Human Pluripotent Stem Cell (**hPSC**) Research

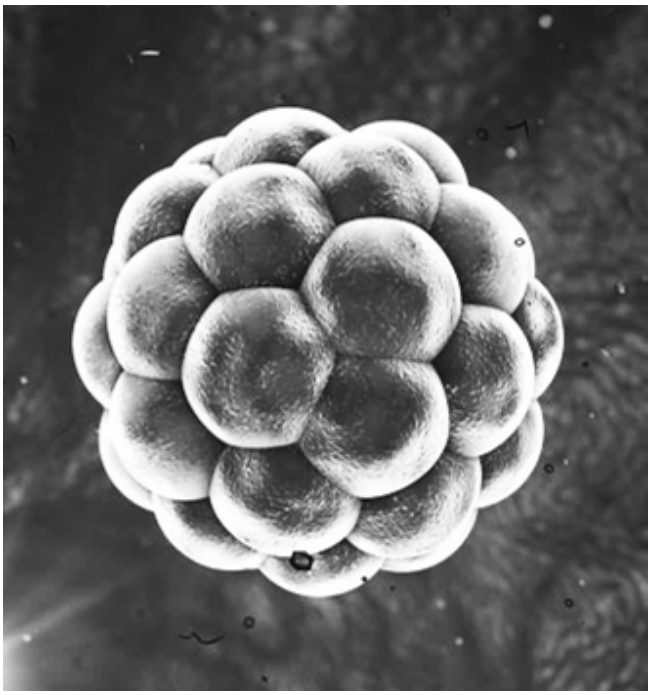
March 2023

University of CINCINNATI

WHAT ARE STEM CELLS?

Stem cells are the foundation cells for every organ and tissue in our bodies.

When a stem cell divides through mitosis, each new cell has the potential either to remain a stem cell (self-renewal) or become another cell type with a more specialized function (differentiation), such as a muscle cell, a red blood cell, or a brain cell.



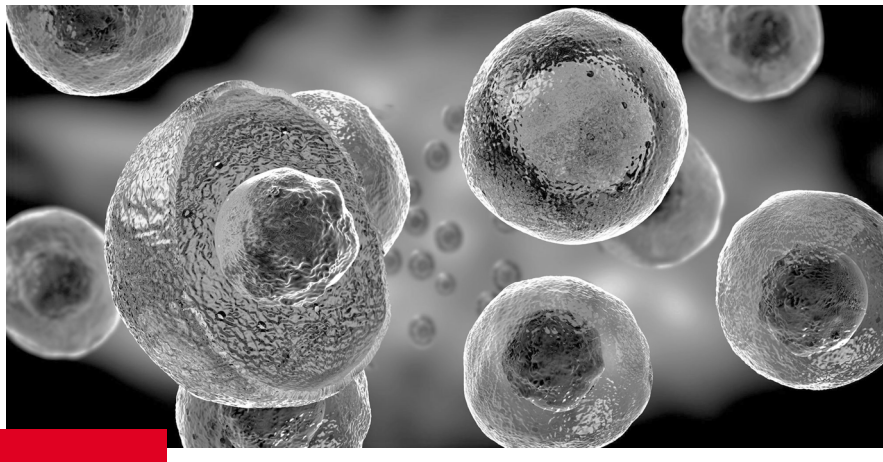
In many tissues, they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells in live humans or animals.

There are many ways in which human stem cells can be used in research and the clinic.

Areas of study include the effectiveness of using human stem cells that have been programmed into tissue-specific cells to test new drugs. For the testing of new drugs to be accurate, the cells must be programmed to acquire properties of the type of cells targeted by the drug.

Stem cell therapy, also known as regenerative medicine, promotes the repair response of diseased, dysfunctional, or injured tissue using stem cells or their derivatives. Stem cell therapy is the next chapter in organ transplantation that will use cells instead of donor organs, which are limited in supply.

By watching stem cells mature into cells in bones, heart muscle, nerves, and other organs and tissues, researchers may better understand how diseases and conditions develop.



