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Unlocking Transformative Potentials of Stem Cells

H. Akhila[1], Dr. G. Nirmala Devi[2] and Dr. Bilquis[3]
[1]Ph.D. Research Scholar (Human Development and Family Studies)
[2]Assistant Professor (Department of Food and Nutrition)
[3]Professor and Head (Human Development and Family Studies)
Corresponding Author's email: arshiyahdfs2026@gmail.com

Stem cells are remarkable cells in the body that have the capacity to divide indefinitely to create new stem cells or to grow into a variety of cell types. They act as the body's internal healing system, crucial for replacing damaged or killed cells resulting from illness or injury. These cells are found in both embryonic and adult species and are the first cells in all tissue lineages (Das and Tyagi, 2014). The human body comprises roughly 200 different cell types, all beginning from a single fertilised egg created by the combination of sperm and ovum. Scientists have been studying stem cells extensively to understand how they specialise into distinct cell types, which is vital for creating novel medicinal treatments targeting injured or missing cells. Stem cell's capacity for regeneration leads to potential uses in the treatment of conditions like diabetes, heart disease, and degenerative disorders (Microbenotes, 2023).

Definition

Stem cells are unique cells that can develop into numerous cell types or replicate endlessly to produce new stem cells. They are vital for supplying a continuous supply of new cells that build the tissues and organs of living animals. These cells live in specialised areas called niches, where surrounding cells release fluids and nutrients necessary for stem cell survival.

Properties

All stem cells feature some fundamental properties that can be visualised by clonogenic experiments, where a single cell is tested for its differentiation ability:

1. Self-renewal capacity: Stem cells can divide and regenerate themselves for extended durations while maintaining an undifferentiated state. They undergo cell proliferation while conserving their undifferentiated properties, which is necessary for maintaining a consistent supply of stem cells throughout an organism's existence (Los *et al.*, 2019).

2. Unspecialised nature: Unlike mature cells, which have specialised structures and functions, stem cells are found as undifferentiated cell masses. This unspecialised status allows them to later evolve into numerous specialised cell types as needed (Lin and Talbot, 2014).

3. Differentiation potential: Stem cells contain the unique ability to develop into specialised cells that together produce distinct tissue types. Depending on their classification, they can be either pluripotent (able to produce multiple cell types) or multipotent (able to form several related cell types) (Hammond-Browning, 2012).

4. Tissue homeostasis: The balance between self-renewal and differentiation is critical for sustaining tissue health and function. According to Neman *et al.* (2010), stem cells constantly regenerate and replace lost or damaged cells and their capacity to react to certain signals aids in maintaining the integrity and balance of the entire tissue.

Classification Based on Potency

Based on their potency or ability to differentiate, stem cells are divided into five main categories. Potency refers to the range of cell types that a stem cell can potentially become.

1. Totipotent Stem Cells

Totipotent stem cells represent the most versatile type of stem cells, capable of developing into any cell type in an organism, including both embryonic and extraembryonic tissues (such as the placenta and umbilical cord). The Latin word "totus," which means "entire," is where the word "totipotent" originates, signifying their whole potential for development. The fertilised egg or zygote is the quintessential totipotent cell. The zygote divides after fertilisation and retains its totipotency until it reaches the eight-cell stage of embryonic development. These cells have the remarkable capacity to evolve into a whole, functioning organism. Beyond the eight-cell stage, cells begin losing their totipotent potential and become pluripotent instead. All 210 cell types in the human body, along with placental tissues, eventually grow from this totipotent stage (Lin & Talbot, 2014).

2. Pluripotent Stem Cells

Pluripotent stem cells may generate cells from all three embryonic germ layers: ectoderm (which forms skin and nerve tissue), mesoderm (which forms muscle, bone and blood), and endoderm (which forms internal organs like the liver and pancreas). "Pluripotent" is derived from the Latin word "plures," which means "several" or "many." These cells exhibit traits necessary for embryonic development and under particular laboratory settings and they can differentiate into any form of foetal or adult cell. However, because pluripotent cells are unable to generate extraembryonic organs like the placenta, they are unable to produce whole embryos, in contrast to totipotent cells. Embryonic stem cells (ES), induced pluripotent stem cells (iPS), germline pluripotent stem cells (gPS), parthenogenetic embryonic stem cells (pES) and foetal stem cells are a few examples (Hammond-Browning, 2012).

