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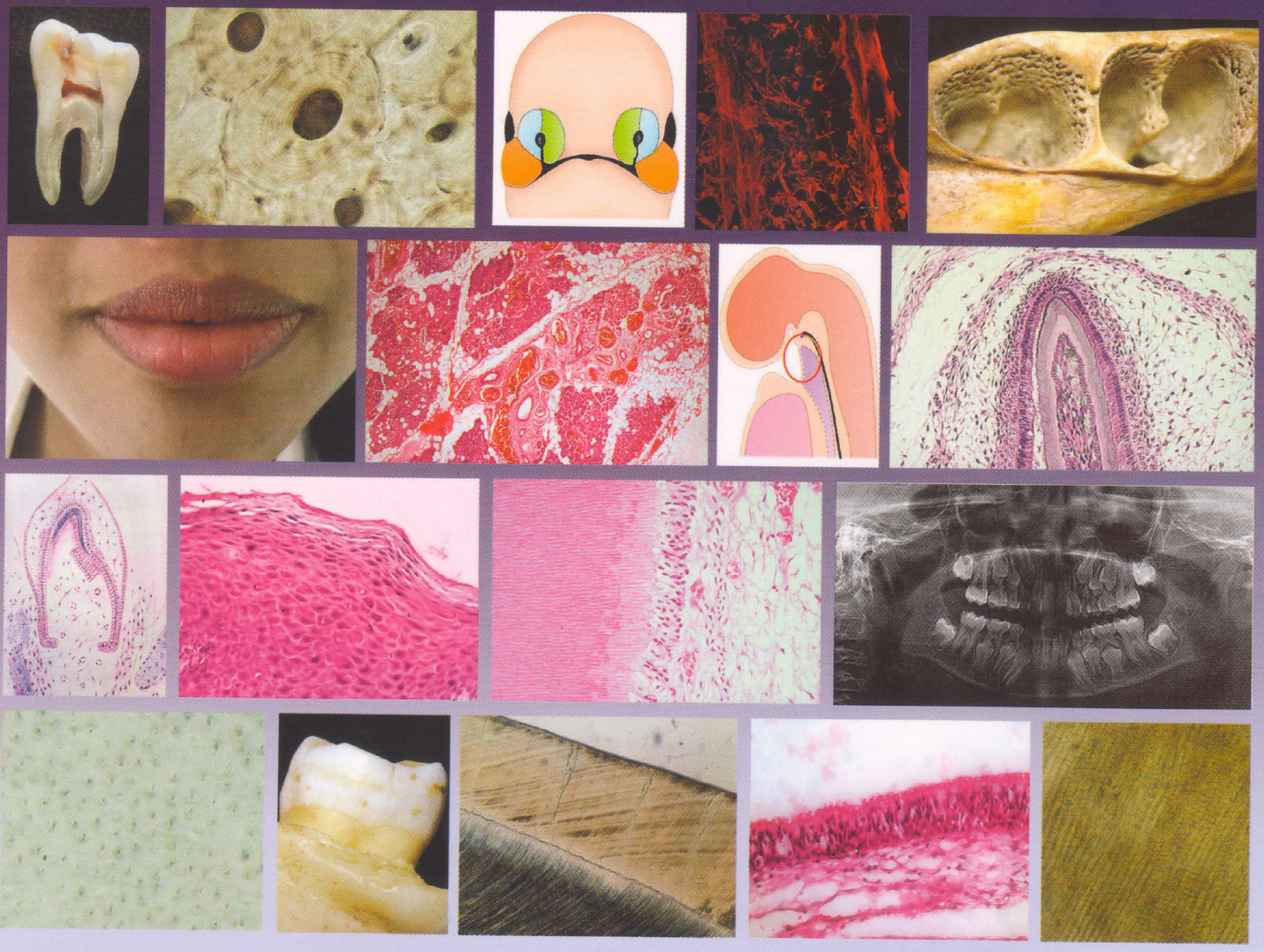
Textbook of Oral Embryology & Histology



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Stem Cells

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CHAPTER OUTLINE

Stem cells are unspecialized cells in the human body that are capable of becoming specialized cells, each with new specialized cell functions. The name "*stem cell*" denotes stemness of multiplication. Basically, a stem cell remains uncommitted until it receives a signal to develop into a specialized cell. Stem cells have the remarkable properties of developing into a variety of cell types in the human body. They serve as a reservoir of repair system by being able to divide without limit to replenish other cells. When stem cells divide, each new cell has the potential to either remain as a stem cell or become another cell type with new special functions.

Classically the stem cell possesses three properties:

1. *Self-renewal:* The ability to go through numerous cycles of cell division while maintaining the undifferentiated state.
2. *Potency:* The capacity to differentiate into specialized cell types. In the strictest sense, stem cells are to be either totipotent or pluripotent—to be able to give rise to any matured cell type. Multipotent or unipotent progenitor cells are sometimes referred to as stem cells.

