Chapter

Human Teeth Is Useful Even after Its SHED! So, Why Discard It?

Meghna Bhandary, Rachaita Chhabra, K. Ananya Rao and Mohammed Shahid

Abstract

A few decades ago, if one underwent a knee injury that makes walking painful or had an atrophied kidney, then, he/she was condemned to a life hooked on to machines, or on constant medications. However, in today's era, teeth can be grown in a Petri dish; heart and liver replacements are possible with no risk of rejection because the organs are made of the patient's own cells. This is the promise of regenerative medicine and tissue engineering. The entire idea of regenerative medicine is based on the presence of stem cells in the body or the ability to introduce stem cells into the body without causing harm. These can be obtained from a variety of body and dental tissues. Deciduous teeth often discarded as biological waste is proven to possess Stem cells (SHED) that have promising applications in tissue engineering and regenerative medicine. Hence, their contribution toward the field of regenerative medicine and dentistry is immense. This chapter summarizes SHED's regenerative potentials and therapeutic applications; and also focuses on its potential future scope in regenerative dentistry. Furthermore, procedures involved in SHED-induced therapy, from SHED collection to SHED banking, have also been explained.

Keywords: stem cells, stem cell from exfoliated deciduous teeth (SHED), tooth, regeneration, repair, dentin pulp regeneration, therapeutic, biodentine

1. Introduction

> *"The Regenerative Medicine revolution is upon us. Like iron and steel to the industrial revolution, like the microchip to the tech revolution, stem cells will be the driving force of this next revolution".*
>
> *-Cade Hildreth.*

Staggering progress in the field of regenerative medicine has sowed the seeds of cell-based therapies for various diseases which cannot be cured by conventional methods. Stem cell therapy deals with the functional revival of specific tissue and/or organ in patients who are suffering from severe injuries / chronic diseased conditions, in a state where the body's own regeneration feedback is not satisfactory.

The entire argument of regenerative medicine is built on the presence of stem cells in the body, or the ability to institute stem cells into the body without causing harm. Given that stem cells can be obtained from a variety of sources, the search for an ideal source that offers excellent therapeutic potential while requiring less invasiveness and immune rejection is unending. Even a tooth which is naturally discarded can be used as a great source for stem cells. Hence a better understanding of the nature and mechanism of stem cell is crucial for their application in cell-based therapy.

1.1 What are stem cells and why is stem cell therapy so much of interest?

Our bodies are the ultimate factory. Every cell has its own function to play, and the fate of each cell is determined at the embryo stage which then cannot be changed. However, the discovery of stem cells has paved the way for regenerative medicine. Stem cells are those immature cells which can differentiate into any type of cells as they are not specialized [1]. Therefore, they can be used in the repair and regeneration of dysfunctional tissues. For instance, they can help treat neurological diseases by making new brain cells to treat people with Parkinson's disease, or they could be used to repair the damaged immune system, or even reverse paralysis/regrow lost limbs.

Therefore, stem cell research can help to:

- Understand how diseases and ailments manifest by observing the maturation of stem cells into cells of bone, heart muscle, neurons, and other organs and tissues.

- Stem cells can be directed to differentiate into specific cells capable of regenerating and repairing damaged or diseased human tissues (regenerative medicine). Hence, Stem cell therapy may benefit people with spinal cord injuries, type 1 diabetes, Parkinson's disease, Battens disease, Amyotrophic lateral sclerosis, Alzheimer's disease, heart disease, stroke, burns, cancer, and osteoarthritis [2].

- Scientists can employ some types of stem cells to evaluate the drugs' quality and safety before administering investigational medications to humans. This form of testing will most likely immediately affect medication development for cardiac toxicity testing first. New fields of research examine the efficacy of employing human stem cells that have been engineered to differentiate into tissue-specific cells for testing new medications. For the testing of novel medications to be accurate, the cells must be programmed to acquire the characteristics of the type of cells the drug is designed to target. For example, nerve cells could be generated to test a novel medicine for a nerve disorder. Tests could determine if the new medicine had any effect on the cells or if they were damaged.

2. Classification of stem cells

Depending on the origin/source of the stem cells, stem cells are divided into various types;

2.1 Embryonic stem cells (ESCs)

They are pluripotent stem cells derived from the blastocyst's inner cell mass. The blastocyst stage, with 50–150 cells, occurs 4–5 days after conception. Embryonic stem cells are able to develop into any type of cell, except those of the placenta (**Figure 1**).

ESCs derived from mouse blastocysts have been studied for 2 decades and shown to differentiate into various cell types including fat cells, brain, nerve, insulin-producing cells of the pancreas, bone cells, endothelial cells, and heart muscle cells [3]. Human ESCs under appropriate culture conditions have demonstrated remarkable abilities to self-renew and produce multipotent cells. The cells are studied extensively in the treatment of diabetes, heart diseases, genetic disorders spinal cord injury, muscular dystrophy, heart illness, and vision/hearing loss. However, it possesses the risk of developing adverse effects such as tumors and unwanted immune responses. Hence the scope of ESCs is still under debate and research to understand how to prevent the rejection of transplanted cells is fundamental.

2.2 Adult stem cells/ somatic or tissue-specific stem cells (ASCs)

ASCs, also called somatic stem cells, are undifferentiated cells that can self-renew and generate all the cell types of the organ from which they originate. An adult stem cell is derived from adult tissue samples. Hence, their use in research and therapy is not considered to be controversial unlike embryonic stem cells derived from embryos.

ASCs are the gold standard for clinical applications and are being tested and accepted for a growing number of conditions. ASCs have been shown to have therapeutic benefits in clinical trials and progress towards fully tested and approved treatments. Phase I/II trials conducted suggest potential cardiovascular benefits from bone

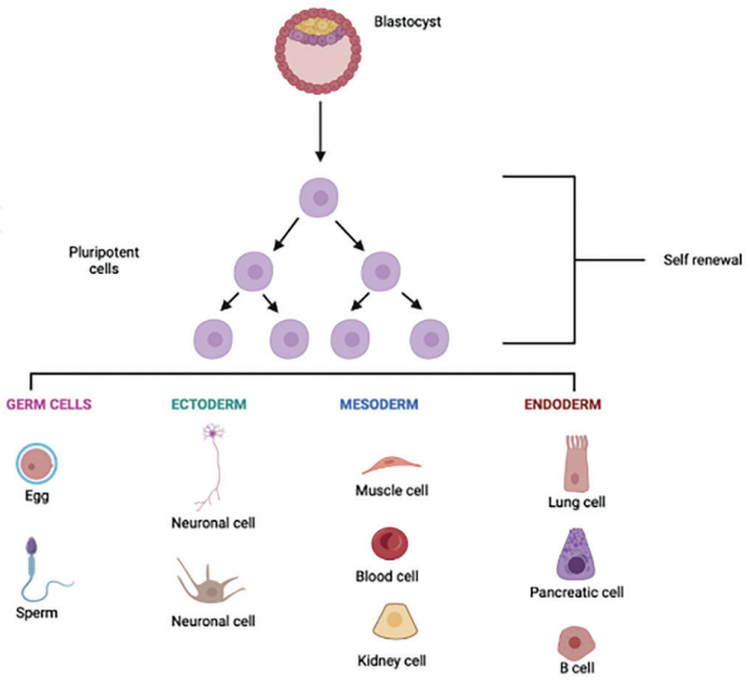


Figure 1.
Human embryonic stem cells differentiation. Image source: Biorender.com.

marrow–derived adult stem cells and umbilical cord blood–derived cells. Striking results have been reported using adult stem cells to treat neurological conditions, including chronic stroke. Positive long-term progression-free outcomes have been seen, including some remission for multiple sclerosis, as well as benefits in early trials for patients with type I diabetes mellitus and spinal cord injury. ASCs are also being used as vehicles for genetic therapies, such as for epidermolysis bullosa. One of the limitations of ASCs includes that they cannot be manipulated to produce all cell types, which limits their use in treating diseases (**Figure 2**).