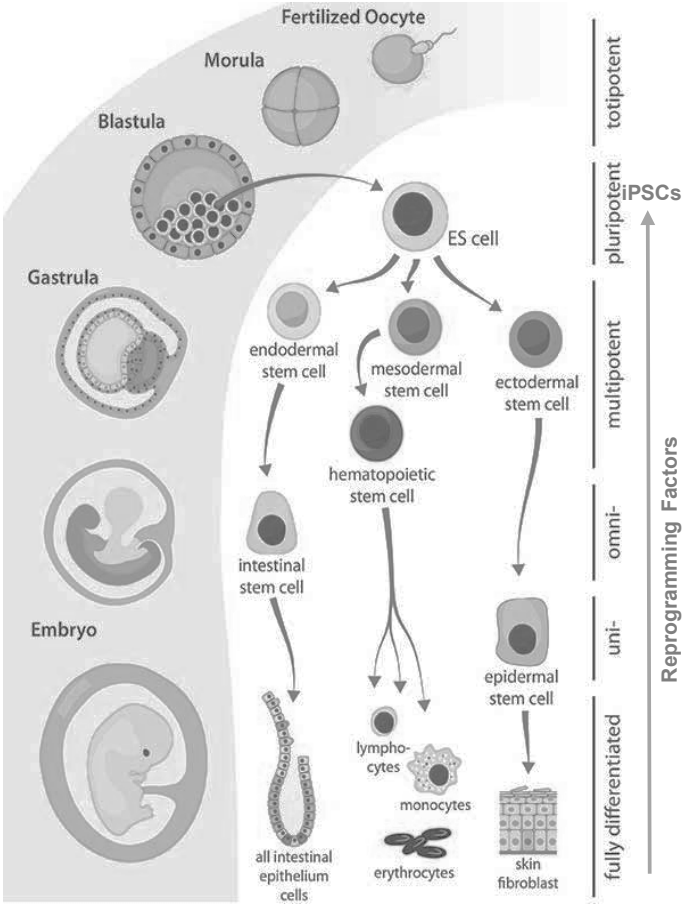
CLASSIFICATION OF STEM CELL POTENCY

Cells in the embryo and adult stem cells have different abilities to differentiate into specialized cell types. The embryonic inner cell mass (ICM) found in the blastocyst 4-7 days post-fertilization has the capacity to generate all the tissues of the embryo proper (ectoderm, endoderm, mesoderm and the germline), but not the extra-embryonic tissues (i.e., placenta and umbilical cord). During organ development, cells are set aside in each tissue to form the adult stem cells that maintain life-long tissue homeostasis after birth. Stem cells derived from the ICM and those found in adult tissues have different capacities for self-renewal and differentiation. Embryonic stem cells (ESCs) cultured *in-vitro* have the greatest potential for self-renewal and differentiation; they are immortal and maintain the ability to form all cell types of the organism. In contrast, adult stem cells found *in-vivo* have a more restricted potency than ESCs; adult stem cells can only generate cells of the organ in which they reside. The full range of potencies exhibited different cell types are defined below.

Totipotent cells can give rise to all tissues of the body and the germline, plus all extraembryonic tissue. This most primitive cell type is found immediately after fertilization.

Pluripotent cells can give rise to all tissues of the body and the germline. In the embryo, the cells of the ICM are pluripotent. ESCs derived from the ICM and cultured *in-vitro* as well as induced pluripotent stem cells (iPSCs) that are engineered/reprogrammed from adult differentiated cells such as fibroblasts and peripheral blood mononuclear cells, are also pluripotent. Pluripotent cells are unable to generate extra-embryonic cell types.

Multipotent cells can give rise to multiple related cell types, but they are more restricted in potential than pluripotent cells. Adult stem cells found in specific tissues are most often multipotent. They self-renew to maintain the stem cell pool, but also develop into cells and tissues. One example is the hematopoietic stem cell, which can develop into all types of blood cells.