   There exists a hierarchy in the stem cell compartment, depending on their "potency" or fate restriction. Totipotent stem cells give rise to embryonic as well as the extraembryonic tissue. Pluripotent stem cells can give rise to cells of any of the three germ layers of the embryo, namely—ectoderm, endoderm and mesoderm. Multipotent stem cells can give rise to other types of cells but its ability to differentiate is limited. Adult stem cells are considered to be multipotent, since their their specialization potential is limited to one or more cell lines. Monopotent or unipotent stem cells are tissue-committed stem cells that give rise to cells of one lineage, *e.g.* hematopoietic stem cells, epidermal stem cells, intestinal stem cells, neural stem cells, liver stem cells or skeletal muscle stem cells.
3. *Homing:* Whenever the stem cells are given *in vivo*, they have a tendency to get along with that tissue or organ accordingly.

Another essential property of stem cells is the flexibility in the use of their functional potentials. Further stem cells are characterized by their ability to respond to actual needs of the system. For this, the cells require communication between each other and with their microenvironment. Some of the adult stem cells possess the property of transdifferentiation or plasticity. Transdifferentiation is the ability of the cell to transform from one cell lineage to another completely different cell lineage. However, transdifferentiation is not a unique property of all stem cells; it is found that some cells such as pancreatic cells can be converted to hepatocytes and vice versa.

HISTORY OF STEM CELLS

The history of stem cell research began in the mid-1800s with the discovery that some cells could generate other cells. Now stem cell research is embroiled in a controversy over the use of human embryonic stem cells for research. In the early 1900s, the first real stem cells were discovered when it was found that some cells generate blood cells.

A revolution in medical science happened in the year 1968, with the first successful bone marrow transplant discovered in human umbilical cord blood. Another major breakthrough was derivation of embryonic stem cells (ESCs) from the inner cell mass (ICM) of mouse blastocysts. In 1997, evidence of cancer stem cells (CSC) was presented with leukemia as an example of a stem cell-derived cancer. Human ESC line derived from the ICM of human blastocyst promised great potential for establishing stem cell-based therapies and much effort has since been put into work with ESCs. However, due to ethical considerations and technical difficulties when working with ESCs, other avenues were continuously explored. Hence, in 2006, Japanese researchers Takahashi and Yamanaka published a report of induced pluripotent stem cells (iPSCs) from adult mouse fibroblasts. The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B Gurdon and Shinya Yamanaka "for the discovery that mature cells can be reprogrammed to become pluripotent".

Dental Pulp Stem Cells

The presence of stem cells in dental pulp tissue primarily has been demonstrated in 1985 and these cells have osteogenic and chondrogenic potential *in vitro*, and could also differentiate into dentin, *in vivo*. Dental pulp stem cells isolated from adult human dental pulp, had the ability to regenerate a dentin-pulp-like complex. Stem cells even in inflamed pulp have the capacity to form mineralized matrix both *in vitro* and *in vivo*.

TYPES OF STEM CELLS

On the basis of source or origin, stem cells are typed as embryonic stem cells and adult stem cells.

Embryonic Stem Cells

These cells are pluripotent stem cells that have the potential to differentiate into any cell in the body except the placenta. Pluripotent stem cells have the ability to differentiate into cells of all the three germ layers namely—ectoderm, endoderm and mesoderm. ESCs can be derived from a very early stage in human development, *i.e.* from the ICM of the blastocyst stage embryos.

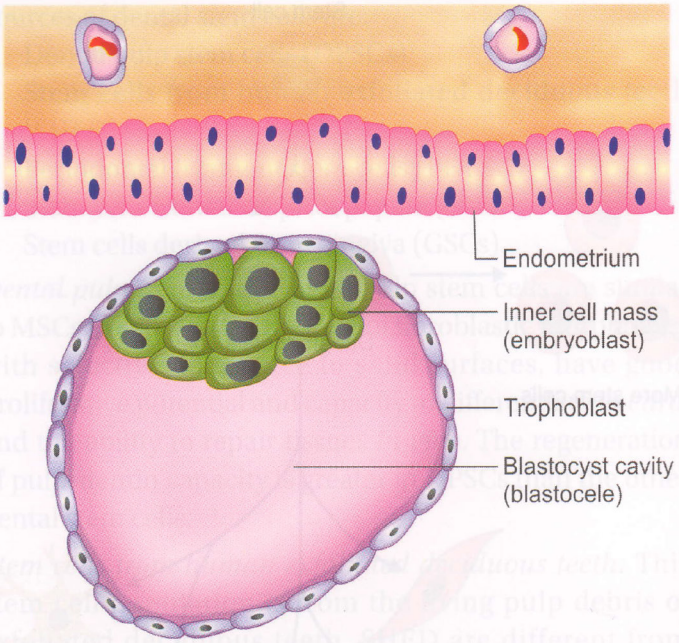


Figure 19-1. Embryonic stem cell.