2.3 Induced pluripotent stem cells (iPSCs)

The limitations in ASCs led to the creation of novel pluripotent cells termed induced pluripotent cells from the adult cells by the process of reprogramming the genes. ASCs can be fused with embryonic stem cells to generate induced pluripotent stem cells. Other somatic cells can also be altered to become pluripotent. iPSCs can differentiate from ESCs. Their gene expression and chromatin differ from ESCs. These cells are important because they may be utilized to create cells from almost all organs for each patient in therapeutic treatment. Besides, they also prevent the use of more ESCs which might cause ethical issues. It also helps to study new genetic diseases by generating iPSCs from their adult or somatic cells (**Figure 3**).

Hepatocyte–like cell derivatives, dendritic cells, macrophages, insulin–producing cell clusters similar to the duodenal islets of Langerhans, and hematopoietic and endothelial cells are currently produced from murine and human iPSCs, in addition to the already listed types of differentiated cells [4–7]. Reprogrammed iPSCs derived from peripheral blood cells could effectively develop into hematopoietic lineage cells [8]. Human β cell-derived iPSCs possess epigenetic memory and may develop into

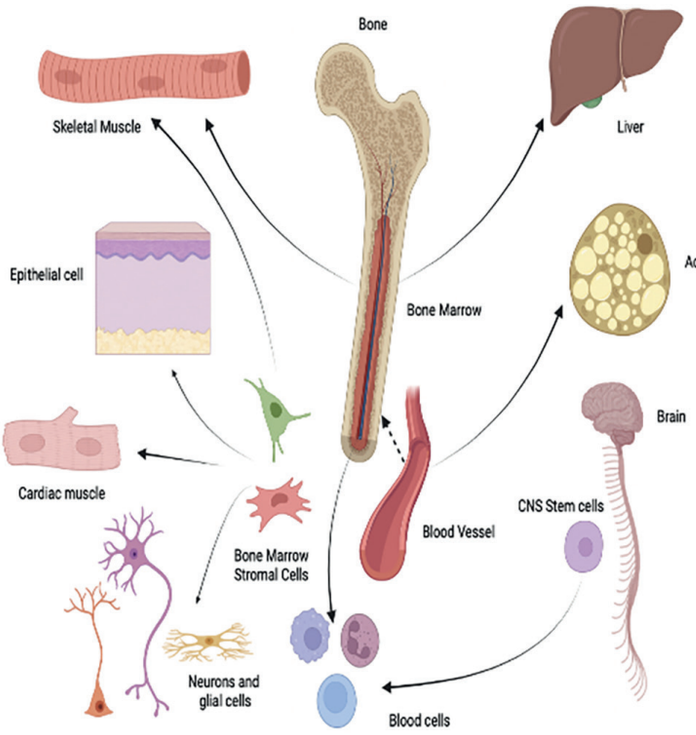


Figure 2.
Sources of adult stem cells. Image source: Biorender.com.

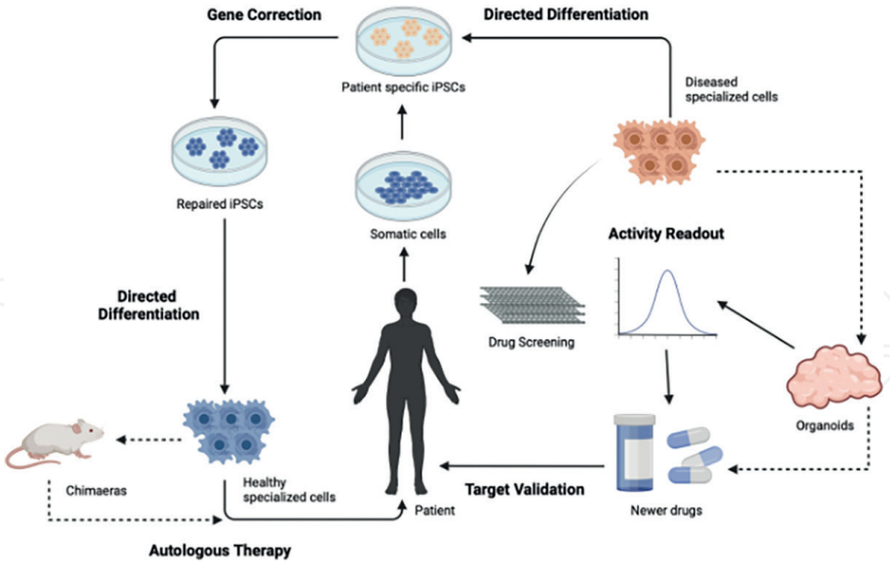


Figure 3.
Evolution of induced pluripotent stem cells. Image source: Biorender.com.

insulin-producing cells more readily [9]. Dopamine and motor neurons can also be produced from human iPSCs by directed differentiation *in vitro* [10, 11].

2.4 Mesenchymal stem cells (MSCs)

MSCs are a type of adult stem cell that can develop into mesodermal (osteocytes, adipocytes, and chondrocytes), ectodermal (neurocytes), and endodermal cell lines (hepatocytes). Some of the potent sources of MSCs include bone marrow, adipose tissue, synovial fluid, umbilical cord tissue, peripheral blood, placental tissue and dental pulp. MSCs can be extracted readily and yield more than other stem cells, making them beneficial for cell proliferation, differentiation, and tissue regeneration under severe immunological circumstances. These also have immunomodulatory features as they secrete cytokines and immune receptors, which regulate the microenvironment in the host tissue. MSCs can treat chronic diseases by producing cells of diverse cell lines, immunomodulating, and secreting anti-inflammatory chemicals. Thereby, showing promising results in preclinical studies for various medical conditions. Research continues to explore their potential in regenerative medicine (**Figure 4**).

MSCs have been studied for a wide range of therapeutic applications, including tissue repair, regenerative medicine, and cell-based therapies for various medical conditions. Some of the medical conditions that MSCs have been studied for include:

1. Myocardial infarct ion: MSCs have been studied for their potential to promote heart tissue repair following a heart attack [12].

2. Spinal cord injury: MSCs have been investigated for their potential to promote the repair of damaged nerve tissue and spinal cord injury [13].

3. Autoimmune diseases: MSCs have been demonstrated to have anti-inflammatory and immunomodulatory properties, which may make them helpful in treating autoimmune diseases, such as multiple sclerosis and lupus [14].

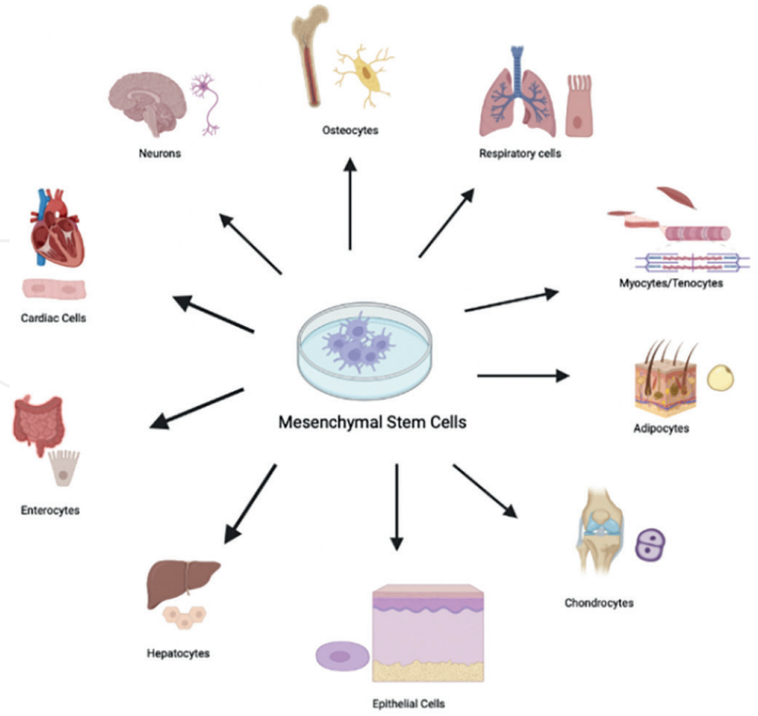


Figure 4.
Regenerating abilities of mesenchymal stem cells. Image source: Biorender.com.