3. Multipotent Stem Cells

Progenitor cells that can differentiate into a limited number of cell types, usually within a related family of cells, are known as multipotent stem cells. These cells are more constrained in their differentiation potential compared to pluripotent cells but nevertheless exhibit great plasticity within their respective lineages. This category include somatic (adult) cells including neural stem cells, which produce different kinds of brain cells and haematopoietic stem cells (HSCs), which are located in bone marrow and produce a variety of blood and immune cells. Throughout an organism's existence, multipotent stem cells are essential for tissue upkeep and repair. They are particularly valuable for therapeutic applications because they may be obtained from adult tissues, avoiding the ethical difficulties associated with embryonic stem cells (Loya, 2014).

4. Oligopotent Stem cells

Oligopotent stem cells can differentiate into a few specific cell types within a single tissue. Usually, these cells stay in their original tissue and grow into the specialised cells required for that particular tissue. For instance, studies have shown oligopotent stem cells on the surface of the mammalian eye that are limited to that tissue system but can differentiate into many types of eye cells (Britannica, 2021).

5. Unipotent Stem Cells

Unipotent stem cells, despite exhibiting self-renewal capability, can develop into only one cell type. The term "unipotent" stems from the Latin "unus," meaning "one." High specialisation is indicated by these cells, which are usually present in mature organisms. Under normal conditions in healthy, undamaged organs, adult stem cells are often unipotent, generating only one specific cell type essential for tissue maintenance. This method permits tissues to maintain a constant state of self-renewal with suitable cell types. However, pluripotent stem cells may be activated when various cell types need to be replaced due to injury. Spermatogonial stem cells, which create exclusively sperm cells and muscle stem cells, which only make muscle cells, are two examples (Neman *et al.*, 2010). It is crucial to remember that embryonic stem cells have a unique quality called pluripotency,

which allows them to differentiate into cells from all three germ layers. Recent research has successfully revealed the relevance of unipotent progenitor cells in postnatal development, such as in prostate development (Microbenotes, 2023).

Types by Source

Embryonic Stem Cells

Derived from the inner cell mass of blastocysts (4-5 days post-fertilization), these pluripotent cells can develop into around 250 cell types but not extraembryonic cells. They were first documented in people (1998) and mice (1981) and they are being cultivated more and more for genetic disease testing and regenerative medicine. Leukaemia inhibitory factor (LIF)-cultured mouse embryonic stem cells have created thousands of genetic mouse lines for disease study and can be kept alive indefinitely.

Adult Stem Cells (Somatic/Tissue-Specific)

Adult stem cells are less powerful than embryonic cells and are limited to certain lineages. They are found in particular tissues and serve to repair and create tissue-specific cells. Located in niches generated by supporting cells, they're discovered in continuously renewing tissues like epidermis, bone marrow and intestinal lining.

While epithelial and neural stem cells have specific roles in their respective tissues, haematopoietic stem cells in bone marrow differentiate into three blood cell types and immune cells.

Induced Pluripotent Stem Cells (iPSCs)

Generated by reprogramming adult cells through gene introduction, iPSCs resemble embryonic stem cells in differentiation capacity but differ in gene expression and chromatin state. crucial for therapeutic treatment, allowing physicians to create patient-specific cells for almost any organ while avoiding ethical problems with embryonic stem cells. In 2008, researchers successfully created retinal cells from reprogrammed adult skin cells, enabling thorough embryonic development inquiry and innovative eye disease therapeutics. By 2009, cardiac stem cells were created through dedifferentiation for heart disease treatment.

