Original Article

STEM CELLS AND ITS MULTIMODALITY - A REVIEW

[1]Sutharshan G S, [2]Dr.Priyadharshini R*, [3]Dr.Palati Sinduja
[1]Undergraduate, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.
[2]*Assistant Professor, Department of Pathology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai - 77, TamilNadu.
[3]Assistant Professor, Department of Pathology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai 77, TamilNadu,

Abstract

Stem cells are unique human cells that have the ability to grow rapidly, usually from a single cell, and differentiate into many cell and tissue types. It can differentiate into any sort of cell, which can then differentiate into daughter cells, which can then differentiate into hematopoietic stem cells. To avoid the creation of teratomas, hESCs are derived from traditional culture to laser-assisted methods or microsurgery, and their differentiation must be determined. Inner cell mass (ICM) and trophectoderm are the two cell types that make up blastocysts (TE). Pluripotent cells have the ability to proliferate and differentiate into any type of cell, and they can be a limitless source of cells. In stem cell therapy, iPSCs eliminate the requirement for embryos.The self-renewal property of ESC, Hwang et al. (Hwang, Varghese and Elisseeff, 2008) the optimum culture method for hESC-based cell and tissue treatment would be a defined culture free, according to the researchers. Certain diseases that are currently incurable, such as neurodegenerative diseases, may be able to be treated with stem cell therapy. We are better able to extend human life than at any other point in history thanks to stem cell therapy and all of its restorative effects. This review paper on stem cells and their control, together with insightful remarks on clinical application and future outcomes, gives stem cell renewal a lot of optimism.

Key words: pluripotent, regenerative medicine, Innovative technique, self renewal, stem cells.
DOI: 10.47750/pnr.2022.13.S04.151

INTRODUCTION

Stem cells are unique human cells that have the ability to grow rapidly, usually from a single cell, and differentiate into many cell and tissue types. (Kolios and Moodley, 2013) It can differentiate into any sort of cell, which can then differentiate into daughter cells, which can then differentiate into hematopoietic stem cells. (Avasthi, Srivastava and Singh, 2008), (Daley, 2015). It has two key characteristics: self-renewal and infinite potency. Potency refers to a stem cell's differential potential. Totipotent stem cells are created by fusing an egg and a sperm cell. Morula cells are totipotent, meaning they can transform into any tissue. Adult bodily tissues and embryos are the two main sources of stem cells, each with slightly distinct features. Embryonic stem cells have the ability to renew and repair tissues. All three embryonic germ layers, ectoderm, mesoderm, and endoderm, can be formed from it. Nanong protein controls the pluripotency of embryonic stem cells. Adult stem cells can be found all over the body and have a limited ability to give rise to various bodily cells (Haas, Weidner and Winkler, 2005). The adult stem cells differentiation is regulated by Bimi, Notch, Sonic hedgehog & Wnt and plasticity. The clinical application and potential use of embryonic and adult stem cells are genetic therapy (Mitsui *et al.*, 2003), drug testing, cancer (Beachy, Karhadkar and Berman, 2004), brain damage, muscle damage, heart damage, and spinal cord injury.

Therapeutic in certain fields like organ replacement and transplantation of lost tissues are having a lot of hope from stem cell research (Crop *et al.*, 2009). Stem cells can also be used in immuno-modulation or reconstitution methods in cancer therapy (Crop *et al.*, 2009; Soltanian and Matin, 2011). Stem cells replace diseased cells with healthy cells by regenerative and reprogramming techniques. Researchers believe that stem cell-based therapies may one day be used to treat serious illnesses like paralysis and Alzheimer's disease etc (Zaret, 2008). Exosomes derived from mesenchymal stem cells used in the treatment of autosomal disorders. Hematopoietic stem cells are used in ischaemic vascular diseases of the heart (Ajani *et al.*, 2015). Our team has extensive knowledge and research experience that has translate into high quality publications(Rao and Kumar, no date; Krishnan and Lakshmi, 2013; Devi and Gnanavel, 2014; Varghese *et al.*, 2015; Samuel *et al.*, 2019)(Kamisetty *et al.*, 2015; Patturaja and Pradeep, 2016; Felicita, 2017; Jain, 2017; Kumar, 2017)

This review article on stem cells and their regulation with enlightening comments on clinical application and future outcomes have huge hope in stem cell renewal.