In the embryo, three kinds of mammalian pluripotent stem cell types have been described—ESCs, embryonic germ cells derived from primordial germ cells, and embryonal carcinoma cells. ESCs are derived from 4-day-old to 5-day-old embryo (blastocyst). Each blastocyst consists of 50–150 cells and includes three structures—an outer layer of cells (the trophoblasts), a fluid-filled cavity (the blastocyst), and a group of about 30 pluripotent cells at one end of the cavity, called the ICM (the embryoblasts) (Figure 19-1).

Adult Stem Cells

Adult stem cells are undifferentiated cells, found throughout the body after embryonic development. These cells play a major role in replacement of dying cells and regeneration of damaged tissues. They are also known as somatic stem cells.

The bone marrow contains two types of stem cells. One population is called hematopoietic stem cell (HSC) that differentiates into all the blood cells. The other population of adult stem cells is called bone marrow stromal cells that differentiate into bone, cartilage, fat, and connective tissue. These bone marrow stromal cells are also called as mesenchymal stem cells (MSCs).

An adult stem cell undergoes replication by mitosis to produce two daughter cells, one daughter cell differentiates into a cell with characteristic morphology and specialized function and the other cell retains the property of mother cell for long-term cell renewal (Figure 19-2).

The other sources of adult stem cells are umbilical cord blood, chorionic villi of the placenta, amniotic fluid, peripheral blood, fetal liver, lung, adipose tissue, and exfoliated deciduous teeth and dental pulp.

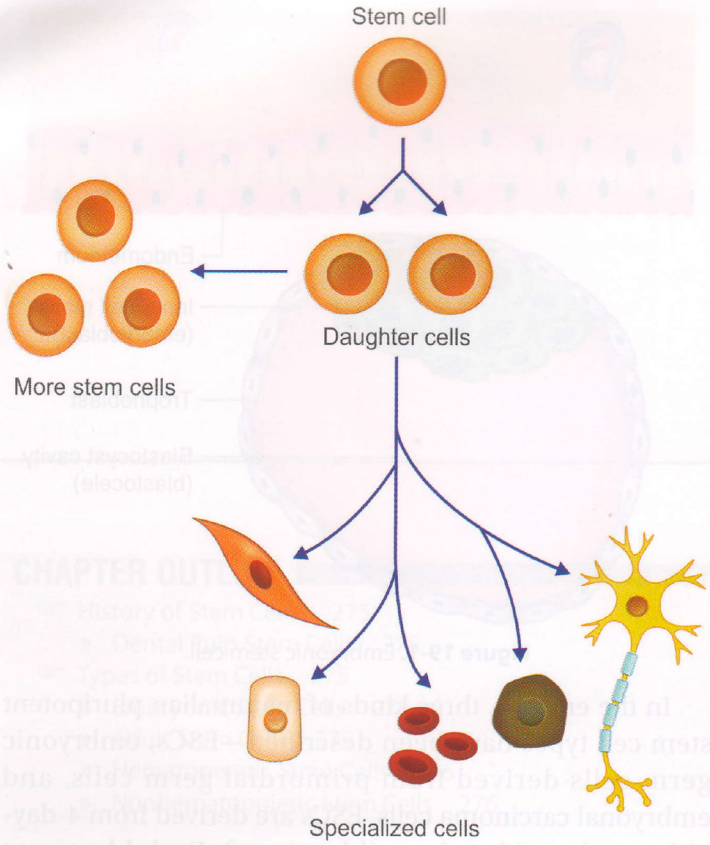


Figure 19-2. Replication of an adult stem cell.

Hematopoietic Stem Cells

The hematopoietic stem cells have the ability to self-renew continuously in the marrow and differentiate into the blood cells and its components. HSCs reside in bone marrow but migrate through blood to various organs on proper signaling. They are also found in liver, spleen, umbilical cord, and blood. There are evidences that these HSCs also have plasticity, *i.e.* they tend to form tissues other than blood systems. This property may be useful in life-saving regenerative therapies.

Nonhematopoietic Stem Cells

These nonhematopoietic stem cells are also termed as multipotent marrow stromal cells or MSCs.

Mesenchymal stem cells are multipotent adult stem cells that can differentiate into connective tissue, bone, cartilage, marrow-stroma, and adipocytes. It is also claimed that MSCs may even give rise to sarcomeric muscle, endothelial cells, and even cells of nonmesodermal origin, such as hepatocytes and neural cell.

With this wide range of differentiation potential, MSCs find the possibility in engraftment and immunosuppressive effect. Their expansion through culture led to increasing clinical interest in the use of MSCs, through either intravenous infusion or site-directed administration, in numerous pathologic situations.

During embryonic development, mesenchyme or the embryonic mesoderm contains stem cells that differentiate into virtually all connective tissue phenotypes such as bone, cartilage, bone marrow stroma, interstitial fibrous tissue, skeletal muscle, and dense fibrous tissues such as tendons and ligaments, as well as adipose tissue.

Mesenchymal stem cells have been isolated from various tissues. The different sources could be umbilical cord blood, chorionic villi of the placenta, amniotic fluid, peripheral blood, fetal liver, lung, and exfoliated deciduous teeth. Increased number of reports are available describing their presence in adipose tissue and dental pulp.