4. Type 1 diabetes: MSCs have been examined for their potential to help preserve insulin-producing cells in type 1 diabetes patients

5. Lung diseases: MSCs have been reviewed for their potential to help repair lung tissue in conditions such as chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome (ARDS).

6. Multiple Sclerosis (MS): MSCs have been shown to have anti-inflammatory and immunomodulatory properties, which may make them helpful in treating MS. This autoimmune disorder affects the central nervous system.

7. Lyme disease: MSCs have been analyzed for their potential to help repair tissue damage and reduce inflammation caused by Lyme disease, a bacterial infection transmitted by ticks.

8. Parkinson's disease: MSCs have been examined for their potential to help protect and repair nerve cells in the brain that are damaged in Parkinson's disease, a degenerative disorder that affects movement.

9. ALS (Amyotrophic lateral sclerosis): MSCs have been investigated for their potential to help protect and repair nerve cells in the spinal cord damaged in ALS, a progressive neurodegenerative disease that affects nerve cells in the brain and spinal cord [15].

10. Rheumatoid arthritis: MSC-based therapies via administration of exogenous MSCs or targeting of the endogenous MSCs in the joint are strategies that are being pursued to trigger/enhance repair of the damaged joint tissues, with the aim to restore joint homeostasis.

11. Osteoarthritis: Intra-articular injection of infrapatellar fat pad-derived mesenchymal stem cells is effective for reducing pain and improving knee function in patients being treated for knee osteoarthritis [16].

Various research on adult stem cells led to their discovery in different dental tissues. Stem cells extracted from dental tissue have been shown to possess similar properties to MSCs derived from other sources. Hence, considering dental stem cells are easily accessible. Currently, there is extensive research focusing on dental stem cells and their clinical applications.

2.5 Dental stem cells

Dental stem cells offer a very promising therapeutic approach to restoring structural defects. To date, eight unique populations of dental tissue-derived MSCs have been isolated and characterized. Postnatal dental pulp stem cells **(DPSCs)** were the first human dental MSCs to be identified from pulp tissue. Gradually, other dental MSC-like populations were also reported (**Figure 5**).

- **Dental Pulp Stem Cells (DPSCs):** The first dental MSCs from the dental pulp tissue of impacted third molars were isolated two decades ago [17]. These adhering cells are fibroblast-like and MSC-like [18]. They are valuable cells in regenerative medicine because of their strong proliferation capacity and multi-lineage differentiation potential [19].

- **Periodontal Ligament Stem Cells (PDLSCs):** PDL is a specialized issue located between the cementum and alveolar bone. Fibers of PDL, attach the tooth to the jaw and plays a vital role in maintaining and supporting the teeth. PDLSCs may be recovered from dental roots or the perivascular space of the periodontium. They possess characteristics of MSCs to self-renew and develop into cementum, PDL, alveolar bone, peripheral nerves and blood vessels. In vitro PDLSCs were able to differentiate into osteogenic, adipogenic and chondrogenic cells [20].

- **Dental Follicle Stem Cells (DFSCs):** The dental follicle surrounds the tooth germ before the eruption [21]. It has progenitor cells of the periodontal ligament,

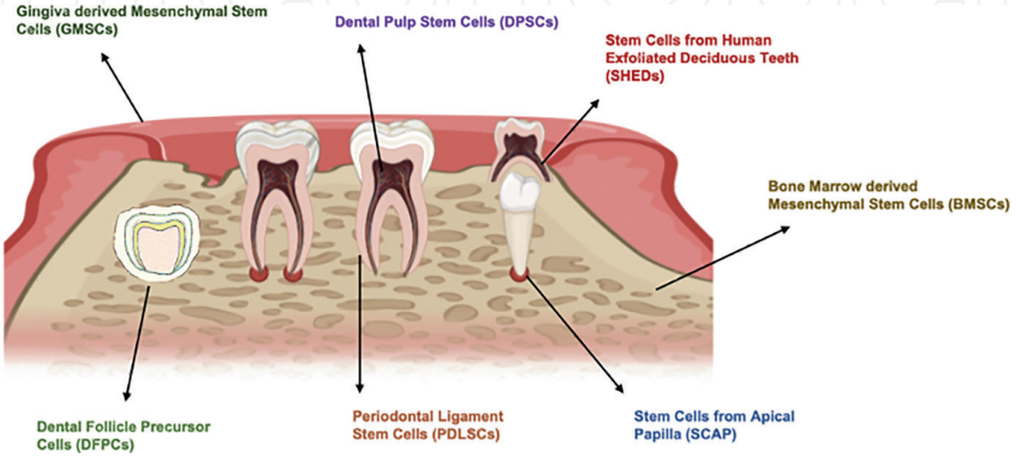


Figure 5.
Sources of dental stem cells. Image source: Biorender.com.

cementoblast, and osteoblast [20]. DFSCs are isolated from 3rd molars and expressed various biomarkers such as Notch 1, STRO-1 and Nestin. They demonstrated multilineage potential to undergo osteogenic, adipogenic, and neurogenic potential in vitro.

- **Alveolar Bone-derived MSCs (ABMSCs):** Alveolar bone is embryonically produced from the dental follicle and resembles a thickened ridge with tooth sockets [21]. ABMSCs show a similar osteogenic differentiation capacity to BMMSCs but lower chondrocyte and adipocyte differentiation [22]. They express surface markers CD73, CD90, CD105, and STRO-1 but do not express hematopoietic markers CD14, CD34 and CD45 [21]. They are used along with bioceramics scaffolds for bone tissue engineering applications.

- **Stem Cells from Apical Papilla (SCAP):** Stem cells from apical papilla (SCAP) from incompletely developed teeth were extracted in 2006 [23]. The apical papilla is loosely attached to the apices of immature permanent teeth and has fewer cells and vessels than pulp tissue [24]. These stem cells have higher proliferative potential than PDLSCs and DPSCs [25], self-renewal ability, and low immunogenicity. After implantation of SCAP into immunocompromised mice, in a carrier matrix, due to the presence of odontoblast-like cells typical dentin pulp-like structure was formed. In regenerative dentistry, SCAPs can generate osteogenic, odontogenic, neurogenic, adipogenic, and chondrogenic cells [26].

- **Tooth Germ Progenitor Cells (TGPCs):** TGSCs are found in the dental mesenchyme of the third molar tooth germ at the late bell stage [21]. TGSCs have similar multilineage differentiation capacity to other dental MSCs like differentiating into osteoblast/ odontoblast, chondrocytes, and neurons. TGPCs can differentiate into cells with morphological, phenotypic and functional characteristics of hepatocytes in vitro suggesting that TGPCs can be used to treat liver diseases [27, 28].

- **Gingival MSCs (GMSCs):** Gingival-derived mesenchymal stem cells (GMSCs) originated in the spinous layer of the human gingiva. They are multipotent, self-renewing, and immunomodulatory [29]. Regenerative dentistry uses gingiva stem cells because it is easily accessible during dental operations [20].

- **Stem Cells from Human Exfoliated Deciduous teeth (SHED):** SHED is a unique source of stem cells as deciduous teeth are usually discarded as biological waste, they can be readily accessible without any invasive procedures. Over the last decade, SHED have been identified to be highly proliferative, clonogenic cells capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts [30].

