Journal of Experimental and Basic Medical Sciences 2024;5(1):7-11

Review

Stem Cell Production: Processes, Practices, and Regulation

Ece Kaya[1], Oytun Erbaş[1]

ABSTRACT

Stem cells possess the unique ability to develop into different types of cells. Utilizing stem cells allows for the restoration of cells that have malfunctioned or lost their function entirely, presenting a promising approach to treating numerous diseases and repairing impaired biological mechanisms. Stem cells occur naturally within our bodies through spontaneous processes, and nowadays, they can also be artificially generated in laboratory settings for therapeutic applications. Depending on the intended treatment, diverse techniques are employed to produce stem cells utilized in various diseases. This chapter aims to provide insights into the techniques employed for the production of stem cells.

Keywords: Stem cell, stem cell cure, stem cell isolation.

Stem cells possess the unique capacity to undergo self-renewal and differentiation into specialized cell types. Their remarkable feature lies in their ability to both regenerate and divide, leading to the formation of different types of cells. They exhibit elevated telomerase activity, enabling them to undergo multiple divisions. Depending on the type of stem cell, they have the ability to differentiate into different cell types and create an entire organism.[1-4]

CLASSIFICATION OF STEM CELLS

Stem cells can be classified into two main categories based on their differentiation potential and the sources from which they are obtained.

a) Classification of stem cells according to differentiation potential

Stem cells can be classified into four subtypes based on their differentiation potential: totipotent, pluripotent, multipotent, and unipotent.[5] The term "totipotent" is derived from the combination of the Latin words "totus" (whole) and "potentia" (power).[6] Totipotent stem cells are the stem cells with the highest potential. These cells are present in the early stages of the zygote, at the two or four-cell stage, and have the ability to form an entire embryo. Totipotent stem cells can differentiate into all cell types and also have the ability to differentiate into extra-embryonic cells such as the amniotic sac and placenta. Due to these characteristics, they have the ability to create a new organism.[7]

Pluripotent stem cells, while similar to totipotent stem cells in their ability to form all tissue and organs in the body, differ in that they do not have the ability to form extra-embryonic cells and thus cannot create a new organism. They have the potential to differentiate into approximately 200 cell types under appropriate conditions and are therefore an important source of cells for many damaged tissue therapies.[5-8]

Multipotent stem cells, which were first isolated from bone marrow by Friedenstein et al.[9] and characterized by Caplan, are stem cell types that occur in the later stages of embryonic development. In humans, multipotent stem cells can differentiate into all blood cells and many tissue cells, and in adults, they can differentiate into groups of cells with similar functions.[10] Multipotent stem cells play an important role in tissue repair.[5,11]

[1]ERBAS Institute of Experimental Medicine, Illinois, USA & Gebze, Türkiye

Correspondence: Ece Kaya. Institute of Experimental Medicine, 41470 Gebze-Kocaeli, Türkiye

E-mail: ecekaya1995@gmail.com

Cite this article as: Kaya E, Erbaş O. Stem Cell Production: Processes, Practices, and Regulation. JEB Med Sci 2024;5(1):7-11.

doi: 10.5606/jebms.2024.1067

Received : September 23, 2023
Accepted : October 7, 2023
Published online : February 26, 2024

©2024 Journal of Experimental and Basic Medical Sciences. All rights reserved.

Some scientists consider unipotent stem cells, which are only able to differentiate into a specific type of cell, as precursor cells rather than stem cells. However, others classify them as stem cells.[12-15]

b) Classification of stem cells according to their source

According to their sources of origin, stem cells can be classified into two subcategories: embryonic and non-embryonic stem cells. Embryonic stem cells are pluripotent stem cells that are present in the blastocyst stage (4-5 days) of the embryo and have the ability to differentiate into various cell types that originate from the endoderm, mesoderm, and ectoderm layers.[16,17]

Non-embryonic stem cells include fetal stem cells, as well as stem cells derived from the umbilical cord, placenta, bone marrow, adipose tissue, and cadavers.[18,19]

Usage Areas of Stem Cells

Due to their ability to regenerate and differentiate into various cell types stem cells hold promise in the treatment of many diseases. Stem cell therapy is used in the treatment of diabetes[20], sickle cell anemia[21], human immunodeficiency virus infection[22], and various types of cancer.[23] Additionally, stem cells are used in veterinary medicine to treat damaged bones and tissues in animals, as well as to treat diabetes in cats and dogs, and to treat central nervous system disorders.[24-26]

Stem Cell Division

Asymmetric and symmetric divisions can be observed in stem cells. In symmetric division, stem cells give rise to two identical daughter stem cells or two progenitor cells that will differentiate in the future. In asymmetric division, one stem cell and one progenitor cell are formed. The generation of progenitor cells or stem cells in symmetric division is random.[4,27]

