Chapter

Mesenchymal Stem Cells and Tissue Engineering in Dentistry

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Abstract

Conventional treatments rely on the removal of damaged or impaired tissues, followed by the use of restorative materials. However, the inability to regenerate a functional tooth complex simulating its original structure remains a major unmet treatment objective. Tissue engineering is an amalgamation of engineering and biological principles that aims to not just remove diseased tissue but also replicate and repair lost structures. This evolutionary concept draws from three key elements: cells, an extracellular matrix scaffold, and signaling molecules. Though tissue engineering has come a long way in regenerative medicine, its future in dentistry is promising too. Tissue engineering approaches in dentistry harbor the potential of inducing mesenchymal stem cells (MSCs) of dental origin to combine with biocompatible scaffold, and growth factors to create a three-dimensional environment for regeneration and repair of a fully functional tooth complex. This chapter summarizes the application of mesenchymal stem cells and tissue engineering in dentistry.

Keywords: tissue engineering, regenerative medicine, mesenchymal stem cells, scaffold, dentistry

1. Introduction

For a field as dynamic as medicine, the most significant challenge lies in regenerating or restoring missing or damaged organs or tissues. In the past few decades, miscellaneous regeneration techniques have been applied to rebuild tooth structure destroyed due to dental caries, pulpitis, fractures, and periodontal disorders. Conventional therapies work to remove harmed or impaired tissues and replace missing tissue with a variety of restorative substances. However, the capacity to repair the injured tissues still represents an unmet target. Therefore, the primary objective of regenerative dental medicine continues to be the predictable three-dimensional regeneration and repair of a healthy, functioning tooth complex that mimics its predisease structure [1].

Recently, it has been understood that the processes occurring during regeneration of a tissue mimic those occurring during the natural development of that tissue. This has led to the development of the concept of tissue engineering. Primarily used in the field of medical specialties for replacing vital structures damaged due to disease or trauma; the application of mesenchymal stem cells and tissue engineering in dentistry

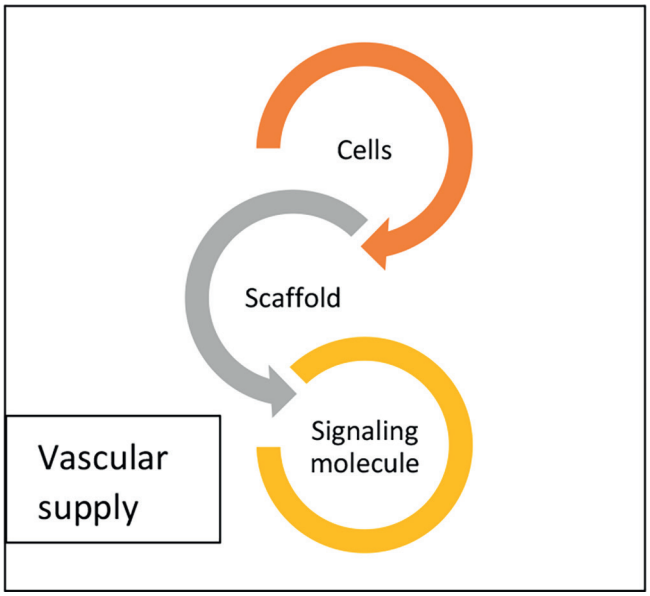


Figure 1.
Tissue engineering triad.

presents a potential solution to achieve predictable three-dimensional regeneration and repair of a fully functioning tooth complex [1, 2].

The field of tissue engineering was first described as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ" by chemist R. Langer and surgeon J.P. Vacanti in the 1980s [3]. It is a science based on fundamental principles that involve the identification of appropriate cells with the ability to differentiate into specialized regenerative cells, certain signaling molecules required to induce cells to regenerate a tissue or organ, and a conductive scaffold with vascular networks to provide nutrition for tissue growth. In the last few years, medicine has begun to explore the possible applications of stem cells and tissue engineering toward the repair and regeneration of body structures.

Accordingly, tissue engineering triad combines three key elements (**Figure 1**) [4].

- Stem cells
- Scaffold or supporting matrix
- Signaling molecules

One of the most important factors in tissue engineering is the choice of scaffold and optimal stem cell population to employ.

2. Stem cells

Tissue regeneration requires specialized cells capable of synthesizing the extracellular matrix specific to each tissue. In this sense, stem cells have been extensively

used in regenerative medicine [5]. Stem cells are immature progenitor cells capable of both self-renewal and multi-lineage differentiation through mitosis into one or more types of specialized cells. They can be isolated from various sources, such as fetuses, embryos, or adult tissues, and their differentiation capability depends on the cell source.

Characteristics of stem cells [2]:

- Totipotent: these are embryonic cells and extra-embryonic cells, which can be differentiated into all cell types.

- Pluripotent: cells that can give rise to all the cell types that make up the body; except extra embryonic tissues such as placenta. For example, embryonic stem cells and induced pluripotent stem cells.