Perinatal Stem Cells

Intermediate cells containing properties of both embryonic and adult stem cells, derived from extra-embryonic foetal membrane tissues, umbilical cord, and amniotic fluid. Possessing immune-privileged features and extensive multipotent plasticity, these non-tumorigenic cells efficiently avoid ethical concerns. Applications include the treatment of spinal cord damage, inflammatory diseases, heart disease, renal disease, and bone regeneration.

Mesenchymal stem cells

Adult stem cells present in muscle, liver, and bone marrow tissues, human MSCs differentiate into mesodermal lines (osteocytes, adipocytes, chondrocytes) plus ectodermal (neurocytes) and endodermal (hepatocytes) lines. Easily extracted with higher yields than other stem cells, MSCs possess immunomodulatory properties through cytokine and immune-receptor release, making them attractive for chronic disease treatment.

Fetal Stem Cells

Including umbilical cord blood (UCB), amniotic fluid (AF), Wharton's jelly, amniotic membrane, and placenta sources, these pluripotent cells contribute to prenatal tissue development. With no ethical implications as tissues are normally discarded post-birth, they display self-renewal and differentiation capacities comparable to embryonic and adult multipotent stem cells.

Stem Cell Identification, Isolation, and Characterization

The goal of stem cell research is to produce populations of pure, lineage-specific stem cells for therapeutic application in diseases that are now incurable. Accurate identification, separation and characterisation of stem cells are therefore important to stem-cell–based regenerative therapy.

Identification of Embryonic and Adult Stem Cells

Embryonic Stem Cells (ESCs)

ESCs are defined by:

• Self-renewal, clonogenicity, and long-term proliferation under established culture conditions.

• Pluripotency, as evidenced by the development of teratomas, embryoid entities, and contributions to all germ layers in vivo.

• Expression of pluripotency markers: OCT4, SOX2, NANOG, SSEA-3/4, TRA-1-60/81.

• Normal karyotype and the ability to resist freeze–thaw cycles.

Adult Stem Cells (ASCs)

Adult stem cells are most rigorously proven by single-cell transplantation, demonstrating long-term tissue reconstitution without prior in vitro multiplication. They are clonal, self-renewing and differentiate to certain tissue lineages. Despite the lack of a universal "stemness signature," they are distinguished by markers peculiar to their ancestry.

Methods of Identification and Characterization

1. Surface Marker Profiling

Flow cytometry / FACS (Fluorescence-Activated Cell Sorting) employing cell surface antigens remains one of the most practical approaches. For instance, Haematopoietic stem/progenitor cells (HSPCs): often used markers include CD34, CD133 (PROM1), CD90, CD45, and combinations of these. MSCs markers such as STRO-1 aid in the isolation of multipotent stromal cells, frequently in conjunction with negative selection against haematopoietic markers. Neural stem/progenitor cells such as neuronal and glial progenitors have been enriched using markers such PSA-NCAM, Nestin and p75NTR (low-affinity nerve growth factor receptor). However, a caveat, which is a surface-marker expression may alter with culture conditions, passage number, or differentiation, therefore marker-based identification must be accompanied by functional assays to guarantee actual stem cell behaviour.

2. Dye-Efflux (Side Population, SP) Assay

- Some stem cells contain significant quantities of ATP-binding cassette (ABC) transporters, especially ABCG2 (also termed BCRP1). These transporters pump out fluorescent dyes (e.g., Hoechst 33342), thus when tissue is labelled and subjected to dual-wavelength flow cytometry, a distinct “Side Population (SP)” develops (cells with limited dye retention). SP cells are generally enriched for stem or progenitor features.
- Potential stem or progenitor cells from bone marrow, skeletal muscle, the heart and numerous other tissues have been isolated with the SP assay. For example, in adult heart tissue, cardiac SP cells that express ABCG2 have demonstrated the ability to proliferate and differentiate.