STEM CELL ISOLATION TECHNIQUES

Stem cells can be isolated through physical techniques based on their size and density or affinity techniques based on their electrical, chemical, and magnetic properties. The separation of stem cells based on size and density relies on the fact that stem cells and other cells have different sizes and densities.[28] Various methods can be used for stem cell isolation, including density gradient centrifugation techniques, pre-coating techniques, conditional expansion medium method, dielectrophoresis, field flow fractionation, flow cytometry, and magnetic-activated cell sorting. These methods have certain advantages and disadvantages compared to each other.[29]

Isolation by Density Gradient Centrifugation

Although the density gradient centrifugation method has advantages such as being able to separate a large process volume, being easily scalable, and being a cheap method, it is disadvantaged by having low purity and high heterogeneity and being a difficult and time-consuming process.[29,30] In this method, the medium containing the cell source is centrifuged for 30 minutes, and the cell layers are separated from each other. The heterogeneous medium containing the cells is separated into different layers according to their densities. In this method, a density gradient medium is added to the cell medium before centrifugation, and the isolation of stem cells is achieved by collecting them in the gradient region where the densities of the cells and medium are equal at the end of the process.[30] Ficoll-paque; Percoll density gradient centrifugation is an example of stem cell isolation using this method. Additionally, density gradient centrifugation with negative selection is also used in stem cell isolation, which can only be applied to whole blood. The advantage of this method is its high process volume, easy scalability, and high purity. RosetteSep can be an example of this method.[29]

Isolation with Pre-Plating Method

The pre-plating method, despite its advantages of easy scalability and high process volume, is disadvantageous due to its low purity and high heterogeneity. This method can be used to obtain human embryonic stem cells, stem cells derived from human adipose cells, and muscle stem cells.[29] This method is based on the different adhesive properties of cells added to a gelatin-coated cell surface. For example, muscle cells adhere to culture surfaces more slowly than fibroblast and epithelial cells. In this way, the cells added to the culture medium can be collected from the medium after approximately one hour, resulting in a 90% yield of stem cells. In a study focused on myogenic markers in mouse myeloblasts, the preplating method yielded approximately 98% pure stem cells.[31,32]

Isolation with Conditional Expansion Environment

The conditional expansion medium method, especially used in the isolation of mesenchymal stem

cells, has the advantages of easy scalability and large process volume, but it has low purity and high heterogeneity.[29]

Mesenchymal stem cells are cells that can renew and differentiate themselves to a limited extent, like fibroblast cells that can adhere. They can age rapidly in culture due to telomere shortening and morphological changes. Different expansion media such as fetal bovine serum and L-glutamine containing Dulbecco's modified eagle's medium (DMEM) have been developed to maintain their properties for therapeutic purposes. The most commonly used expansion medium is the one developed by Catherine Verfaillie. The expansion medium method allows mesenchymal stem cells to be passaged between 19-40 times.[33]

Isolation by Dielectrophoresis

The dielectrophoresis method, which is based on separating cells according to their electrophysical properties, separates cells by taking advantage of the difference in electrical charges between the cells and the liquid they are in. In this method, an alternating current field is used.[34] The most important advantages of this method are that it does not require labeling the cells with any markers to separate them and that it separates the cells based on their viability. However, due to cross-reactivity, low-purity stem cells can be obtained with this method.[29,35] The cell placed in the electric field polarizes and forms a dipole.[29] Depending on the induced electrical momentum polarity, the cells move in two different ways, positive and negative dielectrophoresis. In positive dielectrophoresis, cells approach the electrodes, while in negative dielectrophoresis, they move away from the electrodes. Due to these characteristics, cell separation can be performed in environments containing two different cell groups.[34,35]

Isolation by Flow Cytometry

The flow cytometry method, which is based on the examination of cells with different properties stained with fluorescent dyes in a flowing liquid, is a highly sensitive and high-resolution method. However, this method is expensive and not suitable for large-scale studies.[36,37]

In the flow cytometry method, the size, shape, cytoplasmic content, nucleic acid content, and fluorescent properties of cells are analyzed.[38,39] Cells stained with fluorescent dyes are used to analyze the properties of cells by measuring the wavelengths of light transmitted through the method.[40]

The flow cytometry method is used to analyze the quantitative properties of cells in a heterogeneous cell-containing environment. Monoclonal antibodies are used to isolate stem cells using this method, and specialized markers on stem cells are identified, allowing for the identification of necessary antigenic structures for treatment.[38]

