Cells (PSCs) *In Vitro*

The main pluripotent cell types used *in vitro* have been isolated over a number of decades.

1967 & 1975 Isolation of Mouse and Human Embryonal Carcinoma (EC) Cells [1 & 2]

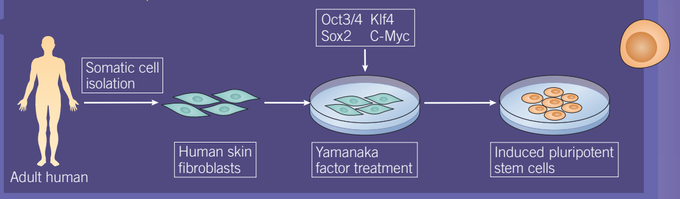


1981 Mouse Embryonic Stem (mES) Cell Isolation [3]



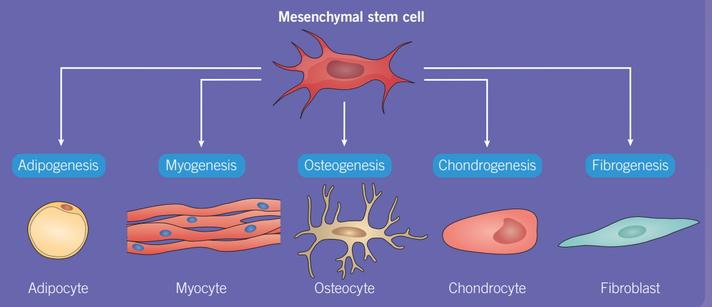
1998 Human Embryonic Stem (hES) Cell Isolation [4]

2007 Induced Pluripotent Stem (iPS) Cell Isolation [5]



Adult Stem Cells (ASCs)

It is thought that most organs contain resident adult (or somatic) stem cell populations, i.e. groups of cells that are able to self-renew and differentiate into a limited number of cell types to repopulate an organ for maintenance of functionality throughout normal cell turnover or upon challenge/injury. Mesenchymal stem cells (MSCs) are a very well characterized somatic stem cell type, due to their ease of isolation from connective tissue and relatively large capacity for differentiation. Recently, the receptor LGR5 has gained provenance as a marker for a range of more specific organ adult cell types, such as in the intestine and liver. Such markers are important in the development of organoid technology.



Maintenance of Stem Cell Phenotype *In Vitro*

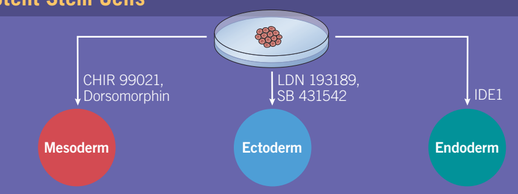
The maintenance of stem cell self-renewal and an undifferentiated status is often overlooked. However stem cells occupy a continuum of developmental states (e.g. "naïve" vs. "primed" pluripotency) that can, in some cases, be defined by the culture environment. Methods of maintaining pluripotency were initially focused around growth on "feeder" layers of fibroblasts. The spotlight subsequently shifted to specialized growth medium formulations and the use of qualified serum and small molecules. LIF for mESCs and bFGF for hESCs, are most commonly used to maintain pluripotency, but there are a range of other compounds, including Y-27632, A83-01 or SB 431542, which can be used to maintain cell phenotype.

More recently focus has shifted further towards the composition of the growth substrate itself; research concerning substrate coatings (including tailored integrins) and substrate topography (hydrogels and other scaffolds) is ongoing for both the maintenance and differentiation of PSCs.

Differentiation

Monolayer Differentiation of Pluripotent Stem Cells

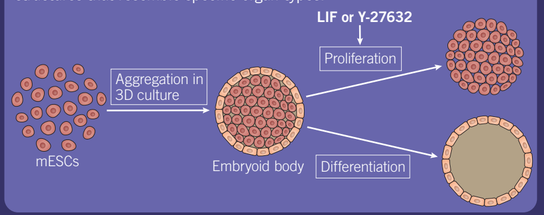
Initial models of differentiation were based upon conventional monolayer cell culture, and facilitated identification of the key biochemical pathways controlling spatial patterning and development: canonical WNT, Hedgehog, TGF and Retinoic Acid. Using this method many key modulators governing these pathways were also revealed, providing well characterized and controllable methods of differentiation. There are a number of known disadvantages of monolayer systems, the main one of which, a lack of biomimetic extracellular architecture, is thought to be the main contributor to the extremely low yields of differentiation plaguing regenerative medicine. There are, however, a number of established and emerging alternative techniques that researchers are now turning to.



Current and Emerging Trends in SC Differentiation

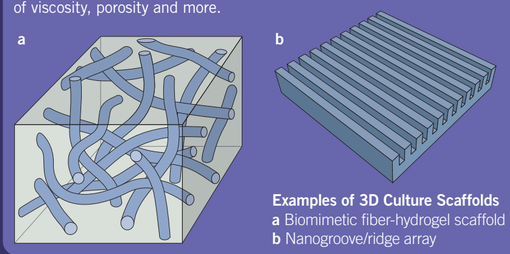
3D Differentiation of PSCs: Embryoid Bodies

Three dimensional (3D) differentiation of PSCs has been utilized since the 1980s, through cell aggregation in the absence of an adhesive culture substrate, which exploits the known PSC dependency upon cell-cell contact (e.g. E-cadherin) signaling. Aggregate formation can be utilized for the expansion and differentiation of pluripotent cells. In the presence of factors to maintain self-renewal, such as LIF (for mouse ESCs), cells aggregate but do not differentiate, an attractive prospect for scaling up cell numbers for regenerative medicine. In the absence of factors used to maintain pluripotency, aggregate, or more specifically, embryoid body (EB) formation occurs resulting in spontaneous cell differentiation. EBs recapitulate developmental processes in a more relevant way than two dimensional (2D) culture and have certain other advantages including scalability. EBs can be formed in a number of ways, including hanging drop culture systems or microwell plates, providing good consistency in aggregate size and so limiting variability within a culture. Disadvantages however include a rather haphazard differentiation profile when not modulated exogenously, and often the formation of a necrotic core, limiting the time span of differentiation studies. Differentiation can be directed generally towards each germ layer, by use of a combination of small molecules and further structural support such as Matrigel® or modulated very precisely to form organoids that represent complex *in vitro* aggregate structures that resemble specific organ types.