3. Why there is such an interest in stem cells from exfoliated deciduous teeth?

Pulp from naturally exfoliated deciduous teeth may be like an umbilical cord providing a rich and distinctive source of stem cells showing a multipotent nature. SHEDs exhibit a much higher proliferation rate, faster population doublings and greater osteoinductive capacity than DPSCs, adult MSCs and PDLSCs. They can differentiate into a variety of cell types including odontoblasts, osteoblasts, adipocytes, chondrocytes,

neural cells, hepatocytes, endothelial cells, β-cells, and dentin and pulp-like tissues. SHEDs express the same cell markers as ESCs such as OCT 4 and NONOG, which makes them have a significant impact on clinical applications. Evidence indicates that functional recovery and remodeling in lesions not only rely on their multipotency but also on their protective and anti-inflammatory action by the paracrine mechanism of grafted SHEDs. In this context, SHEDs have shown to function as an immunomodulator by suppressing T helper 17 cell functions. Transforming growth factors TGF-β1 and β2, fibroblast growth factor FGF-2, and Col I and III are highly expressed in SHED as compared to DPSCs.

The primary difference in the pulp of primary and permanent teeth is the occurrence of physiologic root resorption of the deciduous teeth. The transition from deciduous teeth to permanent teeth is a unique and dynamic process wherein the resorption of the deciduous teeth is coordinated with the development and eruption of permanent teeth. Deciduous teeth without any visible root resorption were unable to proliferate in vitro, whereas those in an advanced state of root resorption showed good proliferation and differentiation potential [31]. Due to its unique stemness of capability of multi-differentiation, self-renewal, developing into other cell lineages and easy accessibility, without major morbidity to host and minimal ethical concern, SHED has been widely investigated in the field of regenerative medicine and tissue engineering (**Figure 6**).

4. Applications of SHED in research

Owing to its multipotent, no/reduced ethical conflicts and minimally invasive to obtain has opened a wide area for research. Research spans across categories—Dental materials, wound healing, dental tissue engineering, treatment of chronic diseases like diabetes, and Wilson's disease, treatment of autoimmune diseases like SLE, and encephalomyelitis, adjunct to surgical treatment e.g., cleft lip and palate, pediatric surgeries like biliary atresia.

4.1 Cell culture studies

- The proliferative, osteogenic, and immunomodulatory potentials of MSCs isolated from the dental pulp of SHED and fragments of the orbicularis oris muscle (OOMDSCs) treated with an inflammatory IFN- γ stimulus were evaluated, and it was determined that SHED and OOMDSCs lack immunogenicity and have immunomodulatory properties that are enhanced by inflammatory stimulation with IFN- γ. This opens new perspectives for the therapeutic use of these cells [32].

- Response of stem cells from human exfoliated deciduous teeth (SHED) to three bioinductive materials conducted in vitro determined functional differentiation

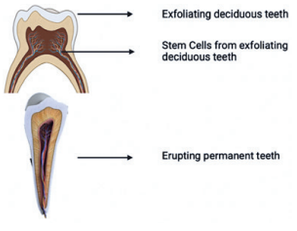


Figure 6.
Exfoliating deciduous teeth with root resorption possess SHED. Image source: Biorender.com.

potential (osteogenic/odontogenic) of various biomaterials on SHED and concluded that all the tested materials are bioinductive to SHED. Enamel Matrix Derivative (EMD) can be used in dentistry for various vital pulp therapies as that of Biodentine and Mineral Trioxide Aggregate (MTA) with predictable as well as enhanced success rates [33].

- Biological interactions of a calcium silicate-based cement (Biodentine™) with SHED were studied. SHED attached effectively to the crystalline surface of Biodentine specimens, exhibiting a spindle-shaped phenotype. Different concentration and time-dependent expression patterns of odontogenic genes were observed under non-inductive and inductive (osteogenic) conditions, with significant upregulation of DSPP and Runx2, BMP-2, BGLAP, and MSX. A gradual increase in the development of mineralized tissue was observed [34].

- Cytotoxicity and bioactivity of various pulpotomy materials on stem cells from human exfoliated primary teeth were investigated and SHEDs showed significant cell viability in the presence of Biodentine when compared to other materials. In addition, SHEDs maintained their mesenchymal phenotype in all conditions although their capacity to migrate was higher in the presence of Biodentine. Cytotoxicity and bioactivity of various pulpotomy materials on stem cells from human exfoliated primary teeth [35].

- The effects of MTA, Biodentine™, and calcium hydroxide on the viability, proliferation, migration, and differentiation of stem cells from human exfoliated deciduous teeth were investigated. The results demonstrated that the three capping materials are biocompatible, maintain viability, and stimulate proliferation, migration, and differentiation in a key dental stem cell population [36].

4.2 Animal studies

- SHED investigated for wound healing effect on mouse model revealed enhanced wound healing promotion [37].

- SHED conditioned medium Ameliorated Experimental Autoimmune Encephalomyelitis in a mouse model of multiple sclerosis [38].

- SHED teeth reduce tissue-infiltrating inflammatory cells, improving clinical signs in experimental autoimmune encephalomyelitis. SHED infusion improved EAE clinical score by reducing the number of tissue-infiltrating IFN-γ + CD8+, IL-4 + CD8+, IFN-γ + CD4+, and IL-4 + CD4+ T cells in the central nervous system (CNS). In addition, SHED can modify CD4+ T cell responses in the periphery, indicating that SHED may be investigated as a component of cellular treatment for autoimmune illnesses related to the CNS [39].

- SHED transplantation into the caudal vein of Diabetic Goto-Kakizaki (GK) rats revealed improved nerve function, thereby alleviating persistent neuropathic pain (mechanical hyperalgesia). SHED transplantation can prevent the development of DPN by participating in tissue regeneration, increasing local blood flow, and conferring neurotrophic protection [40].

- SHED and SHED-converted hepatocyte-like- cells (SHED-Heps) were transplanted into Wilson's disease model Atp7b-mutated Long-Evans Cinnamon (LEC) rats, reduced copper-induced oxidative stress via ATP7B- independent stanniocalcin 1 secretion, suggesting a possible role for paracrine effect. Therefore, SHED-Heps can be a novel effective source to rescue and prevent Wilson's Disease with fulminant hepatic failure by restoring the deficient ATB7B function and diminishing copper-induced oxidative stress [41].

- Intravenous infusions of SHED can effectively alleviate the autistic-like symptoms of impaired social novelty preference (SNP) and obvious social stress in SHANK3 mutant beagle dogs accompanied by an increase in the level of serum IL-10 and a decrease in the level of IFN-γ [42].

- SHED transplanted in carbon tetrachloride (CCl4)-induced liver fibrosis model mice directly transformed into hepatocytes without cell fusion and improved hepatic dysfunction [43].

- The application of implants pre-adhered with SHEDs improved and accelerated early osseointegration around the implant with an improved total bone-to-implant contact (BIC%) and interthread bone, resulting in thicker and denser trabecular bone in adult beagle dogs [44].

5. Therapeutical applications of SHED

It has been found that SHEDs showed alleviating effects on nervous system diseases, including Spinal cord injury, Parkinson's disease, Trigeminal neuralgia, Cerebral ischemia, Alzheimer's disease, and Encephalomyelitis. Owing to the capacity to interact with the local inflammatory microenvironment, SHEDs have also embraced remarkable modulatory effects in various autoimmune and inflammatory diseases such as rheumatoid arthritis, diabetes, acute kidney injury, liver fibrosis/acute liver failure osteoarthritic, heatstroke, and acute respiratory distress syndrome (ARDS) could also benefit from SHEDs for the protective effects underlying immunomodulatory activities.