A standard set of criteria have been formulated to identify MSCs.

First, MSC must be plastic-adherent when maintained in standard culture conditions.

Second, phenotypically, MSCs express a number of markers but unfortunately none of which are specific to MSCs. It is generally agreed that adult human MSCs do not express the hematopoietic markers CD45, CD34, CD14, or CD11. They also do not express the co-stimulatory molecules CD80, CD86, or CD40 or the adhesion molecules CD31 [platelet/endothelial cell adhesion molecule (PECAM)-1], CD18 [leukocyte function-associated antigen-1 (LFA-1)], or CD56 (neuronal cell adhesion molecule-1), but they can express CD105 (SH2), CD73 (SH3/4), CD44, CD90(Thy-1), CD71, and Stro-1 as well as the adhesion molecules CD106 [vascular cell adhesion molecule (VCAM)-1], CD166 [activated leukocyte cell adhesion molecule (ALCAM)], intercellular adhesion molecule (ICAM)-1, and CD29.

Third, MSC must differentiate to osteoblasts, adipocytes, and chondroblasts *in vitro*.

MECHANISM OF ACTION OF STEM CELLS

The basis of stem cell action is the formation of new tissues to promote repair and regeneration of damaged tissues, thereby restoring normal function. This enables the implication of stem cells in degenerative diseases through remodeling of injured tissues. It was originally hypothesized that, stem cells on administration would migrate to sites of damaged area and perform the following functions:

- Differentiation into replacement cell types.
- Rescue of damaged or dying cells through cell fusion.
- Secretion of paracrine factors such as growth factors, cytokines, and hormones.
- Transfer of organelles (*e.g.* mitochondria) and/or molecules through tunneling nanotubes (TNTs), Ca^{2+} (calcium), and Mg^{2+} (magnesium).
- Mediate the transfer of proteins or peptides, RNA, hormones, and/or chemicals by extracellular vesicles such as exosomes or microvesicles.

These functions help to increase angiogenesis, plasticity, pluripotentiality, reduce inflammation, activate neighboring resident stem cells, and aid in remodeling. Hence, these functions form the basis for the development of cell-based therapeutics and regenerative medicine.

DENTAL STEM CELLS

The pulp chamber comprises of fibrous pulp along with dental papilla, vascular network, and nerve bundles. There are strong evidences that cells isolated from this structure of tooth have the ability to form tooth. Recently, dental tissues such as periodontal ligament (PDL), dental papilla or dental follicle have been identified as easily accessible sources of undifferentiated cells (Figure 19-3). The dental stem cell biology provides meaningful visions into the development of dental tissues and cellular differentiation processes. The cells isolated from tooth structure were bioengineered into physiologically whole tooth along with epithelial and mesenchymal population of neural crest germ cells.

The final goal of tissue engineering in dentistry is to develop a functional tooth. Dental stem cells could also be a feasible tool for dental tissue engineering. Constructing complex structures like periodontium, which provide the functional connection between a tooth or an implant and the surrounding jaw, could effectively improve modern dentistry. Dental precursor cells are attractive for novel approaches to treat diseases like periodontitis, dental caries or to improve dental pulp healing, and the regeneration of craniofacial bone and teeth. These cells are easily accessible and, in contrast to bone marrow-derived mesenchymal stem cells, are more closely related to dental tissues.

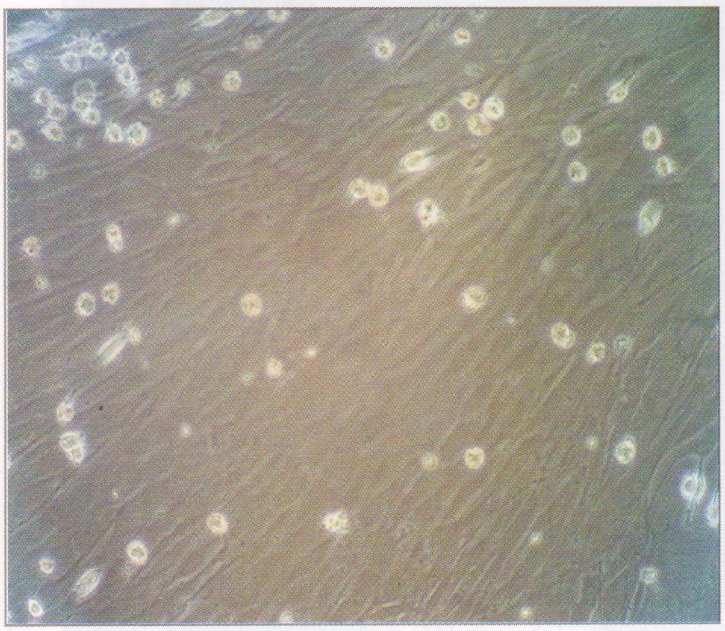


Figure 19-3. Dental pulp stem cell appears spindle-shaped in culture.
Source: Mother Cell Regenerative Centre, Tiruchirappalli, India.