Classification of stem cells

The human body contains stem cells, which are unspecialized cells that can differentiate into any self-renewing cell. Differentiation occurs in both embryonic and adult stem cells, resulting in the formation of specialised cell types. The ability of stem cells to develop into a variety of cells is used to classify them. When compared to multipotent stem cells, unipotent stem cells have less developmental potential. Totipotent stem cells have the ability to differentiate into entire biological entities and divide.

Totipotent stem cells can be found in both embryonic and extraembryonic sites. A zygote, for example, is formed when sperm fertilises the egg, which then forms the germinal layers of the placenta. Totipotent stem cells are multipotent stem cells that can divide. It can also develop into organism-wide cells. The differentiation potential of totipotency is the highest. Cells can generate both embryonic and extraembryonic structures because of it. The creation of a blastocyst, which eventually becomes pluripotent, occurs on the fourth day. The inner cell mass (ICM) of a blastocyst becomes pluripotent after around 4 days. Germ cell layer development is caused by pluripotent stem cells. Pluripotent stem cells come from this organisation. The epiblast layer of implanted embryonal cells is where induced pluripotent cells are produced. Embryonic stem cells can be found in pre-implanted embryos.

Oligopotent, Multipotent, and unipotent are those cells with less number of differentiation. But are capable of differentiating to specialized stem cell lineages like hematopoietic cells resulting in several blood cell formations. After that comes the oligopotent cells like myeloid stem cells which divide to form WBC but not RBC cells. Unipotent stem cells are characterized by narrow and repeated differentiation to one type of cells like dermatophytes.

BIOLOGY OF STEM CELL

A blastocyst is generated when sperm and ovum fertilise each other. Short-lived stem cells coat its inner wall (embryonic stem cells). Inner cell mass (ICM) and trophectoderm are the two cell types that make up blastocysts (TE). Blastocyst is in charge of controlling the ICM. The trophectoderm provides extra embryonic support to the placenta, whereas ICM cells remain undifferentiated, pluripotent, and proliferative. ICM produces human embryonic stem cells (hESCs). The germ layer, which includes endoderm, mesoderm, and ectoderm, is produced during embryogenesis and gives rise to differentiated cells and tissues in the foetus and later in the adult organism. After hESCs differentiate into one of the germ layers, their potency is limited to only the germ layer cells, and this process is short in human development, they become multipotent stem cells. The key skills of pluripotent stem cells were then multiplied by the development of the next generation of stem cells. It also serves as the body's internal mending mechanism. The replenishment and production of new cells are limitless as long as an organism is alive.

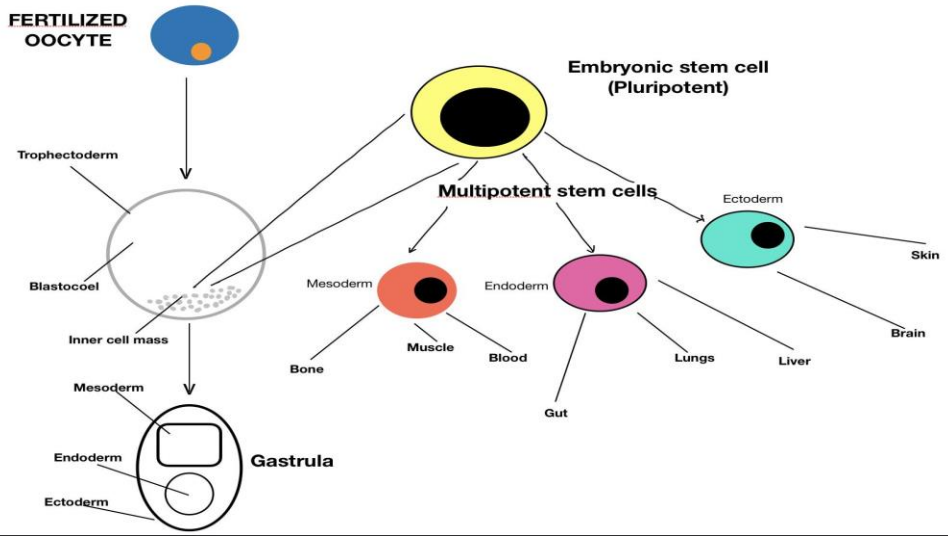

Figure 1: Oocyte development and formation of stem cells; the blastocoel, which is formed from oocyte, consists of embryonic stem cells that further differentiate into mesodermal, ectodermal or endodermal cells, Blastocoel develops into gastrula.