5.1 Evidence supporting SHEDs potential in autoimmune and nervous diseases via paracrine and immunomodulatory

Effects of SHED on Parkinson's disease: Transplantation of neural-like spheres derived from SHEDs into the striatum of parkinsonian rats significantly improved the behavioral disorders, the number of TH-positive (tyrosine hydroxylase) cells and the protective effect on endogenous dopaminergic neurons, indicating SHED spheres were of potential therapeutic value [45].

Effects of SHED on Acute liver failure: Intravenous administration of SHED-CM improved the condition of the injured liver and the animals' survival rate by induction of anti-inflammatory M2-like hepatic macrophages [46].

Effects of SHED on Heatstroke: Intravenous administration of SHED exhibited therapeutic benefits for heatstroke in mice, related to decreased inflammatory response, decreased oxidative stress, and increased hypothalamic pituitary adrenocortical axis activity.

Effects of SHEDs in the treatment of retinal degeneration: It has been confirmed through paracrine secreta that SHEDs exert neurotrophic, angiogenic, immunoregulatory, and antiapoptotic functions in injured tissues. SHEDs and SHED-CM showed therapeutic effects on Retinitis pigmentosa (RP) by improving retinal visual function and delaying the degeneration of photoreceptors by antiapoptotic activity. Therefore, SHEDs may be a promising stem cell source for treating retinal degeneration [47].

5.2 Other therapeutic effects of SHED

- Hair Regeneration: There is increasing evidence that mesenchymal-epithelial interactions in early morphogenesis stages of both tooth and hair follicles show many similarities. In vitro, SHED shortened the hair regeneration cycle and promoted the proliferation and aggregation of dermal cells. When epidermal and dermal cells were freshly extracted and co-cultured with SHED, several signaling molecules in hair follicle regeneration were detected and it was found that the expression of Sonic Hedgehog (Shh) and Glioma-associated oncogene 1 (Gli1) was up-regulated. It seems that SHED may boost the prosperity of hairs by increasing Shh/Gli1 pathway [48].

- Kidney Injury: SHED therapy is useful for ischemic kidney damage. *In vitro* studies showed that SHED significantly could reduce MCP-1 secretion in tubular epithelial cells caused by H_2O_2 and hence could be utilized for acute kidney injury [49].

- On wound healing and wound itching: SHED along with basic fibroblast growth factor (b-FGF) in a nude mouse full-thickness skin defect model significantly accelerated wound healing. SHED-derived exosomes were investigated for their contribution to immune response and wound itching during healing. The effects of SHED-derived exosomes on inflammatory wound healing were examined using lipopolysaccharide (LPS)-induced wounds in a mouse model. SHED-derived exosomes facilitated LPS-induced wound closure and relieved wound itching. SHED-derived exosomes containing miR-1246 also enhanced autophagy by regulating macrophage function through the AKT, ERK1/2, and STAT3 signaling pathways. Therefore, SHED-derived exosomes promote wound healing with less itching in an LPS-induced wound model by stimulating macrophage autophagy, which has implications for the treatment of inflammatory wound healing [50].

- On Photo aging: SHED were investigated for its effect on wrinkles caused by UV-B photodamage, SHED or SHED-conditioned medium injected subcutaneously reduced the wrinkles compared with the control group. In addition, SHED had effects on human dermal fibroblasts (HDFs) by increasing collagen synthesis and by activating the proliferation and migration activity of HDFs, suggesting that SHED or SHED conditioned medium can be used for the treatment of photoaging [37].

- On Type II Diabetes Mellitus: SHED administration reduced Glycosylated serum albumin and hemoglobin level significantly. Further research revealed there was a low reply to SHED administration in hypercholesterolemia and low C-peptide. As serum lipid level and baseline islet activity are major factors for treatment in Type 2 DM patients, it can be concluded that SHED administration is an assured and efficient treatment for islet activity and glucose metabolism recovery in T2DM patients [51].

- On Liver Impairment: Liver transplantation is an end treatment for incurable liver involvement. Stem cells are important as a suitable cell source for liver renewal. SHED administration significantly improved liver disorders and caused anti-fibrotic and anti-inflammatory influences. SHED could directly transform into hepatocytes and be suitable for liver renewal. SHED administration and bio three-dimensional printers that can produce scaffold-free three-dimensional images of the liver and diaphragm are innovative regenerative medicine treatments for uncontrolled pediatric surgery, such as biliary atresia and diaphragmatic hernia [43].

- On nerve impairment: Numerous common therapeutic applications of SHEDs have been identified, although their emphasis has been on neuroprotection rather than neuroregeneration. SHEDs and their medium may impact neural disorders by multiple mechanisms, including cell replacement, paracrine effects, angiogenesis, synaptogenesis, immunomodulation, and apoptosis inhibition. SHED-exos is a suitable regeneration agent for neuronal disorders [52]. SHED transplantation can inhibit peripheral c-Jun in the trigeminal ganglia (TG) and have analgesic effects in trigeminal neuralgia. The phosphorylation of c-Jun improved hyperalgesia and allodynia, suggesting that SHED transplantation could improve trigeminal neuralgia [53]. SHED-conditioned media promoted the regeneration of sciatic nerve defects in rats. SHED-CM promoted axon development, peripheral nerve tissue angiogenesis, SC migration, proliferation, and activation, and neuron survival. Therefore, SHED-CM promotes peripheral nerve regeneration through multiple processes, resulting in functional recovery, and maybe a promising strategy for the clinical treatment of Peripheral Nerve Injury [54].

- On Brain Injury: SHED-Exos could decrease neuroinflammation by replacing microglia M1/M2 polarization in animal models. SHEDs based therapies emerge as a potential therapy option for neurodegenerative disorders because of their homing, engraft, differentiate and generating factors for CNS improvement [55].

- On Bone formation: Transplanted SHED with hydroxyapatite/tricalcium phosphate into calvarial defect restored the parietal continuity dynamically contributing to bone formation [56].

- On Systemic Lupus Erythematosus: SHED administration could reverse SLE-associated defects in MRL/lpr mouse. SHED had significant effects on inhibiting T helper 17 (Th17) cells *in vitro.* At the cellular level, SHED transplantation elevated the ratio of regulatory T cells (Tregs) via Th17 cells. It can be concluded that SHED is an accessible and feasible mesenchymal stem cell source for treating immune disorders like SLE [57].

6. Scope of tissue engineering in dentistry using SHED

Recent advances in stem cell research especially in the field of dentistry have led to the onset of an entirely new era in which even an entire tooth can be regrown. This is just one of the several approaches that hold promise for tooth regeneration.

As far as pediatric dentistry is concerned, decay is one of the most common problems faced. Most often a pedodontist ends up performing a pulpectomy procedure

which involves the complete removal of the pulpal tissue and filling it with an ideal obturating material. Now, with technological evolution, researchers are using stem cell therapy for regenerative pulpotomies which can restore the vital pulp, which bypasses the need of going through painful invasive dental procedures. Following are some scope and potential applications of SHED in regenerative dentistry.