Sources of dental stem cells are:

- Dental pulp stem cells (DPSCs)
- Stem cells from human exfoliated deciduous teeth (SHED)
- Periodontal ligament stem cells (PDLSC)
- Stem cells from the apical papilla (SCAP)
- Stem cells derived from gingiva (GSCs).

Dental pulp stem cells: Dental pulp stem cells are similar to MSCs in some ways; they are of fibroblastic morphology with selective adherence to solid surfaces, have good proliferative potential and capacity to differentiate *in vitro*, and the ability to repair tissues *in vivo*. The regeneration of pulp-dentin capacity is greater in DPSCs than the other dental stem cells.

Stem cells from human exfoliated deciduous teeth: This stem cell population is from the living pulp debris of exfoliated deciduous teeth. SHED are different from DPSCs, in the way that they are "more immature". They are able to differentiate into a variety of cell types, to an extent greater than the DPSCs and are characterized by faster proliferative capacity, higher population doubling efficiency, sphere-like cluster formation, etc. One striking feature that differentiates SHED from DPSCs is their ability to differentiate into bone forming cells.

Periodontal ligament stem cells: Periodontal ligament stem cells have first been introduced by Seo, Miura et al. in 2004. The stem cells of the periodontal ligament occupy the perivascular region in the periodontal ligament and the adjacent endosteal spaces. PDLSCs have been reported to form adherent clonogenic population of fibroblast-like cells in the culture. They express early MSC markers. A further class of dental ectomesenchymal stem cells is PDL stem cells, which were isolated from the root surface of extracted teeth. These cells could be isolated as plastic-adherent, colony-forming cells, but display a low potential for osteogenic differentiation under *in vitro* conditions. PDL stem cells differentiate into cells or tissues very similar to the periodontium.

Stem cells from the apical papilla: Stem cells from dental apical papilla were first identified in human permanent immature teeth. The dental papilla is an embryonic-like tissue that becomes also the dental pulp during maturation and formation of the crown. Therefore, SCAP can only be isolated at a certain stage of tooth development. SCAP have a greater capacity for dentin regeneration than DPSCs because the dental papilla contains a higher number of adult stem cells compared to the mature dental pulp. In addition, SCAP are likely to be less differentiated than DPSCs, as they originate from an embryonic-like tissue. Interestingly, only a combination of SCAP and PDL stem cells induced the formation of a dental connective tissue, namely, the attachment of an artificial tooth crown in the alveolar bone.

ISOLATION OF DENTAL PULP STEM CELLS

There are various methods of isolation of stem cells from dental pulp—(A) enzymatic digestion—where the pulp is digested with collagenase or dispase enzyme and isolated trypsinized cells are plated in culture dishes; (B) explant culture—where small pieces of undigested dental pulp tissue is explanted directly to Petri dishes, and (C) combination of enzymatic and explant cultures—dental pulp tissues are initially trypsinized and then small tissue pieces are explanted to Petri dishes for their outgrowth. Once the cells are isolated, they are cultured in Minimum Essential Medium (MEM) supplemented with 20% fetal bovine serum (FBS) at 37°C with 5% CO_2 and 90% humidity in CO_2 incubator.

