

Science Education Collection

An Introduction to Stem Cell Biology

URL: <https://www.jove.com/science-education/5331>

Abstract

Cells that can differentiate into a variety of cell types, known as stem cells, are at the center of one of the most exciting fields of science today. Stem cell biologists are working to understand the basic mechanisms that regulate how these cells function. These researchers are also interested in harnessing the remarkable potential of stem cells to treat human diseases.

Here, JoVE presents an introduction to the captivating world of stem cell biology. We begin with a timeline of landmark studies, from the first experimental evidence for hematopoietic stem cells in the 1960s, to more recent breakthroughs like induced pluripotent stem cells. Next, key questions about stem cell biology are introduced, for example: How do these cells maintain their unique ability to undergo self-renewal? This is followed by a discussion of some prominent methods used to answer these questions. Finally, several experiments are presented to demonstrate the use of stem cells in regenerative medicine.

Transcript

As their name implies, stem cells are the precursors from which many different cell types "stem." They are characterized by their potency, or ability to differentiate into cells from all three germ layers, as well as their renewability, or capacity to generate more stem cells. In hopes of advancing the fields of developmental biology and regenerative medicine, stem cell researchers are working to understand how these unique cells accomplish such a major feat.

This video covers major discoveries in the field of stem cell biology, key questions asked by scientists in this field, prominent methods used by stem cell researchers, and applications of stem cell research.

Now that we've introduced the stem cell concept, let's dive into the rich history of stem cell research.

In the 1960s, Drs. Ernest McCulloch and James Till discovered some of the first definitive evidence for the existence of hematopoietic stem cells in the bone marrow of adult mammals. These cells have the ability to self-renew and are multipotent, meaning that they can differentiate into multiple, but limited, types of cells—namely the cells of the blood and immune systems.

In 1988, Irving Weissman and colleagues perfected a method for purification of hematopoietic stem cells from mouse bone marrow.

In 1981, Professor Gail Martin coined the term "embryonic stem cell." Unlike hematopoietic stem cells, embryonic stem cells are pluripotent, having the ability to differentiate into all cell types of the body. She and scientists Martin Evans and Matthew Kaufman, simultaneously, but separately, developed methods to extract the inner cell mass from mouse blastocysts and culture them *in vitro* as stem cells.

In 1998, over ten years after the isolation of mouse embryonic stem cells, Dr. James Thomson successfully established the first human embryonic stem cell lines.

In 2006, a major breakthrough occurred with the advent of induced pluripotent stem cells, as devised by Dr. Shinya Yamanaka. Building on the work of John Gurdon, Yamanaka developed a method to reprogram differentiated cells to a pluripotent state by using retrovirus to induce the expression of a small set of transcription factors now known as the "Yamanaka factors." The resultant cells were named "induced pluripotent stem cells," or "iPSCs." These experiments were considered so fundamentally important that Yamanaka and Gurdon were awarded the Nobel Prize in 2012.

At present, we now have iPSC models of several human diseases, and regeneration platforms in multiple tissues.

Today, stem cell research is driven by several overarching questions.

One of the most important of these questions is: how do stem cells maintain pluripotency and renewability? There are two related characteristics of stem cells that confer these properties. First is the expression of specific genes essential for stemness and self-renewal. The second is the responsiveness of stem cells to regulatory factors that affect the expression of these genes.

The next logical question is: how is the differentiation of stem cells directed? As a stem cell develops into a mature cell, activation of specific differentiation pathways induces changes in gene expression, turning off stem cell genes and turning on tissue-specific genes, which results in increasing specialization of cell function and morphology.

Finally, let's address the main question driving stem cell research funding: can stem cells be used to treat disease? Regenerative medicine is tackling this question in two ways: 1) by regrowing organs in the lab, and 2) by delivering stem cells via transplantation to treat tissue degeneration.

Now that we've presented some of the key questions concerning stem cell biology, let's go over some of the prominent methods used to address them.