6.1 Dentin Pulp complex regeneration (DPC)

The DPC consists of the outer hard tissue layer, which is composed of orientated cells (odontoblasts) that secrete a specific matrix to form new dentin, and the inner soft tissue layer, which is composed of vital pulp tissue that comprises a network of microvasculature, nerves, and fibrous elements. Therefore, regeneration of the Dentin Pulp Complex entails a cascade of events involving odontogenesis and angiogenesis. SHEDs have a greater capacity for the formation of Dentin Pulp Complex cells, including osteoblasts, chondroblasts, adipocytes, endothelial cells, nerve cells, and odontoblasts [58–60]. SHEDs have demonstrated the ability to develop into functional odontoblasts and endothelial-like cells [61]. SHED's capacity to develop into odontoblasts is defined by the expression of dentin matrix protein-1 (DMP-1) and Dentin Sialophosphoprotein (DSPP) [60, 62]. The goal of DMP-1 is to maintain dentin mineralization, DSPP stimulates odontoblast development in stem cells by phosphorylation of SMAD 1/5/8 and nuclear translocation via the P38 and ERK1/2 pathways [63, 64]. Regenerating the missing interface between two distinct tissues (dentin and pulp), as seen in the Dentin Pulp Complex, is one of the main challenges of regenerative dentistry. It is crucial to provide a perfect environment that encourages the aggregation, proliferation, and differentiation of these disparate tissues. The ultimate regeneration of the dentin-pulp complex requires successful innervation and revascularization. In this context, various scaffolds have been employed to support cell growth and functionality in the transplanted area. Tissue engineering methods involving SHED, growth factors and scaffolds have been researched to regenerate DPC. SHED has shown the ability to regenerate pulp- and dentin-like tissues utilizing scaffolds and stem cells in animal models. SHEDs are also able to increase the angiogenesis process by forming vascular connective tissue structures and expressing and synthesizing VEGF. This ability is crucial in maintaining pulp viability as it can supply oxygen and nutrients needed for cell metabolism for tissue regeneration. Also, Exosomes extracted from SHED aggregates (SA-Exo) showed to significantly improve pulp tissue regeneration and angiogenesis in vivo, it also promoted endothelial differentiation and enhanced the angiogenic ability of HUVECs [65, 66].

6.2 Dentin Pulp regeneration

Dentin Pulp Regeneration aims to revitalize necrotic, infected, or lost pulp teeth by restoring the morphology and function of the pulp. Ideal pulp regeneration should possess natural structures such as nerve, fibers and blood supply, allowing nutritional, defense, sensation, and immunological functions to be restored. SHED have been utilized for pulp revascularization in regenerative endodontic procedures over the years. The conventional endodontic treatment in an immature tooth with pulpal necrosis is often challenging owing to its open root apex and thin root canal dentin. In addition, there is a risk of obturating material overflowing into the periapex. Regenerative endodontics (pulp revascularization) in an immature tooth aims to

promote continued root development by generating new tissues. SHED seeded onto the synthetic scaffolds formed well-vascularized pulp-like tissue in vivo on a tooth slice model [67].

To restore the vitality of a tooth, elements with regeneration properties in the pulp are required. SHED not only produced mesenchymal stem cell-specific markers, but it also caused odontoblastic differentiation and increased the formation of endothelium and fibroblasts. The regenerated pulp tissue built by SHED had similar cellularity and architecture of the physiological dental pulp [68]. The combination of SHED, Platelet Rich Fibrin, and Chitosan enhanced the migration, proliferation, and odontoblastic differentiation of dental pulp cells. Therefore, the combination of SHED, PRF, and Chitosan scaffold as a new method for pulp regeneration in a clinical environment appears promising as 3D tissue engineering. SHED were implanted in empty root canals of mini pigs to determine whether a full-length dental pulp is regenerated. After 3 months of implantation, the histological analysis showed that full-length dental pulp tissue was regenerated which contained the odontoblast layer and blood vessels. The regenerated pulp showed a similar tissue structure to the normal pulp. Furthermore, blood vessels and nerves were regenerated as confirmed by positive expressions of CD31 and neurofilament (NF) in regenerated pulp tissues. In addition, positive expressions of CGRP and TRPV1 cells indicated the regenerated pulp might have sensory nerves. These results indicated that implantation of SHED was capable of regenerating full-length dental pulp with blood vessels and nerves in a large animal model [69].

6.3 Bio root regeneration

Techniques based on cell-based tissue engineering have made significant advances in the field of tooth regeneration. However, regeneration of the complete tooth has proven to be laborious; consequently, tooth root regeneration is advocated as a more practical and promising alternative for tooth restoration than the regeneration of the entire tooth. The tooth root serves a vital role in chewing and maintaining the tooth's stability, which is the structural foundation of a functional tooth. Bio-engineered tooth root (bio root) mediated by stem cells has shown to be a promising treatment for tooth loss. Multiple studies have demonstrated that Dental Follicle cells are appropriate seeding cells for the development of bioroots. SHEDs can be regarded as a prospective seeding cell for use in bio root regeneration in the future [70]. The comparison of ultrastructure revealed that SHEDs participate in active cell metabolism and the autophagy process, which are essential for stem cell immunological defense, self-renewal, and apoptosis [71, 72]. In addition, they possessed protein synthesis and secretion capabilities that were superior to those of dental follicle cells, resulting in the establishment of a microenvironment that is favorable to tissue repair and regeneration, a breakthrough in the field of nerve regeneration [54, 73, 74].

6.4 Periodontal regeneration

SHEDs treated with dentin matrix can regenerate periodontal tissue composed of periodontal ligament fibers, blood vessels, and new alveolar bone [70]. Due to their high proliferative capacity, the strong immunosuppressive ability of multiple differentiation, and minimal carcinogenic potential, exfoliated human deciduous dental stem cells have been employed to restore periodontal tissue and repair alveolar bone abnormalities [75].

6.5 Reconstruction of Cleft lip/palate (CL/P)

Autogenous iliac bone grafting has been demonstrated to heal alveolar cleft defects; however, surgical intervention is required. Hence, the creation of a less invasive technique is anticipated. Consequently, Alveolar bone regeneration methods in patients with CL/P employing human bone marrow mesenchymal stem cells (hBMSCs) have been attempted, and the transplantation of hBMSC in a canine alveolar cleft model has demonstrated the ability to regenerate bone [76]. SHEDs have higher osteogenic potential compared to bone marrow stem cells [77]. SHEDs, human dental pulp stem cells (hDPSCs), and hBMSCs were utilized to induce bone regeneration in immunodeficient animals with calvarial bone abnormalities. However, animals treated with SHED scaffolds had the greatest amounts of osteoid and the widest distribution of collagen fibers. During cell culture, MSCs can secrete paracrine substances into a conditioned medium (CM). MSC-CM contain the growth factors insulin-like growth factor-1 (IGF-1), transforming growth factor 1 (TGF-1), and vascular endothelial growth factor (VEGF), which influence the features and behavior of regenerating bone cells [78–80]. It is possible that both transplanted MSCs and their paracrine actions contribute to tissue regeneration. SHED-CM demonstrated mature bone development and contained tissue-regenerating factors with functions in angiogenesis and osteogenesis. Deciduous dental pulp stem cells (DDPSC) associated with a hydroxyapatite-collagen sponge showed closure of alveolar defects during the secondary dental eruption in a clinical setting. Thus, SHED could be an ideal source of cells for alveolar cleft reconstruction due to its capacity to regenerate bone with minimal surgical invasion [81].

6.6 Temporomandibular Joint Osteoarthritis (TMJOA)

Exosomes secreted by SHEDs (SHED-Exos) demonstrated to suppress inflammation in TMJ chondrocytes. The anti-inflammatory effects of SHED-Exos were verified using western blotting and RT-qPCR. SHED-Exos down-regulated the expression of IL-6, IL-8, MMP1, MMP3, MMP9, MMP13, and dis-integrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5). Thus, they can be a novel therapeutic agent for TMJ inflammation [82].

7. Limitations / challenges of stem cell research

Even though stem cell offers a wide range of therapeutic potentials in regenerative medicine, one cannot deny the fact that it does possess limitations and challenges because of different ethical and other issues related to stem cell research. Some of them are listed below:

1. The most crucial challenge to stem cell research is the ethical issue related to the use of embryonic stem cells. Due to these issues, there are even political and religious obstructions to stem cell research. Stem cell research has come under some controversy due to the ethical problems associated with how stem cells are obtained. It is known that while obtaining the stem cells from an embryo, the embryo, in the end, is discarded, which raises ethical issues. However, due to the discovery and use of adult stem cells and induced pluripotent stem cells, the use of ESCs has decreased and so have the ethical issues.