FUNCTIONAL DIVISION OF STEM CELLS

Development of the whole body

The presence of distinct stem cells in division is determined by the organism's development. The ICM of the blastocyst, which is a stage of the pre-implantation embryo for 4 days after fertilisation and these cells are placed in a culture dish filled with culture medium, is derived from the ICM of the blastocyst, which is a stage of the pre-implantation embryo for 4 days after fertilisation and these cells are placed in a culture dish filled with culture medium. The passage is a common method of sub-culturing cells to other plates, and it is called pluripotent because it allows cells to differentiate into any cell type in the body. Because the majority of embryonic stem cells are derived from eggs fertilised in an in vitro clinic. Adult stem cells, also known as somatic stem cells, are undifferentiated cells that enable the healing, growth, and replacement of cells that are lost every day. The differentiation range of these cells is limited. There are many different sorts of cells; here are a few examples:

Mesenchymal stem cells differentiate primarily into bone, cartilage, and fat cells in the bone marrow, although they are an exception because they are pluripotent and can specialise in any germ layer cell.

- Nerve cells develop from neural cells.
- HSCs (hematopoietic stem cells) give rise to all types of blood cells.
- Keratinocytes, which form a protective layer of skin, are formed by skin stem cells.

Somatic stem cell growth takes longer than that of ESCs, and adult stem cells can be reprogrammed to become pluripotent again. Later, this can be accomplished by moving the adult nucleus into the cytoplasm of an oocyte or by fusing the pluripotent cell with the adult nucleus. During the cloning of the Dolly sheep, this method was applied. hESCs are engaged in whole-body development and can differentiate into pluripotent, totipotent, multipotent, and unipotent cells. Pluripotent cells can also generate extraembryonic tissues of the embryo and are referred to as totipotent cells.

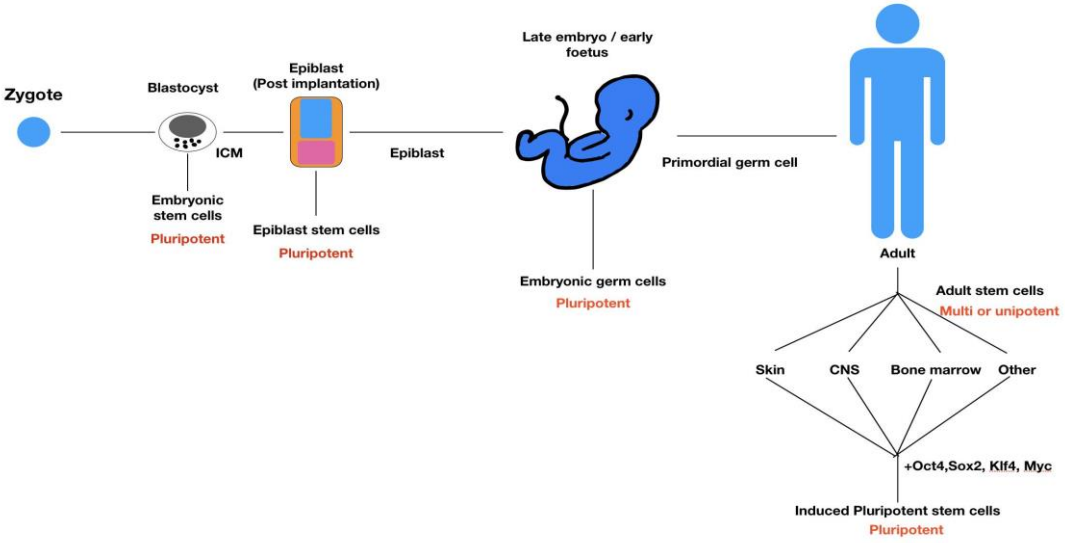

Figure 2: Changes in the potency of stem cells in human body development. iPSC internal control and recognition by morphological differences

Comparing the stem cell lines from different individuals is needed for iPSC lines to be used in therapeutics. Among critical procedures, the following can be distinguished: 1) Short tandem repeat analysis: It is used in measuring an exact number of repeating units. One unit has 2-13 nucleotides repeating many times on the DNA strand. PCR is used to check the length of short tandem repeats, 2) Identity analysis: The unintentional switching of lines, leading to other somatic cell line contamination, requires rigorous assay for cell line identification, 3) Residual vector testing: An appearance of reprogramming vectors integrated into the host genome is hazardous, and testing their presence may be a mandatory procedure and it is a commonly used procedure for generating high-quality iPSC lines, 4) Karyotype - A long-term culture of hESCs can accumulate culture-driven mutations due to that, it's crucial to pay additional attention to genomic integrity. Karyotype tests are often performed by resuscitating representative aliquots and culturing them