Alternative 3D Differentiation Approaches

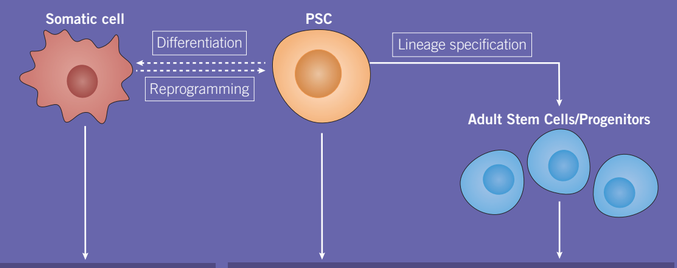
The major alternative mode of 3D differentiation is the use of scaffolds to recapitulate cellular microenvironments. These are produced using naturally occurring or synthetic materials taking a wide variety of physical characteristics. Synthetic methods, such as electrospinning and polyHIPE formation are popular options for cell differentiation, lending themselves well to engineering tissues such as bone, using an often increased substrate stiffness to drive differentiation mechanically. Decellularized extracellular matrix (ECM) scaffolds have shown success when used with MSCs for cartilage engineering, with potential in joint repair. However, methods such as this rely heavily on the maintenance of the native topography of the ECM for effective repopulation, and removal of all cellular material to avoid immune rejection. The final major type of scaffold consists of hydrogels; used to encapsulate a wide range of cell types, including PSCs, progenitors and differentiated populations. This technology is highly tuneable, in terms of viscosity, porosity and more.



Examples of 3D Culture Scaffolds
a Biomimetic fiber-hydrogel scaffold
b Nanogroove/ridge array

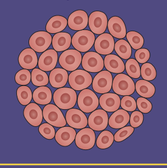
SC-derived Organoids: Promising Tools for a Range of Applications

Since EB formation is controllable by small molecule modulators of key pathways governing differentiation, a similar process could be used to form primitive organs *in vitro*. These structures can be formed from differentiated cell types, more commonly termed spheroids, or using stem cells, including iPSCs, termed organoids. Spheroids are formed by much less complex methods than organoids, usually only capitalizing on the propensity of all cells to aggregate in low adherence conditions, and their functionality reflects this. Organoids are generally much more complex in nature than spheroids, mimicking more closely the *in vivo* condition of a selected cell type. While this research theme is emerging rapidly, and is proving to have a vast range of applications, there are already a number of models of different organs that impressively recapitulate *in vivo* morphology and physiology.



Cells allowed to aggregate in low adherence vessels

Spheroid

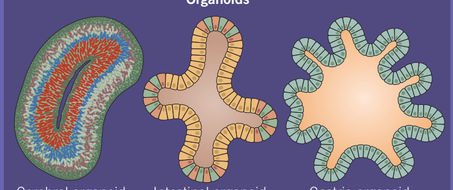


Advantages/disadvantages:
Advantages include:
- Low cost
- Ease of culture

Disadvantages include:
- Lack of complexity
- Potential necrotic core formation

Application of specialized cues to drive differentiation (for example; exogenous small molecules in growth medium for signaling pathway modulation, extracellular scaffolding components (e.g. hydrogels) or introducing fluid flow)

Organoids



Applications:
- Drug screening
- Developmental study
- Personalized medicine
- Modeling of disease states

Future Perspectives

As organoid technology is a rapidly developing field, there are high hopes for its use in a range of applications. In addition to forming complex tissues, organoid formation is also being used to model embryogenesis itself through similarly formed structures known as gastruloids. By modulating the activation of developmental biochemical pathways, coupled with the biophysical cellular microenvironment, it is possible to emulate the early events governing embryo development. It is hoped this area of research will provide never previously achievable insight into such events.

The formation of complex tissue-like systems *in vitro* provides an invaluable tool to decipher the intricate mechanisms that control development among multiple cell types. This may then in turn lead to an increased understanding applicable to the isolation of more difficult differentiated cell populations.

Another major area of interest is that of personalized medicine. Primary cells derived from individuals have also been shown to form these structures, opening avenues into targeted therapies tailored to individuals. This is of great interest to pharmaceutical companies looking at developing high throughput techniques, to provide better predictive validity of candidate drug screens.

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Differentiation

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rhFGF basic (146 aa) Protein
rhGDNF Protein
rhFGF basic (146 aa) Protein
rhLIF Protein

Abbreviations
EC, Embryonal Carcinoma; **ECM**, Extracellular Matrix; **ES**, Embryonic Stem; **iPS**, Induced Pluripotent Stem; **AS**, Adult Stem; **EB**, Embryoid Body; **MSC**, Mesenchymal Stem Cell; **2D**, two dimensional; **3D**, three dimensional

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4 **Thomson, J** *et al* (1998) *Science* **282** 1145
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Recommended Reviews:
Dutta, D *et al* (2017) *Trends Mol.Med.* **23** 393
Clarke, K *et al* (2015) *Tocris scientific review series: Using small molecules to control stem cell growth and differentiation.*

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