2. The source of some stem cell lines might have mutations which increase the chances of mutations in the transplants.

3. It is also difficult to transplant the stem cells produced in the laboratories to the target cells.

4. ESCs also do not permanently renew themselves in vivo, but instead, differentiate soon into different lineage progenitor cells of the three embryonic germ layers.

5. Self-renewal of these cells can be achieved in vitro under artificial conditions, which inhibit their differentiation.

6. It is also challenging to obtain enough stem cells with the ability to differentiate into the desired cell type.

7. The differentiation of embryonic, as well as adult stem cells, even if guided by the addition of differentiation factors, inevitably involves a certain amount of spontaneous differentiation into various cell types

8. Additionally, the differentiation is not synchronizable yet, leading to a mixture of cells in various stages of development.

However, the advent of stem cells derived from less invasive tissues without immune rejection has widened the opportunities for cell-based therapies.

8. Then, why not discard primary teeth after they SHED?

According to the data presented above, SHED have regenerative abilities comparable to umbilical cord stem cells, implying that they have a high potential for treating some life-threatening diseases. Exfoliated teeth are also an unexpectedly unique resource for stem-cell therapies such as autologous stem-cell transplantation and tissue engineering due to their ease of access and lack of ethical concerns. Banking SHED cells is thus extremely beneficial (**Figure 7**).

9. SHED Banking and its advantages

Storing your child's own teeth stem cells could give them access to a huge range of treatment opportunities throughout their lifetime

- It provides a guaranteed matching donor (autologous transplant) for life. There are many advantages of autologous transplant including no immune reaction and

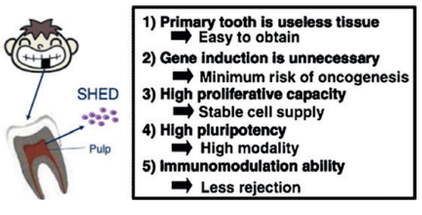


Figure 7.
SHED and its importance.

tissue rejection of the cells, no immunosuppressive therapy needed, and significantly reduced risk of communicable diseases [83, 84].

- Saves cells before natural damage occurs.
  - Simple and painless for both child and parent.
  - Less than one-third of the cost of cord blood storage.
  - SHED are adult stem cells and are not the subject of the same ethical concerns as embryonic stem cells [83, 84].
  - SHED cells complement stem cells derived from cord blood. Even though cord blood stem cells have proven useful in the regeneration of blood cell types, SHED is capable of regenerating solid tissue types that cord blood cannot, such as potentially rebuilding connective tissues, dental tissue, neural tissue, and bone [85–88].

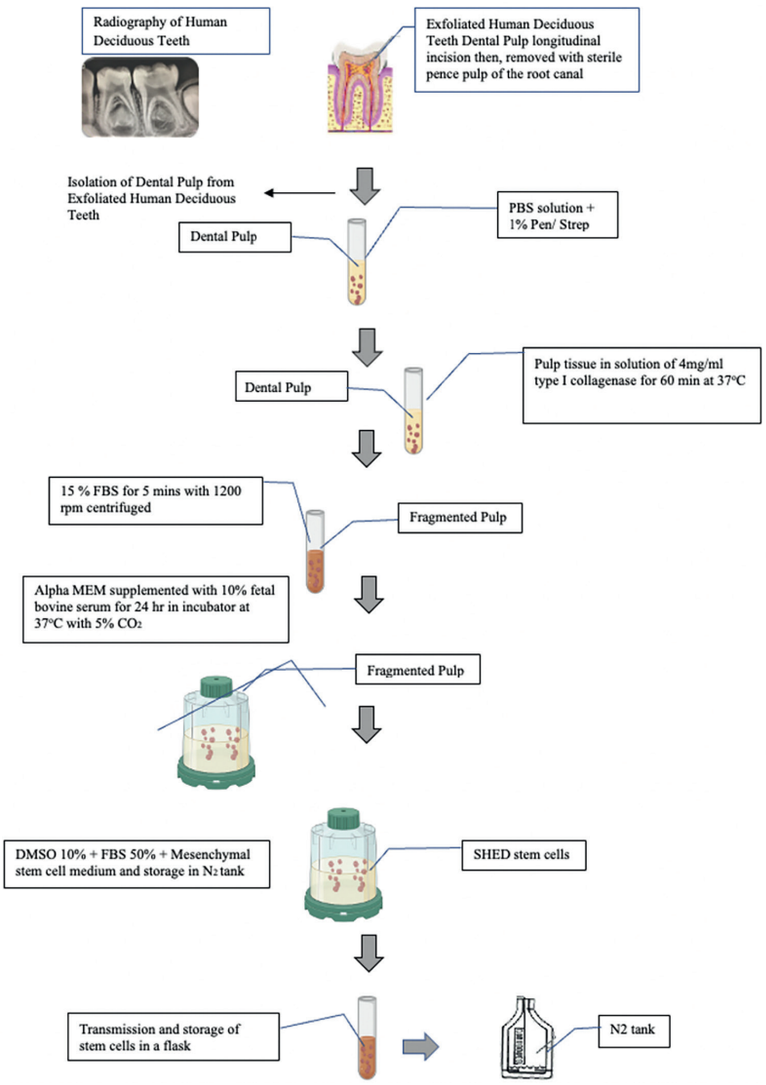


Figure 8.
Isolation protocol of SHED. Image source: Biorender.com.

- SHED may also be beneficial for the donor's immediate relatives, such as grandparents, parents, uncles, and siblings [84].

Certain tooth selection criteria must be followed before SHED banking, which is crucial for the successful isolation and characterization of cell lines to maintain viability.

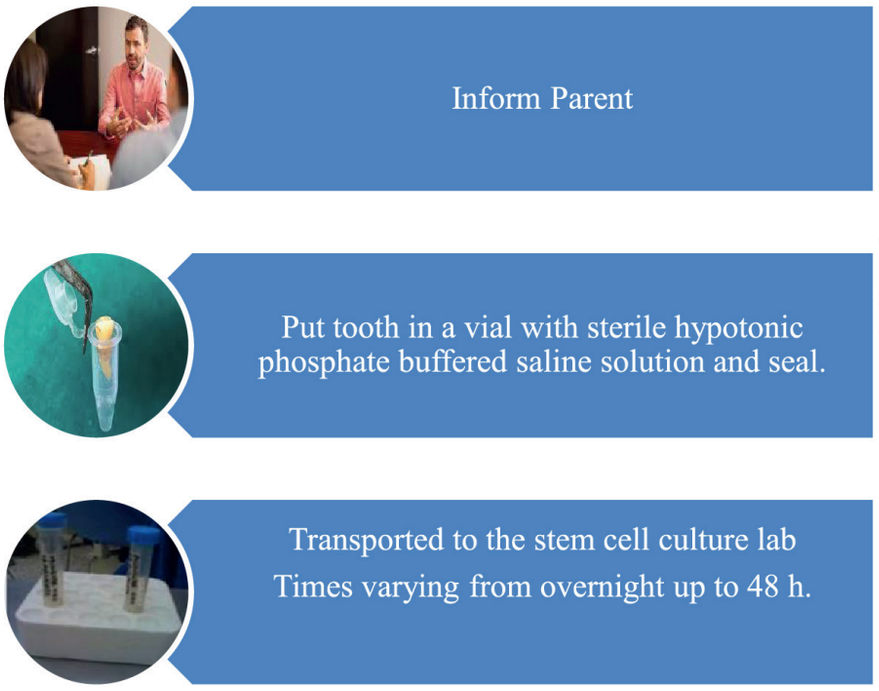
1. A tooth that is extracted for orthodontic purposes. (Extraction to relieve crowding and allow permanent teeth eruption)
2. Tooth extracted/ exfoliated should be vital and devoid of any pulpal necrosis (indication of cell viability)
3. Tooth extracted/exfoliated should be free of any mobility either due to trauma/ pulpal pathology
4. The tooth extracted/exfoliated should be free of any periapical infection (**Figure 8**).