for 48–72 hrs before harvesting cells for karyotypic analysis. The examination must be redone on a new sample if abnormalities are discovered within the primary 20 karyotypes. If this scenario is repeated, the route is deemed irregular.Repeated abnormalities must be recorded, 5) Viral testing - When assessing stem cell quality, all tests for human adventitious agents must be performed (e.g. Hepatitis C or HIV). This procedure must be performed when using non-xeno-free culture agents subsequently. 6) Histone modification and DNA methylation - Quality control is often achieved by using epigenetic analysis tools like histone modification or DNA methylation. The methylation process, when stem cells differentiate, suppresses pluripotency genes and reduces differentiation potential, although other genes may undergo demethylation to become expressed (Kim *et al.*, 2020).

Derivatives and media of hESCs

hESCs are derived from classic culturing to laser-assisted methodologies or microsurgery and its differentiation must be specified to avoid teratoma formation. It differentiates spontaneously into embryonic bodies (EBs), and its effects on early human development can be predicted without relying on embryos or animals. It can be grown by several methods, including bioreactor culture, hanging drop culture, or microwell technology. The important part of these culturing procedures is a separation of ICM to culture future hESCs. After that spontaneous differentiation occurs. Cells must be separated when the colony reaches the appropriate size. The pluripotent cells last for 1-2 days. Chi et al.(Cui *et al.*, 2021) found out that it is also possible to obtain hESCs from four-cell embryos. There are four passing procedures. They are enzymatic dissociation, manual passage, trypsin utilization, ethylenediaminetetraacetic acid (EDTA).

Culture methods used for hESCs are mouse embryonic fibroblasts (MEFs) as a feeder layer and bovine serum as a medium. To ensure that stem cells will differentiate and develop properly, the medium should be changed each day. The first feeder layer-free culture is supplemented with serum replacement combined with laminin. Initial culturing media can be serum artificial replacement such as synthetic serum substitute (SSS), stem pro. The culture medium contains eight essential elements: DMEM/F12 medium, selenium, NaHCO3, L- Ascorbic acid, transferring, insulin, TGFβ 1, and FGF2. However, it is not yet clear whether culture systems developed for hESCs can be used (Ko *et al.*, 2021).

iPSCs

Pluripotency can only occur naturally in embryonic stem cells, however it is possible to create pluripotency in terminally differentiated cells. The reprogramming process is aided by the downregulation of genes that promote genomic stability, such as p53. Histone modification is involved in cell reprogramming, and all of these processes have the potential to be mutagenic, resulting in an increase in the frequency of mutations. Quinlan and colleagues discovered that current reprogramming approaches can generate fully pluripotent iPSCs with no changes. Some modifications in the genetic organisation of these cells arise during the development phase from pluripotent hESCs to differentiated somatic cells.(Azami *et al.*, 2021).

Sources of iPSCs

Pluripotent cells have the ability to proliferate and differentiate into any type of cell, and they can be a limitless source of cells. In stem cell therapy, iPSCs eliminate the requirement for embryos. iPSCs were created using fibroblasts as a source. Renal epithelial cells, peripheral blood cells, and urine keratinocytes are among the additional cells used in the procedure. Stimulating a patient's endogenous stem cells to divide or differentiate, which occurs naturally when skin wounds heal, is a common alternative to somatic cell transplantation. Fibroblasts are the best stem cell source because they can be stimulated quickly and with more precision (Sung *et al.*, 2021).

Teratoma formation assay

Because of their ability to self-renew and differentiate, iPSCs have attracted a lot of attention in the field of regenerative medicine. A quality-control assay, such as a teratoma development assay, is required to investigate their abilities. Teratomas are benign tumours that can grow quickly in the body and develop into tissues from all three germ layers at the same time. Because of teratomas' great pluripotency, the formation assay is considered a test of iPSC capacities. When compared to hESCs, the rate of teratoma formation in human iPSCs was shown to be higher. This discrepancy is linked to distinct differentiation processes and cell sources. Examined iPSC(Jo *et al.*, 2021)s which are given subcutaneously or under the testis in immune-deficient mice are the commonly done assays in teratoma. Following injection, immature but discernible tissue is visible. Teratoma has formed at the injection site. In this experiment, three groups of markers are employed to differentiate germ layer cells. Teratoma development assays under physiological settings are used to demonstrate the efficacy of human IPSC and are regarded as the gold standard. Because of their real tissue creation, they are also employed to characterise various cell lineages.