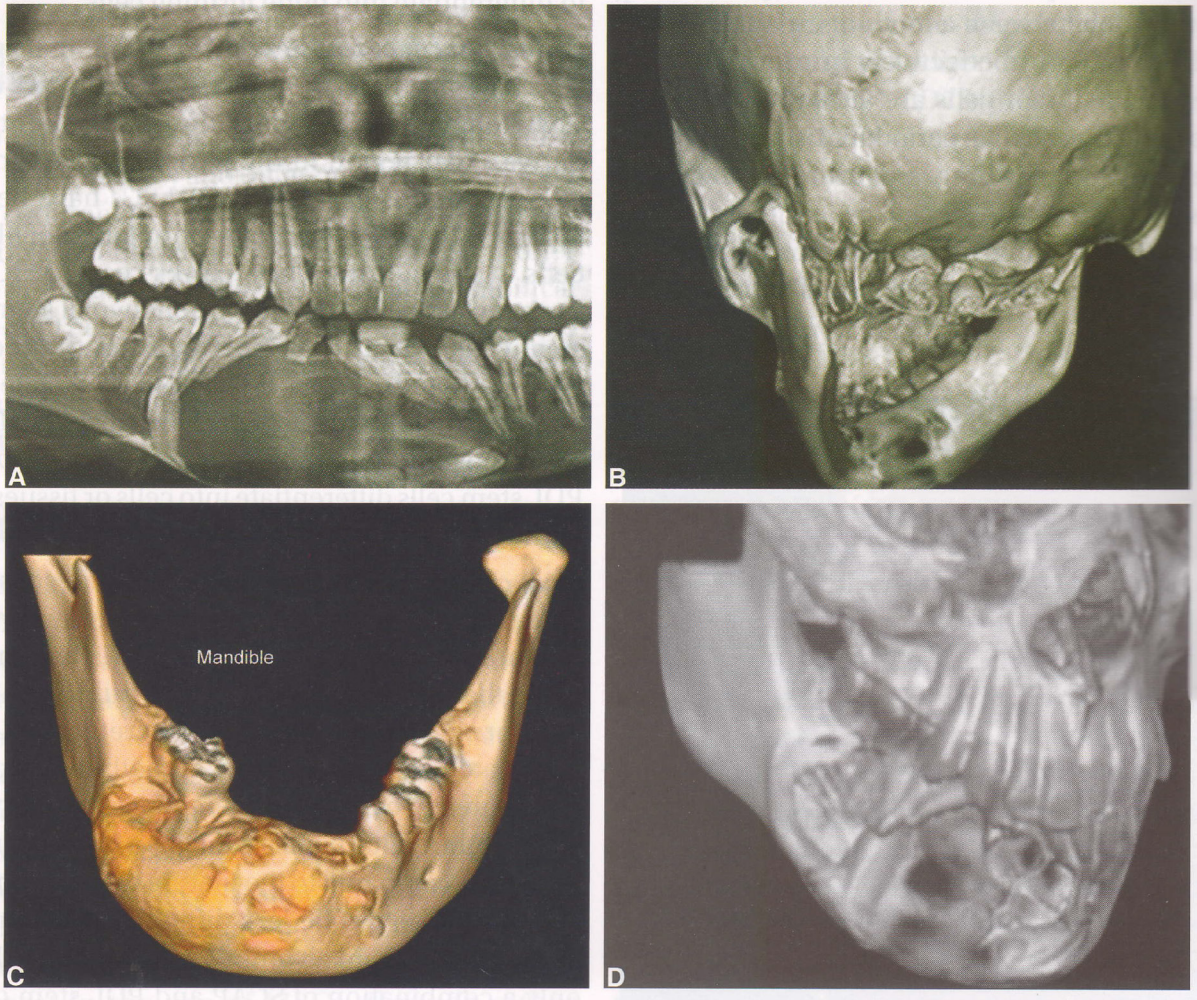
APPLICATIONS OF DPSCS IN REGENERATIVE MEDICINE

At present, treatment for dental pulp degradation is done by conventional methods such as dental pulp capping or by root canal therapy. However, with advancements in dental research, dental scientists are focusing on use of tissue engineering approach with cells, biological, and growth factors. They can use biocompatible material as direct capping agents that can supply growth factors or molecules to stimulate reparative dentin formation.

Dental pulp stem cells are being used as stem cells for regenerative therapies for bone-related diseases and orthopedic surgeries, as dental pulp stem cells have the potential to differentiate into osteoblasts and chondrocytes. The clinical studies by d'Aquino, De Rosa et al. (2009) have shown the use of DPSCs cells in oral and maxillofacial (OMF) bone repair. They have used DPSCs along with collagen sponge for providing optimal support in OMF repair. Thus autologous transplantation of DPSCs can be used in a low-risk and effective therapeutic strategy for the repair of bone defects.