Isolation by Field Flow Fractionation

The area flow fractionation method, which is based on the separation of live cells in the environment where cells are taken according to their morphological and biophysical properties into different fractions, relies on the interaction of the sample, which will be analyzed vertically into the mobile phase in a capillary containing laminar flow and an empty area.[29,41,42] In addition to traditional methods, more recent isolation techniques also exist. For example, the isolation of stem cells is achieved using a temperature-sensitive polymer, poly(N-isopropylacrylamide) (PNIPAAm), which conjugates with antibodies specific to stem cell markers using a two-phase system method.[29] Another stem cell isolation method is the systematic ligand development with exponential enrichment. This method, which is typically used for the isolation of erythrocytes, cancer cells, and stem cells, is an isolation method that uses aptamers with high sensitivity and selectivity due to their three-dimensional structure.[43]

In conclusion, due to their unique properties such as the ability to regenerate and create new tissue and organisms, stem cells are used primarily for therapeutic purposes in serious diseases such as cancer. The isolation of these cells and their subsequent multiplication for use in therapy is crucial in the field of medicine. Stem cells can be obtained from various sources, including embryonic and non-embryonic sources (such as adipose cells, fetal cells, cord blood, etc.). To isolate stem cells in a healthy and pure manner, many methods have been developed. The choice of which isolation method to use depends on the characteristics of the stem cell and the treatment method to be used. The isolated stem cells can then be multiplied using different methods *in vitro* under cell culture conditions and applied to the patient in therapy.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Thore CB, Sudheer S, Janke D, Jagodzinska J, Jung M, Adjaye J. The origins of human embryonic stem cells: a biological conundrum. Cells Tissues Organs. 2008 May;188:9-22.
2. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001 November;414:105-11.
3. Can A. A concise review on the classification and nomenclature of stem cells. Turk J Haematol. 2008;25:57-9.
4. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Research & Therapy. 2019 February;10:1-22.
5. Sobhani A, Nikoo S, Jafari F, Arjmand B, Mohamadnia A, Naderi Nabi H, et al. Multipotent stem cell and current application. Acta Medica Iranica. 2017 February; 55:6-23.
6. Morgani SM, Canham MA, Nichols J, Sharov AA, Migueles RP, Ko MSH, et al. Totipotent embryonic stem cells arise in ground-state culture conditions. Cell Rep. 2013 March;3:1945-57.
7. Ghazimoradi MH, Khalafizadeh A, Babashah S. A critical review on induced totipotent stem cells: Types and methods. Stem Cell Res. 2022 February;55:102857.
8. Donovan PJ, Gearhart J. The end of the beginning for pluripotent stem cells. Nature. 2014 March 6;507:93-4.
9. Friedenstein A, Gorskaja J, Kulagina N. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol. 1976 July;4:267-74.
10. Erdost H, Cerci E. Immunohistochemical localization of Ki67 antibody derived by adipose tissue. Int J Immunopathol Pharmacol Res. 2018 July-September;32:2058738418789699.
11. Mirzaei H, Sahebkar A, Sichani LS, Moridikia A, Nazari S, Sadri Nahand J, et al. Therapeutic application of multipotent stem cells. Journal of Cellular Physiology. 2018 April;233:2815-23.
12. de Kretser D. Totipotent, pluripotent or unipotent stem cells: a complex regulatory enigma and fascinating biology. Journal of law and Medicine. 2007 October;15:212-8.
13. Slack, J. M. Stem cells in epithelial tissues. Science. 2000 February;287:1431-3.
14. Dulak J, Szade K, Szade A, Nowak W, Józkowicz A. Adult stem cells: hopes and hypes of regenerative medicine. Acta Biochim Pol. 2015;62:329-37.
15. Visvader JE, Clevers H. Tissue-specific designs of stem cell hierarchies. Nat Cell Biol. 2016 Apr;18:349-55.
16. Ramalho-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. " Stemness": transcriptional profiling of embryonic and adult stem cells. Science. 2002 September;298:597-600.
17. Trounson A, McDonald C. Stem cell therapies in clinical trials: progress and challenges. Cell Stem Cell. 2015 July;17:11-22.
18. Hortu I, Ozceltik G, Sahin C, Akman L, Yildirim N, Erbas O. Granulocyte Colony-Stimulating Factor Prevents Ischemia/Reperfusion-Induced Ovarian Injury in Rats: Evaluation of Histological and Biochemical Parameters. Reprod Sci. 2019 Oct;26:1389-94.
19. Aragona M, Maisano R, Panetta S, Giudice A, Morelli M, La Torre I, et al. Telomere length maintenance in aging and carcinogenesis. Int J Oncol. 2000 Nov;17:981-9.
20. Helman A, Melton DA. A stem cell approach to cure type 1 diabetes. Cold Spring Harbor Perspectives in Biology 2021;13:a035741.
21. Bernaudin F, Socie G, Kuentz M, Chevret S, Duval M, Bertrand Y, et al. Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. Blood. 2007 Oct 1;110:2749-56.
22. Allers K, Hütter G, Hofmann J, Loddenkemper C, Rieger K, Thiel E, et al. Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. Blood. 2011 Mar 10;117:2791-9.
23. Hu Y, Fu L. Targeting cancer stem cells: a new therapy to cure cancer patients. American Journal of Cancer Research 2012;2:340.
24. Schnabel LV, Mohammed HO, Miller BJ, McDermott WG, Jacobson MS, Santangelo KS, et al. Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. J Orthop Res 2007;25:230-40.
25. Ribitsch I, Burk J, Delling U, Geißler C, Gittel C, Jülke H, et al. Basic science and clinical application of stem cells in veterinary medicine. Bioreactor Systems for Tissue Engineering II: Strategies for the Expansion and Directed Differentiation of Stem Cells, 2010 March 219-63.
26. Fortier L A, Travis A J. Stem cells in veterinary medicine. Stem Cell Research & Therapy, 2011 February 2:1-6.
27. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature. 2006 June, 441:1068-74.
28. Radisic M, Iyer RK, Murthy SK. Microand nanotechnology in cell separation. International Journal of Nanomedicine. 2006;1:3.
29. Zhu B, Murthy SK. Stem cell separation technologies. Current Opinion in Chemical Engineering. 2013;2:3-7.
30. Majekodunmi SO. A review on centrifugation in the pharmaceutical industry. American Journal of Biomedical Engineering. 2015;5:67-78.
31. Park YG, Moon JH, Kim J. A comparative study of magnetic-activated cell sorting, cytotoxicity and preplating for the purification of human myoblasts. Yonsei medical journal. 2006;47:179.
32. Bozkurt MF, Bhaya MN, Dibekoğlu C, Akat A, Ateş U, Erbaş O. Mesenchymal stem cells have ameliorative effect on the colitis model via Nrf2/HO-1 pathway. Acta Cir Bras. 2022 Oct 10;37:e370704.
33. Apel A, Groth A, Schlesinger S, Bruns H, Schemmer P, Büchler MW, et al. Suitability of human mesenchymal stem cells for gene therapy depends on the expansion medium. Exp Cell Res. 2009 Feb 1;315:498-507.
34. Pethig R, Menachery A, Pells S, De Sousa P. Dielectrophoresis: a review of applications for stem cell research. Journal of Biomedicine and