STEM CELL DIFFERENTIATION

In order to be used in therapy, stem cells must be transformed into the required cell types. Because undifferentiated ESCs can develop teratomas in vivo, differentiation of ESCs is essential. The use of signalling channels to differentiate cells is an important strategy in regenerative medicine. An extracellular microenvironment influences cell behaviour. In vitro, it is possible to halt specific differentiation pathways and establish cultures rich in specific precursors, but doing so in vivo is more difficult. Because of ESC's ability to self-renew, Hwang et al. (Hwang, Varghese and Elisseeff, 2008) suggested that a defined culture free technique would be excellent for hESC-based cell and tissue therapy. During the gastrulation process, pluripotent stem cells differentiate into mesodermal or endodermal, ectodermal progenitors. Growth factors cause stem cells to become progenitor cells, which then give rise to the chosen cell type. FGFs; Wnt family, or superfamily of transforming growth factors - (TGF) and bone morphogenic proteins are examples of signal intensities and molecular families that may alter the germ layer in vivo (BMP). Each component is evaluated at different concentrations, and the lengths of time that developing embryonic cells are impacted during differentiation remain unknown. For ESC ectoderm development, molecular inhibitors of endogenous BMP and Wnt signalling can be employed. It later triggers mesodermal differentiation (Cheng, 2015) . Wnt and a reduced quantity of the TGF family stimulate mesodermal differentiation. The efficiency, functional maturity, and eventually the transfer of generated cells to their in vivo counterpart determine the differentiation factor's efficacy. Future cell phenotypes are influenced by shear stress, topography, and substrate rigidity. Controlling biochemical and biophysical signals, the biophysical environment, and a good guidance for hESC differentiation are all key variables in cultured stem cells.

STEM CELLS AND THEIR TISSUES BANKS

iPS cells may be kept in tissues, making them a valuable source of human tissue for medical research. Mesenchymal stem cells are abundant in the umbilical cord. Stem cells are kept and employed in therapies to prevent a patient's future life-threatening disease due to their cryopreservation action after birth. SHED (Stem cells of human exfoliated deciduous teeth) discovered in deciduous teeth have the potential to develop into a variety of bodily tissues. (Arora, Arora and Munshi, 2009) Collection, isolation, and storage techniques are simple and non-invasive. Advantages of banking, SHED cells are:

- Simple and painless for both child and parent.
- There is no subject to the same ethical concerns as embryonic stem cells.
- Confirmed donors match autologous transplant that causes no immune reaction and rejection of cells. (Mao, 2008)
- SHED cells can regenerate into solid tissues such as neural, dental, connective, or bone tissues. (Arthur *et al.*, 2008)

SHED type and its role in regenerative medicine

- Adipocytes play a vital role in heart muscle regeneration, treatment of the spine, cardiovascular disease prevention, and orthopedic conditions, congestive heart failure, Crohn’s disease. (García-Olmo *et al.*, 2005; Perry *et al.*, 2008)
- Chondrocytes play a vital role in cartilage growth, suitable for transplants.
- Osteoblasts play an important role in bone regeneration, bone tissues suitable for transplant, teeth growth, craniofacial defects.
- Mesenchymal play an important role in the restoration of feeling and movement in paralyzed patients, spinal cord injury repair, treatment of Alzheimer’s and Parkinson’s diseases (Kerkis *et al.*, 2008)

Use of stem cells in dentistry

Teeth are a tough material to replicate in regenerative medicine because of their functions in elements such as mastication and articulation, as well as their intricate structure. Periodontal ligaments contain stem cells that can develop into osteoblasts. Gene therapy, which is performed using adenoviruses-containing growth factors, is one of the successful approaches in tissue engineering and a viable method in periodontology for treating periodontal disease. Enamel regeneration is more difficult because the enamel is deformed once ameloblastoma cells differentiate into it, making restoration impossible.(Shi *et al.*, 2005).