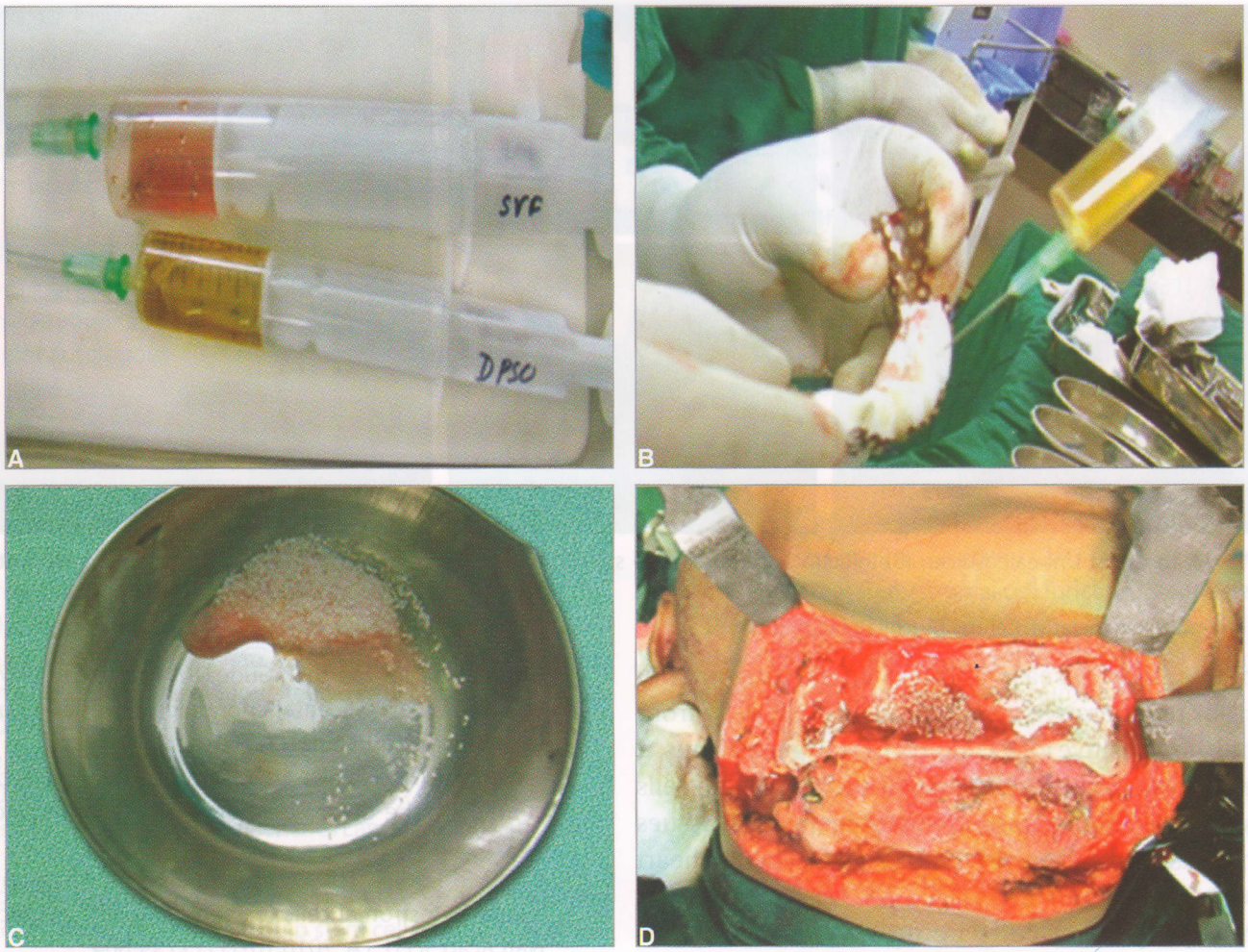
Manimaran, Sharma et al. (2016) has shown bone regeneration in a mandibular ameloblastoma defect with the help of autologous DPSC and buccal pad of fat with more than 1 year of follow-up proving the safety and bone formation potentials of DPSC. Tricalcium phosphate was used as scaffold and platelet-rich fibrin as growth factors in that case (Figures 19-4 to 19-6).

Side population (SP) of dental pulp cells has the property of vasculogenesis. Vasculogenesis is a potential treatment



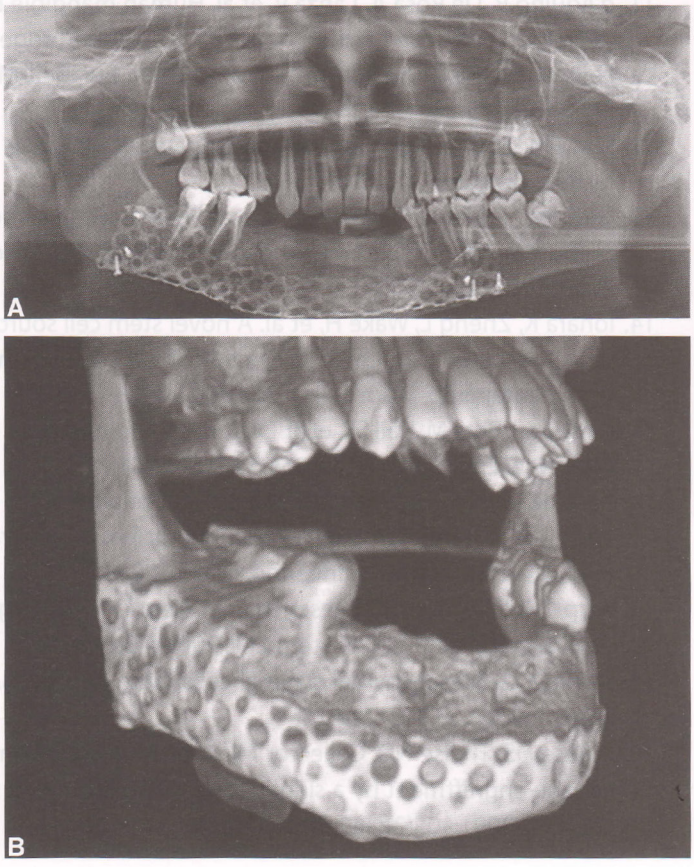
Figures 19-4A to D. (A) Preoperative orthopantamograph; (B) Computed tomography of the mandible showing lingual perforation; (C) Labial expansion; (D) Lateral view of the mandible.