Microarray technology can be employed to discover which genes confer potency and renewability in stem cells. In this technique, total RNA is isolated from a population of cells, which acts as a snapshot of current gene expression. In a series of steps, this mRNA is converted to a

fluorescently labeled probe and hybridized to a chip containing transcripts of the entire human genome. Scanning this chip provides a readout of relative gene expression profiles. As you can see, a stem cell expresses a specific set of genes that differs from a differentiated cell.

Another assay for pluripotency genes involves the Oct4-GFP detection system. Oct4 is required for self-renewal, and is quickly down-regulated during differentiation. Therefore, its expression acts as a reliable indicator of "stemness." In this experiment, cells express green fluorescent protein under the control of the Oct4 promoter. These cells can then be experimentally manipulated, and changes in GFP expression are analyzed to identify new genes or soluble factors that modulate self-renewal.

In order to study stem cells *in vitro*, we must first understand how to culture them. Stem cells require a particular microenvironment to maintain stemness. This can be achieved by co-culturing stem cells with feeder cells, such as mouse embryonic fibroblasts, or MEFs. MEFs secrete a complex mixture of necessary pluripotency and self-renewal factors.

At times, it is desirable to have feeder-free cultures of stem cells. The main method to maintain feeder-free cell lines is to supplement cell culture media with stock reagents of growth and inhibitory factors.

Differentiating stem cells *in vitro* is accomplished using several methods. Differential gene expression is ultimately responsible for specialization of cells. In the two-step method you see here, cultured mouse embryonic stem cells are primed for a neuronal fate before being further differentiated with motor neuron induction medium. These factors activate specific pathways of gene expression, resulting in morphological and proteomic changes characteristic of motor neurons.

One major disadvantage of traditional *in vitro* differentiation is that flat plates restrict the 3D growth of cells. The hanging drop method and microcapsule methods circumvent these issues. In the hanging drop technique, small drops of stem cell suspensions are plated on the lid of a petri dish and cultured upside down to form aggregates of stem cells known as embryoid bodies.

In microencapsulation methods, stem cells are mixed with a biocompatible semipermeable membrane called alginate, and deposited as beads into cell culture plates. Both methods allow for further differentiation into specialized cells, such as dopaminergic neurons and cardiomyocytes.

Knowing how to direct differentiation is a major step towards using stem cells in regenerative medicine. Stem cell transplantation therapy aims to treat and cure degenerative diseases by repairing damaged tissues with stem cells. In this experiment, somatic cells from patients are reprogrammed into iPS cells via lentiviral infection of the Yamanaka factors. From their pluripotent state, cells are differentiated into specific cell types and returned to the host to repair damaged tissue.

Now that you know some of the methods used to investigate stem cells, let's take a look at how these methods are applied in specific experiments.

In this experiment using a mouse model of multiple sclerosis, neural stem cells are injected intravenously into affected mice. Brain slices of treated mice are collected and imaged under a microscope to assess success of the transplantation. Cells derived from donor neuronal precursor cells are tracked using the reporter gene LacZ. As you can see, a number of donor stem cells have differentiated and integrated into the central nervous system of diseased mice.

Not every ailment can be treated by systemic injections. Cartilage injuries, for example, require a specialized scaffolding to rebuild around. In this experiment, a mixture of mesenchymal stem cells and coagulation factors are cultured together to form a clot. The clot is then placed into the damaged knee cartilage of a rabbit and allowed to integrate. Following this procedure, remodeling of the knee cartilage to a smooth and functional joint can be observed.

Sometimes, stem cell researchers and tissue engineers team up to rebuild entire organs. In this experiment, primate lungs are washed to decellularize the organ, leaving behind only non-cellular structural components. This "ghost" lung is then transferred to a bioreactor, where it is seeded with vascular and epithelial stem cells. To further mimic the pressure and behavior that a natural lung experiences, the bioreactor circulates media, maintains pressure and gas levels, and inflates the lungs.

You've just watched JoVE's stem cell biology overview. To recap, in this video we have discussed stem cells and their history, maintenance, differentiation and delivery methods, and stem cell applications. Thanks for watching!