10. Procedure

We have outlined the detailed process involved in SHED banking for its application in stem cell therapy.

10.1 Collection, isolation, and preservation of SHED

1. Tooth collection



1. Stem cell isolation: Step by step of Stem cell Isolation is explained below.
2. Stem cell verification and viability testing

Figure 9.
Stem cell verification and validation.

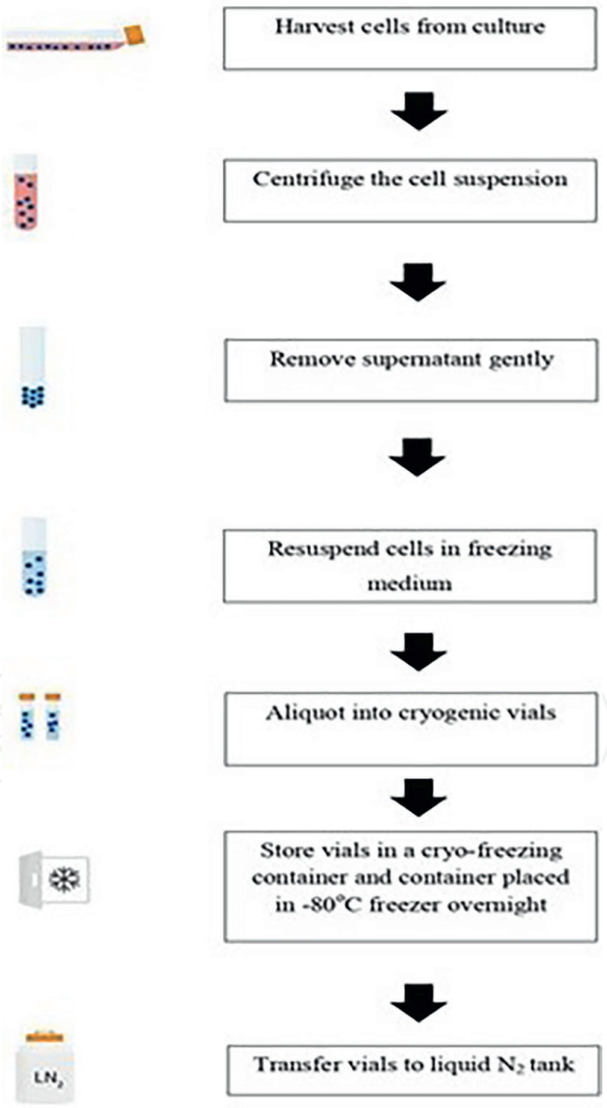


Figure 10.
Typical step-by-step cryopreservation protocol. Image source: Cryopreservation Basics: Protocols and Best Practices for Freezing Cells. Stem cell technologies.

The viability of the isolated cells is then tested (**Figure 9**).

3. Stem Cell Storage

Stem cell storage refers to the collection and cryopreservation of stem cells from source tissue for use in stem cell treatments or clinical trials in the future. Methods used for Stem cell storage are cryopreservation or magnetic freezing [89].

10.2 Cryopreservation

This routine procedure generally involves slow cooling in the presence of a cryoprotectant to avoid the damaging effects of intracellular ice formation (**Figure 10**).

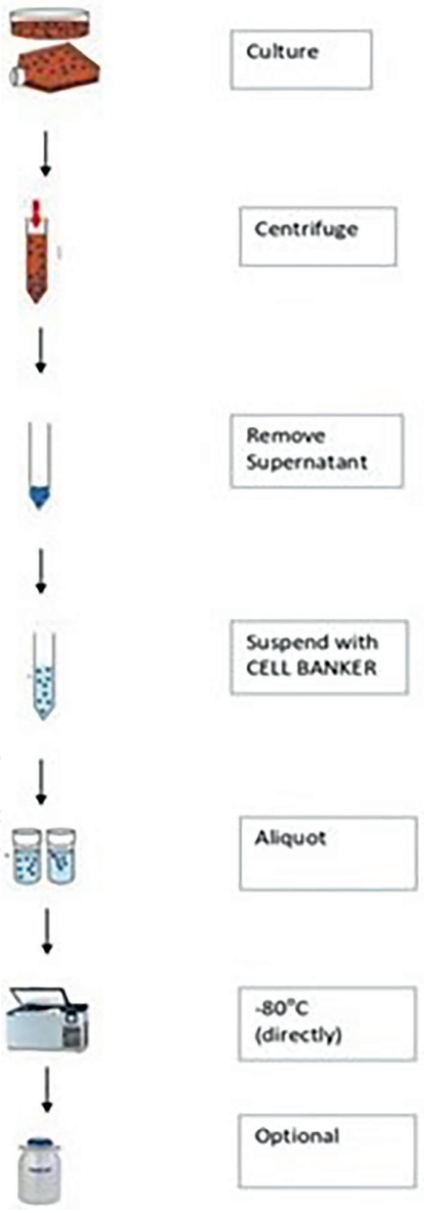


Figure 11.
Magnetic freezing step by step protocol.

10.3 Magnetic freezing

These above steps are followed by Stem cell differentiation, characterization, and validation of required cell types prior to their application in cell-based therapies (**Figure 11**).

11. Conclusion

Stem cell therapy has made significant advances in regenerative medicine and dentistry. It has simplified the treatment of many diseases that were previously difficult to treat. Cord blood stem cells are used to treat over 85 different blood and immune diseases, including Leukemia and Neuroblastoma. Considering the scope of stem cells in regenerative medicine, it is imperative to opt a source of stem cell which is less invasive and has promising future in regenerative therapies without immune rejection. SHED has been shown to be as distinct as cord blood stem cells. Multiple studies demonstrate that SHED can differentiate into odontoblasts, neurons, hepatocytes, endothelial cells, β-cells, and other cell types. This vast array of cell types generates an abundance of options for the application of SHED in tissue regeneration processes. SHED has demonstrated a wide range of therapeutic applications due to its ease of harvesting, lack of bioethical concerns, and excellent expansibility. Also, using one's own stem cells (SHED) reduces, if not eliminates, the risk of developing immune reactions or rejection after transplantation, as well as the risk of contracting disease from donor cells. However, Prior to SHED-based therapies becoming a clinical reality, it is necessary to have a deeper understanding of the mechanisms underpinning differentiation processes. Considering the tremendous use of SHED in stem cell therapy, will banking of SHED or the cost of stem cell banking justified and reasonable? The answer is—Why not, if money is not an issue? If a naturally discarded tooth can have such broad therapeutic applications in the future, why not save it? Regardless, the ultimate fate of SHED cell banking will be decided by the patient or parent.

Acknowledgements

We are extremely grateful for the generous contributions of our colleagues and friends to this work. Specially, Dr. B. Mohana Kumar, Associate Professor, Nitte University Centre for Stem Cell Research and Regenerative Medicine (NUCSReM), K. S. Hegde Medical Academy, Nitte (Deemed to be University), and his team for their expertise in stem cell isolation and characterization. Dr. Veena Shetty, PhD, Additional Professor, Department of Microbiology, K. S. Hegde Medical Academy, for providing emotional and scientific support for this work and also, Nitte (Deemed to be University) for providing access to all of the educational material required for this work.

DOI: http://dx.doi.org/10.5772/intechopen.110769

Author details

Meghna Bhandary[1*], Rachaita Chhabra[2], K. Ananya Rao[1] and Mohammed Shahid[3]

1 Department of Pediatric and Preventive Dentistry, AB Shetty Memorial Institute of Dental Sciences, India

2 Pediatric Dental Practitioner, Mumbai, India

3 Department of Oral Pathology and Microbiology, A.J. Institute of Dental Sciences, India

*Address all correspondence to: meghnabhandary92@gmail.com

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