Biotechnology. 2010;182581.

35. Ismail A, Hughes MP, Mulhall HJ, Oreffo ROC, Labeed FH. Characterization of human skeletal stem and bone cell populations using dielectrophoresis. Journal of tissue engineering and regenerative medicine. 2015;9:162-8.
36. Alexander CM, Puchalski J, Klos KS, Badders N, Ailles L, Kim CF, et al. Separating stem cells by flow cytometry: reducing variability for solid tissues. Cell Stem Cell. 2009 December;5:579-83.
37. Preffer F, Dombkowski D. Advances in complex multiparameter flow cytometry technology: Applications in stem cell research. Cytometry Part B: Clinical Cytometry: The Journal of the International Society for Analytical Cytology. 2009;76:295-314.
38. Nery AA, Nascimento IC, Glaser T, Bassaneze V, Krieger J E, Ulrich H. Human mesenchymal stem cells: from immunophenotyping by flow cytometry to clinical applications. Cytometry Part A. 2013 January;83:48-61.
39. Adan A, Alizada G, Kiraz Y, Baran Y, Nalbant A. Flow cytometry: basic principles and applications. Critical Reviews in Biotechnology. 2017;37:163-176.
40. Karaboz İ, Kayar E, Akar S. Flow cytometry and its applications. Electronic Journal of Microbiology. 2008;6:1-18.
41. Roda B, Lanzoni G, Alviano F, Zattoni A, Costa R, Di Carlo A, et al. A novel stem cell tag-less sorting method. Stem Cell Rev Rep. 2009 Dec;5:420-7.
42. Roda B, Zattoni A, Reschiglian P, Moon MH, Mirasoli M, Michelini E, et al. Field-flow fractionation in bioanalysis: A review of recent trends. Anal Chim Acta. 2009 Mar 9;635:132-43.
43. Didar TF, Tabrizian M. Adhesion based detection, sorting and enrichment of cells in microfluidic Lab-on-Chip devices. Lab on a Chip. 2010;10:3043-53.