3. Functional (Assay-Based) Validation

- **Self-renewal experiments** such as neurosphere creation (for neural stem cells), mammosphere assays (for mammary progenitors), or other “sphere assays” allow in vitro development of presumed stem cells in suspension culture. Single-cell generated spheres (clonal) and serial passage (secondary spheres) support the presence of self-renewing stem cells. However, sphere assays often encounter criticism such as not all sphere-forming cells are real stem cells and clonal purity or differentiation potential may not be equal to in vivo behaviour.
- **Slow-cycling / label-retaining cell experiments** by pulse-labeling dividing cells (e.g., with BrdU or ^{3}H-thymidine), then chasing for a lengthy period, cells that retain the label are likely quiescent stem cells (slow-cycling), whereas differentiated progeny dilute the label. This technique aids in locating populations of quiescent adult stem cells, such as those found in skin.
- **Lineage-tracing and fate-mapping** is the gold standard for verifying self-renewal and differentiation capacity in physiological contexts is still genetically tagging individual

cells (e.g., via inducible Cre-lox systems, retroviral or barcoding approaches, CRISPR-based lineage barcodes), then monitoring their progeny over time in vivo. Recent improvements integrate high-throughput scRNA-seq or epigenome profiling to resolve lineage links at single-cell resolution.

- **Genomic and epigenetic quality monitoring notably for pluripotent cells** continued culture might lead to chromosomal defects, copy-number variations, or epigenetic drift. Modern techniques therefore incorporate karyotyping, whole-genome sequencing / copy number variation (CNV) analysis, DNA methylation profiling and chromatin accessibility (ATAC-seq), to assure stability and safety for downstream applications.
- **Proteomic and functional assays**: these are used to verify the expression of lineage-specific markers (proteins), evaluate metabolic and physiological behaviour and measure functional characteristics (such as contractility for cardiomyocytes and neurotransmitter release for brain cells).

Stem Cell Culture

Growing and preserving stem cells outside of the body in a lab setting is known as "stem cell culture" (in vitro). This field has attracted considerable attention not just for basic research into stem cell biology but also due to the potential therapeutic applications of cultivated stem cells. Because of their potential to repair damaged cells, stem cells are cultivated artificially to create bigger quantities for research and medical uses. However, cultivating stem cells is more challenging than cultured conventional cells because stem cells must maintain their delicate balance between remaining undifferentiated and having the capacity to differentiate when needed.

Culturing Different Types

Embryonic stem cells are more routinely grown than adult stem cells because they display greater potency, they can develop into a wider number of cell types (about 250 kinds). Adult stem cells, while valuable, are more restricted in their differentiation potential and can typically only form cells within specific lineages or tissue families.

Culture Requirements

Stem cell culture parameters must be carefully modified according to the unique stem cell type. For instance, the needs for different types of adult stem cells are very different from those for embryonic stem cells. Similarly, culture settings need adjustment dependent on the final purpose, whether cells are being kept in their stem state or being guided to differentiate into specific cell types.

Because stem cells constantly balance self-renewal and differentiation, the culture procedure needs to be refined. Certain stem cells, in contrast to other cells, need specific, non-standard culture reagents like:

• **Feeder cell layers:** Supporting cells that release substances that aid in the survival of stem cells

• **Conditioned media:** Culture medium previously utilised by other cells, containing favourable secreted substances

• **Growth matrices:** specialised surfaces that replicate stem cell's natural surroundings

Standard Culture Conditions

Stem cells are normally kept in controlled incubators at 37°C (body temperature) with 5% CO_2 and 95% O_2. Cells are typically handled under sterile laminar flow hoods and closely observed during culture. Maintaining exact control over pH and oxygen pressure, which are essential for stem cell survival and appropriate behaviour, is one of the key hurdles.

Conclusion

Stem cells represent one of medicine's most promising frontiers. These amazing cells continue to transform therapeutic modalities, from well-established bone marrow transplants to state-of-the-art iPSC therapies. Cord blood banking provides accessible stem cell supplies,

while continuous research enhances therapeutic potential. Despite obstacles, new developments in biology and technology hold out the possibility of more advances in regenerative medicine and the treatment of illness.

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