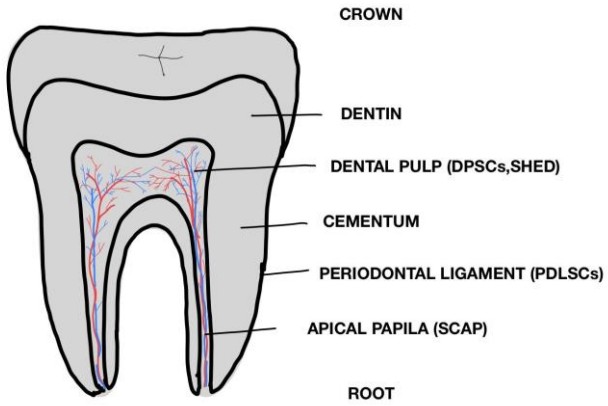

Figure 3 : Localisation of stem cells in dental tissues. DPSC - Dental pulp stem cells and Stem cells of human deciduous teeth (SHED) are located in the dental pulp. PDL stem cells are located in the periodontal ligament. Apical papilla consists of stem cells from the apical papilla (SCAP).

Dental pulp stem cells(DPSC): It was the first dental stem cell to be isolated from human dental pulp, which is found within the pulp of the teeth. It has the ability to be osteogenic and chondrogenic. When dental pulp MSCs are separated, they appear to be quite clonogenic. They can be isolated from adult and foetal tissue, and they can develop into odontoblast-like cells and osteoblasts in order to generate dentine and bone (Bansal and Jain, 2015). The third molar is their finest source place. Due to their cryopreservation ability, easy surgical accessibility, higher synthesis of dental tissue relative to non-dental stem cells, and anti-inflammatory qualities, DPSCs are a useful dental source of tissue engineering. SHEDs proliferate at a faster rate than DPSCs. They divide into a larger number of cells, including brain cells. They have one important drawback: in vivo, they form a partial dentin-like complex. In vivo, DPSCs and SHED can create bone-like tissue, which is employed for periodontal, pulp regeneration, and dentin regeneration. Such neutral deficits can be treated with both DPSCs and SHED. DPSCs were investigated and employed satisfactorily for alveolar bone and mandible restoration.

Periodontal ligament stem cells (PDLSCs): Because of their safety and efficacy, it is used for PDL regeneration therapy. In vitro, they can develop into collagen-forming cells, cementum tissue, Sharpey's fibre, and osteoblast-like cells by dividing into key mesenchymal cell lineages. It can be found on the alveolar bone as well as the root surfaces.

Stem cells of the apical papilla (SCAP): These cells are mesenchymal entities isolated from human immature permanent apical papilla and are found within immature roots. SCAP, which produces apexogenesis, is the source of odontoblasts. In vitro, these cells can be injected to create odontoblast-like cells and neuron-like cells. SCAPs have a stronger proliferative ability than DPSCs, making them a superior alternative for tissue regeneration.

Dental follicle stem cells (DFCs): It is present in the loose connective tissue that surrounds the tooth germ. It has the ability to transform into osteoblasts, cementoblasts, and periodontal ligament cells. It is retrieved from a third molar's sac. DFCs can create a root-like tissue with a pulp-dentin complex and generate tooth roots when they join with the dentin matrix.(Guo *et al.*, 2012)

Endodontics pulp regeneration: Dental pulp stem cells can develop into odontoblasts, and some procedures allow for pulp regeneration. The first method is an ex vivo procedure in which stem cells are cultivated on a scaffold before being injected into the root canal. The following method is an in vivo procedure that involves injecting stem cells into disinfected root channels once the in vivo apex has been opened. The potential of dental stem cells is primarily related to the regeneration of injured dentin and pulp or the healing of any perforation; in the future, it looks to be possible to regenerate the entire tooth. This result would pave the way for implant therapies to be phased out gradually.(Yang *et al.*, 2021).

CONCLUSION

This review focuses on stem cell classification, function, biology, differentiation, and usage of dentistry. After multiple trials, stem cell therapy is proving to be a game changer in medicine. The capacities of stem cells are expanding with each experiment and study, despite the fact that there are still numerous challenges to overcome. The impact of stem cells on regenerative medicine and transplantology is enormous. Certain untreatable disorders, such as neurodegenerative diseases, may be treated using stem cell therapy. We are better able to extend human life than at any other point in history thanks to stem cell therapy and all of its restorative effects.

ACKNOWLEDGMENTS

We would like to thank Saveetha Institute of Medical and Technical Sciences, Saveetha Dental College and Hospitals, Saveetha University for providing us support to conduct this study.

CONFLICT OF INTEREST

The author declares that there were no conflicts of interests in the present study.

SOURCE OF FUNDING

The present project is supported by

- Saveetha Institute of Medical and Technical Sciences
- SaveethaDental College and Hospitals, Saveetha University
- Jeevan clinic, Madurai.

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