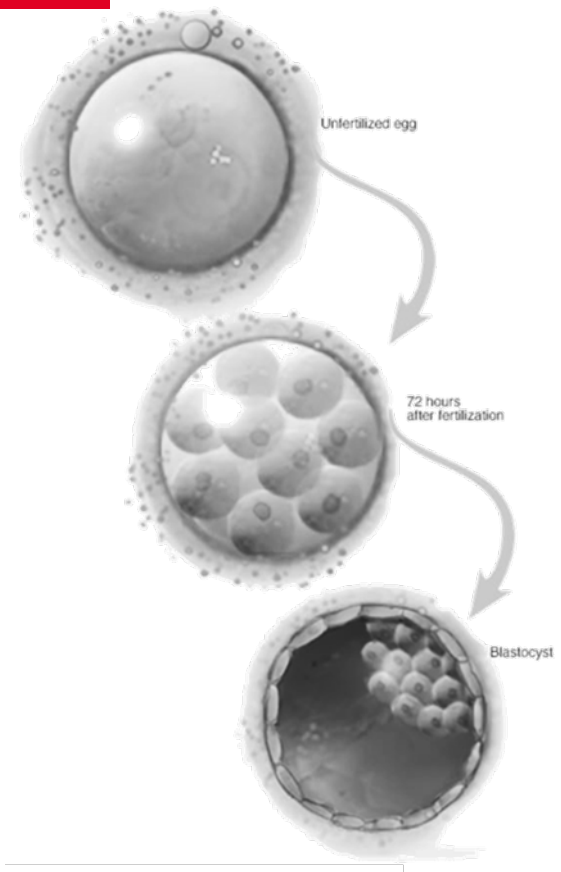


Unipotent cells can give rise to only one type of daughter cell. Some adult stem cells are unipotent (e.g., epidermal stem cells, muscle stem cells).

HUMAN EMBRYONIC CELLS (hESCs)

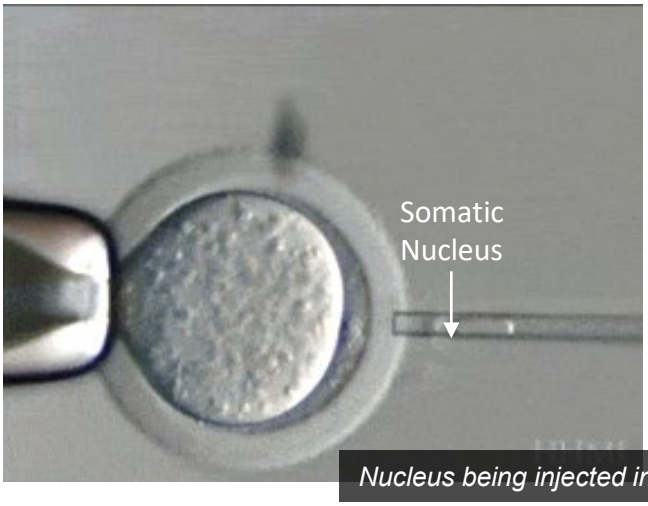
Embryonic stem cells can be obtained from the blastocyst, the very early stage of development, that consists of a hollow ball formed 4-7 days following fertilization. The blastocyst consists of approximately 150-200 cells and is barely visible to the naked eye.

Unlike tissue-specific (adult) stem cells, embryonic stem cells have the potential to generate every cell type found in the body. Just as importantly, these cells can, under the right conditions, be grown and expanded indefinitely in this unspecialized or “undifferentiated” state. These cells help researchers learn about early human developmental processes that are otherwise inaccessible, study diseases and establish strategies that could ultimately lead to therapies designed to replace or restore damaged tissues.



hESCs SOURCES

Human embryonic stem cells are derived primarily from the inner cell mass of blastocysts that were created by *in vitro* fertilization (IVF) for assisted reproduction but never implanted in a uterus. These stem cells are donated with informed consent from donors. Sometimes, cells are obtained from fresh embryos produced specifically for research purposes.



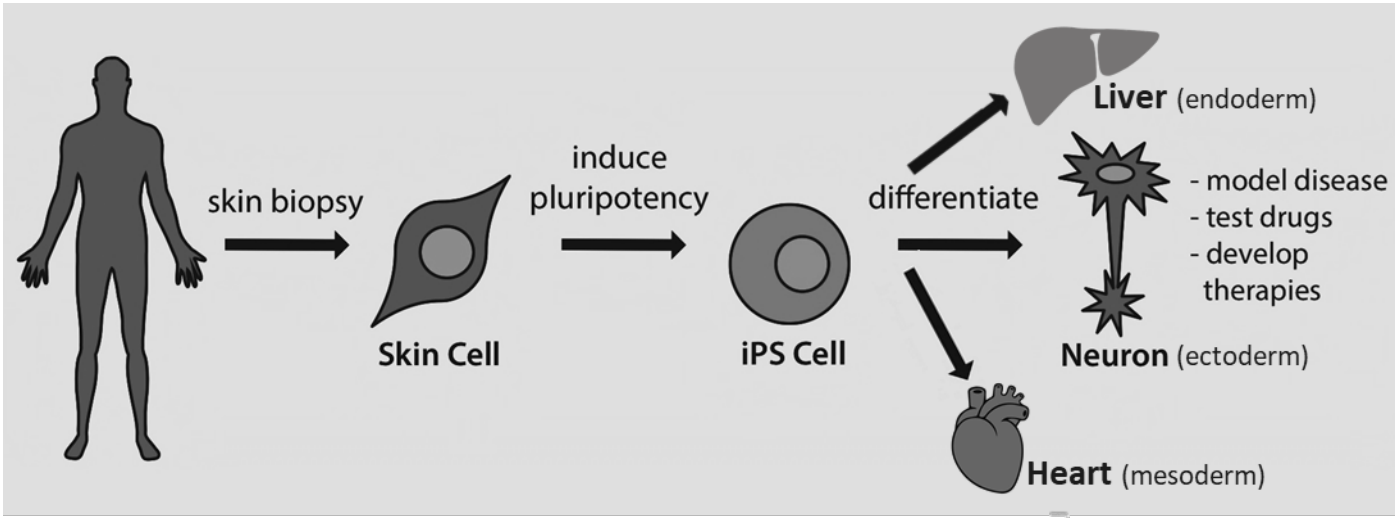
Nucleus being injected into an egg cell in Somatic Cell Nuclear Transfer (SCNT)

