Stem cells (SCs) have immense potential as a limitless source of cells and tissues for research and treatment of various diseases, as well as for investigating early embryonic development. Small molecules can be used at all stages of the stem cell workflow, and their use has several advantages over standard techniques: small molecules are chemically-synthesized, versatile, animal-free, and are cell permeable. In addition, their effects are rapid and reversible, so their use can reduce the duration of reprogramming and differentiation protocols.

### Reprogramming

Reprogramming is the regeneration of a specialized cell to a simpler state resulting in cells with stem-like properties known as induced pluripotent stem cells (iPSCs). Takahashi et al. (2007) first reported reprogramming of specialized human adult cells by introducing the transcription factors Oct3/4, Sox2, Klf4 and c-Myc (known as the Yamanaka factors or OSKM) into adult human dermal fibroblasts via retroviral transduction. The resulting iPSCs had similar properties to ESCs and could differentiate into all three embryonic germ layers. Similar techniques are still widely used.

Small molecules can be used to enhance reprogramming efficiency. Valproic acid (VPA) can increase reprogramming efficiency by Yamanaka factors by 100 fold. Using a cocktail of small molecules and growth factors to reprogram cells dispenses with the need for retroviral transduction and increases the efficiency of reprogramming. Hou et al. (2013) first described the generation of iPSCs using only small molecules. The researchers discovered that a combination of six compounds, VPA, CHIR 99021, RepSox, Tranylcypromine, Forskolin (these 5 compounds are together known as VC6TF) and 3-Deazaneplanocan A, followed by culture in 2i medium (see Self-Renewal panel), could be used to reprogram mouse somatic cells at a frequency of 0.2% (compared with 0.01 – 0.02% by the Takahashi method).

Subsequently, Yamanaka factors by >100 fold. Using a cocktail of small molecules and growth factors to reprogram cells dispenses with the need for retroviral transduction. The resulting iPSCs had similar properties to ESCs and could differentiate into all three embryonic germ layers.

### Verification

The characterization of differentiated cells is a necessary step before the cells can be used in assays or therapy. Immunohistochemistry or Western blotting, using antibodies against specific markers expressed by the target cells, are techniques widely used to verify the identity of differentiated cells, as it is quantifiable RT-PCR, which is used to assess gene expression. Small molecules play an important part in the verification of cell function, by pharmacological or electrophysiological techniques.

Following conversion of fibroblasts into cardiomyocytes, Carai et al. (2015) established the presence of genes involved in cardiomyocyte function. Electrophysiological analysis of the cells revealed ventricular-like action potentials. The researchers then looked at the effect of small molecules on action potential firing and found that Caffeine and the non-selective β-adrenoceptor agonist Propranolol increased firing rate, while the muscarinic agonist Carbachol slowed the firing rate, helping to establish the identity of the differentiated cells as functioning cardiomyocytes.

Kutukov et al. (2016) describe the verification of functional neurons differentiated from iPSCs. iPSCs’ electrophysiological analysis revealed spontaneous action potential firing, which was eliminated by application of Tetrodotoxin, indicating the presence of functioning sodium channels. Application of caffeine and inhibitory postynaptic currents that could be blocked by bicuculline, indicative of the formation of a functional neural network. Calcium imaging using FURA-2AM, revealed Ca²⁺/influx in response to application of neurotransmitters GABA, Glutamate or Glicine. Techniques such as these provide evidence for the presence of mature functioning cells.

### Clinical Applications

Application of neurotransmitters, antagonists, toxins, etc.

### Differentiation

Cells can also be reprogrammed directly from one specialized cell type to another, without first being converted to iPSCs, a process known as transdifferentiation or direct-lineage reprogramming. Cao et al. (2016) described a method to convert human fibroblasts into a cocktail of 11 small molecules and proteins. Aktin A, CHIR 99021, Notchic Acid, SANT-1, LDN 193189, Phorbol 12,13-dibutyrate, T3, Compound E, RepSox, Heparin, Basic Fibroblast and KGF and produces functional β-cells in 28 days, which when transplanted into diabetic mice are seen to restore insulin viability.

Qiu et al. (2017) have developed a protocol using a cocktail of six small molecules to derive cardiac neurons from iPSCs. When transplanted into postnatal mouse cortex after 8 days of differentiation the resulting neurons are functional and establish long term connections. In addition, cells remaining in culture exhibit functional electrophysiological properties by day 16.

### Storage

Cryopreservation is used to store cells, including stem cells, for in vitro experiments or long-term storage. However after freeze-thawing the survival rate of cells can be poor. Li et al. (2008) found that treatment of iPSCs with the ROCK inhibitor Y-27632 prior to freezing significantly increases the viability of cells after thawing. Y-27632 also improves the survival of cells that have been differentiated from iPSCs, facilitating their use in disease modeling and therapy.

### Self-Renewal

Stem cells self-renew and proliferate by the division of a parent cell into two identical daughter cells. Conventional culture methods for PSCs require ‘feeder’ cells, serum products and growth factors, such as LIF and bFGF. More recently, chemically-defined media have been developed to replace the requirement for feeder cells and serum products, and small molecules can be used to maintain self-renewal of stem cells.

Chen et al. were the first to show that a small molecule, SC 1 (also known as Purpurin), which inhibits proliferation, can be used to maintain pluripotency in the absence of growth factors. Ying et al. (2008) subsequently identified a combination of two small molecules (known as 2i), the MEK inhibitor PD 0325901 and GSK-3 inhibitor CHIR 99021, which can sustain ESC self-renewal. ESCs grown in 2i medium show lower levels of spontaneous differentiation compared with standard SC culture methods. 2i can also be used to rescue cultures that have started to deteriorate.

The ROCK inhibitor Y-27632 also has an important role in stem cell maintenance as it increases closing efficiency of ESCs without affecting pluripotency, enabling the survival of stem cells in culture over the long-term (Habib et al., 2007).

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