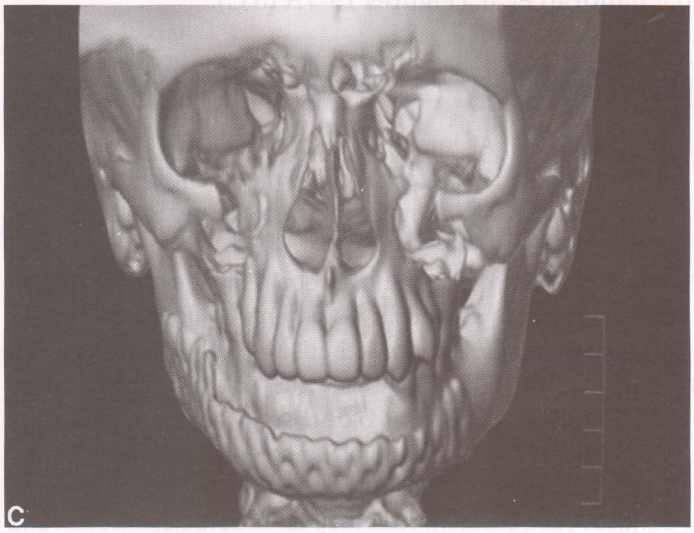
Source: Mother Cell Regenerative Centre, Tiruchirappalli, India.



Figures 19-5A to D. Intraoperative procedures: (A) Syringes with stromal vascular fraction and dental pulp stem cells; (B) Placing stromal vascular fraction and dental pulp cells in syboGraft; (C) Stromal vascular fraction mixed with granules; (D) Placing mixed granules over lingual cortex

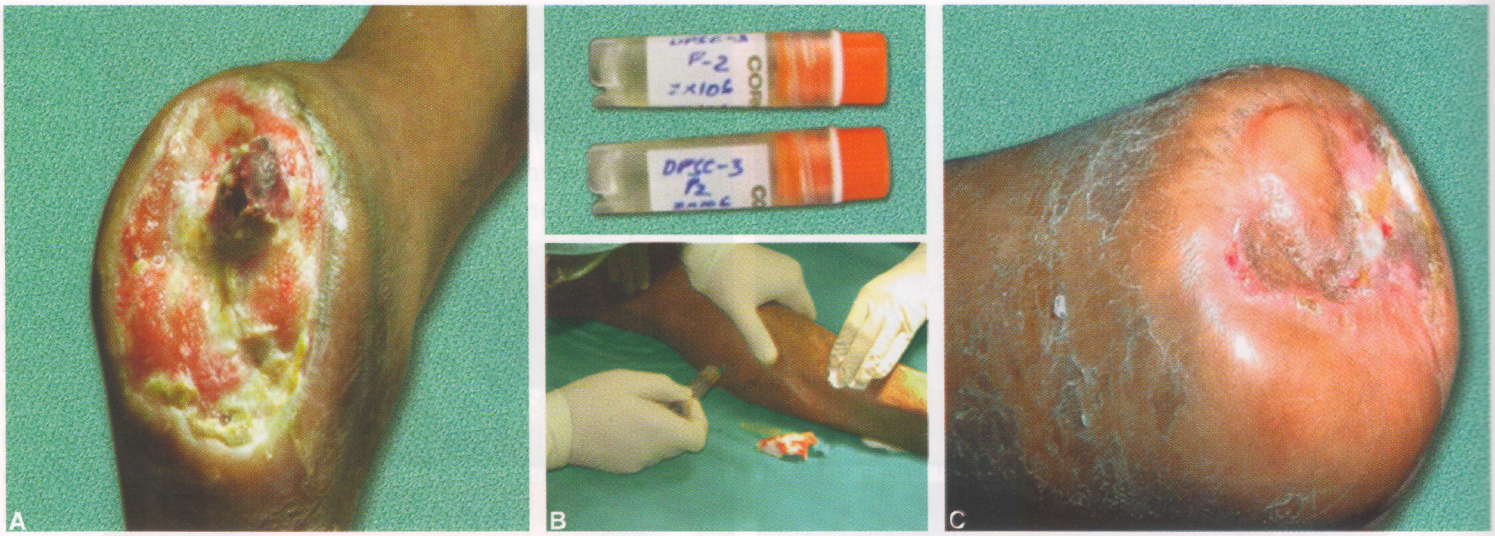
Source: Mother Cell Regenerative Centre, Tiruchirappalli, India.





Figures 19-6A to C. (A) Postoperative orthopantomogram at 10th month; (B) Computed tomography of the mandible at 10th month AP view; (C) Mandible lateral view.

Source: Mother Cell Regenerative Centre, Tiruchirappalli, India.



Figures 19-7A to C. (A) Unhealed ulcer; (B) Injection of dental pulp stem cells along the course of artery; (C) Healed stump postoperative 6 months.

Source: Mother Cell Regenerative Centre, Tiruchirappalli, India.

for ischemic heart disease and it is an exciting area of research in regenerative medicine. These SP cells from dental pulp are positive for CD31 and CD146 genes. Thus, it is suggested that the SP cells are a new source of stem cells which stimulate angiogenesis or vasculogenesis in tissues and can be used in cell-based treatment of ischemic heart diseases.

Dental pulp stem cells being a source for cell-based therapy to stimulate angiogenesis during tissue regeneration, an unhealed diabetic ulcer was treated with DPSC and platelet-rich plasma by Sankaranarayanan S, Ramachandran et al. (Figures 19-7A to C).

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