Somatic cell nuclear transfer (SCNT) is a process that produces stem cells with ESC characteristics by replacing the nucleus of an unfertilized egg with the normal diploid nucleus from a somatic cell and then inducing the new cell to proliferate. This process has been successful in several species but is not fully established/robust in humans yet. This method results in a clonal stem cell population that is essentially genetically identical to the somatic cell donor.

INDUCED PLURIPOTENT STEM CELLS (iPSC)

Although pluripotent cells exist only in the inner cell mass of the blastocyst and as embryonic stem cells, it is possible to induce terminally differentiated cells to become pluripotent again. The process of direct reprogramming converts differentiated somatic cells into iPSC lines that can form all cell types of an organism.

First demonstration that adult cells could be reprogrammed to the pluripotent state was in 2006 with Shinya Yamanaka and Kazutoshi Takahashi who conducted a retrovirus-mediated transduction of mouse fibroblasts with four transcription factors (Oct-3/4, Sox2, KLF4, and c-Myc) that are mainly expressed in embryonic stem cells and induce the fibroblasts to become pluripotent. One year later, the experiment also succeeded with human cells. This method created new ways to study the process of human embryonic development, to produce new tools for studying disease, and potentially a way to generate cells that can be used in transplantation therapies. Recently, studies have focused on reducing carcinogenesis and improving the neuronal system.



In addition to bypassing the need for embryos to create pluripotent stem cells, a major advantages of iPSCs, and one of the reasons that researchers are very interested in studying them, is that they are a good way to make pluripotent stem cells that are specific to any individual patient with a genetic disease. Disease-specific stem cells are powerful tools for studying the cause of a particular disease and then for testing drugs or discovering other approaches to treat or cure that disease. The development of patient-specific stem cells is also very attractive for cell therapy, as these cell lines are from the patient themselves and may minimize some of the serious complications of rejection and immunosuppression that may occur.

The table below describes the level of differentiation and source of stem cells that have the potential for regenerating various tissues in the body.

Mammalian Stem Cells

Stage or Category	Sources / Examples	Level of Differentiation	Description
Undifferentiated Cells	Fertilized egg or zygote	Totipotent	Cells that can generate all cells including those of the placenta and embryo.
Embryonic Stem Cells (ESC)	Inner cell mass (ICM) of blastocyst	Pluripotent	Cells isolated from the inner cell mass of excess embryos produced as a result of IVF.
Adult Stem Cells	Bone marrow and peripheral blood	Multipotent	Used for years in a wide spectrum of clinical transplants for hematopoietic diseases and a limited number of metabolic or genetic disorders.
	Umbilical cord blood & placenta	Multipotent	Used in transplantation protocols in place of bone marrow when fewer cells may suffice or if an appropriate bone marrow donor is not available.
	Mesenchymal stem cells	Multipotent	Derived from a variety of tissues and have reparative characteristics and may have an important role in graft vs. host diseases.
	Tissue specific stem cells or restricted precursors	Multipotent and (few) Unipotent	Many tissue specific stem cells are multipotent and form multiple cell types. A few, such as spermatogonial stem cells or epidermal stem cells are unipotent and only form one cell type.
Induced Pluripotent Stem Cells (iPSCs)	Skin cells or other adult cells transfected or otherwise manipulated to be reprogrammed to act like ESC	Pluripotent	Process whereby key genes are introduced into somatic cells to re-program adult cells into an embryonic stem cell-like state. These cells are genetically identical to the adult tissue donor.
Somatic Cell Nuclear Transfer (SCNT)	ICM of a blastocyst produced from an enucleated egg transplanted with an adult donor nucleus from a somatic cell	Pluripotent	Process that results in a clonal stem cell population identical to the somatic cell donor. These have characteristics of embryonic stem cells and are genetically characteristic of the adult nuclear donor.

hESCs RESEARCH: AN ETHICAL DILEMMA

Human embryonic stem cells (hESC) research offer hope for new therapies, but their use has been hotly debated. Different countries have chosen to regulate embryonic stem cell research in very different ways.

Some people see destroying a blastocyst for its cells as destroying an unborn child while others feel that a blastula is not exactly a child just yet, because unless a blastula is embedded in the uterus wall, it will never have the chance to develop into a baby.

Every year, fertility clinics create many blastula that after the reproductive needs of the donors are met, some may be "left over" and while some are kept in freezers for potential future use, other may be donated to couples in need of an embryo for reproductive purposes, offered for research, or simply destroyed as medical waste. In recent surveys, most infertility patients would permit their excess embryos to be donated for research rather than for other uses. Supporters of hESC research generally feel that using cells from surplus embryos for research and developing medical treatments, which could help improve and save people's lives, is much better than throwing them away.

Debates and discussions about the moral and ethical status of hESCs help establish the rules and regulations that govern scientific research and the development of medical treatments using stem cells.

It is important to realize that, although people may have very strong opinions on what is "best" for society, groups on both sides of this discussion are interested in helping and protecting human lives. Understanding this can greatly help people to respect each other's differences in opinions and work to find the middle ground.

US FEDERAL REGULATION: HISTORY

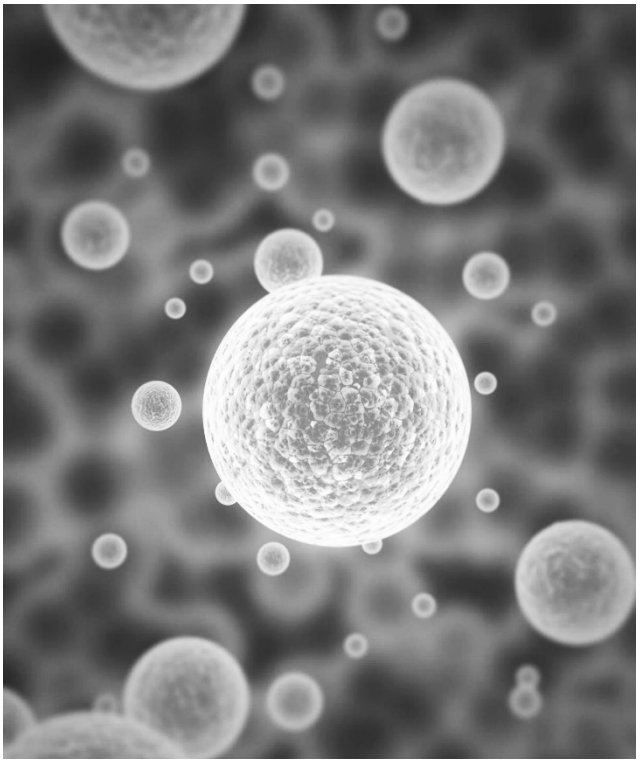
Given the fluctuating regulatory requirements for research using human embryonic stem cells, it is critical that investigators proposing hESC research become familiar with current federal guidelines, as well as state law, and local institutional policies.

US FEDERAL REGULATION: HISTORY

The history of federal statutory constraints on the use of federal funding for research with human embryonic tissues dates back to 1995 with adoption of the Dickey-Wicker amendment (The Balanced Budget 1996) where embryo could not be either produced or destroyed for research purposes using any form of federal funding (grants, buildings, people, etc).

On August 9, 2001, President George W. Bush announced that, for the first time, federal funds would be used to support research on human embryonic stem cells. However, Bush's decision limited research funding to the use of 21 stem cell lines that were created prior to the 08/09/2001 announcement.

On March 9, 2009, soon after assuming office, President Barack Obama issued Executive Order (EO) 13505 that removed President Bush's limitations on hESC research and allowed NIH financial support for "scientifically worthy human stem cell research, including human embryonic stem cell research, to the extent permitted by law" and also called for the NIH to issue a new policy regarding hESC research.



The "new" NIH Guidelines for Human Stem Cell Research were released in final form on July 7, 2009. These revised guidelines allow the use of federal funds for hESC research with hESC lines that are posted on the NIH Human Embryonic Stem Cell Registry. The Registry also contains basic information about organizations intending to submit lines to the Registry, lines pending NIH review and lines not approved for NIH funding eligibility .

For more information, go to the NIH Stem Cell website.