- Multipotent: these cells can develop into more than one cell type, which can give rise to tissues belonging to only one embryonic germ layer (ectoderm or mesoderm or endoderm), for example, cord blood stem cells and adult stem cells

- Clonogenicity: a stem cell is clonogenic as it can proliferate to form colony of cells.

Depending on the developmental stages of the tissues from which the stem cells are isolated, stem cells are broadly divided into embryonic stem cells and adult stem cells (**Figure 2**) [6].

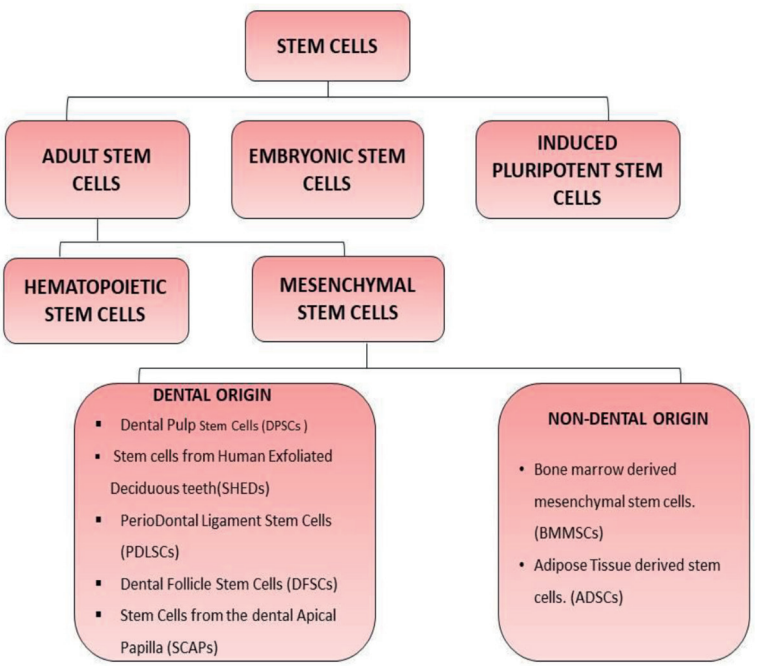


Figure 2.
Classification of stem cells.

2.1 Embryonic stem cells (ESCs)

Embryonic stem cells (ESCs) are derived from the cell of early-stage embryos, during the blastocyst stage. These cells are considered pluripotent type as they can differentiate into any cell type in the body [7]. ESCs have been the focus of much research due to their potential to treat a spectrum of diseases, including Parkinson's disease, diabetes, and heart disease. However, their use has been limited due to ethical concerns and the risk of teratoma formation and tumorigenicity.

2.2 Induced pluripotent stem cells (iPSCs)

Induced pluripotent stem cells (iPSCs) are obtained from somatic cells, such as skin cells, through the reprogramming of gene expression. These cells have similar properties to ESCs and can differentiate into any cell type in the body. iPSCs were first generated in 2006 and have since been used in disease modeling and drug screening, as well as in regenerative medicine. However, their use is still limited due to the risk of genetic abnormalities resulting from the reprogramming process [8].

2.3 Adult stem cells (ASCs)

Adult stem cells (ASCs) are multipotent stem cells and depending upon their origin, they are further classified into hemopoietic stem cells and mesenchymal stem cells.

2.3.1 Hematopoietic stem cells (HPCs)

An immature cell that gives rise to different types of blood cell types, including platelets, red blood cells, and white blood cells. Bone marrow and peripheral blood both contain hematopoietic stem cells. They are also known as blood stem cells. Many cancers (such as leukemia, lymphoma) and non-malignant conditions (such as sickle cell disease) are treated with HPCs in order to repair or rebuild the patient's hematopoietic system. This type of treatment is known as bone marrow or stem cell transplant [9].

2.3.2 Mesenchymal stem cells (MSCs)

Mesenchymal stem cells are nonhematopoietic and multipotent cells that can differentiate into an array of cell types comprising varieties of tissues. They were first identified in aspirates of adult bone marrow by Friedenstein in 1976 [10].

These cells demonstrate specific properties [11]:

1. MSCs have ability to adhere to plastic tissue-culture surfaces.

2. They have potential to differentiate into osteoblasts, adipocytes, chondrocytes, etc.

3. Immunoregulatory properties.

4. MSCs are positive for the surface antigens CD73, CD90, and CD105.

MSCs can be isolated from various sources, including bone marrow, blood vessels, skeletal muscle, umbilical cord, amniotic fluid, placenta, adipose tissue, and teeth.

Owing to their presence in teeth, and relatively easy availability of tooth-derived MSCs from deciduous and permanent teeth than other anatomical sites; dental mesenchymal stem cells are of great interest for research in the field of tissue engineering and regenerative medicine [12].

3. Mesenchymal stem cells of dental origin

MSCs are the main cell source. They are multipotent cells that can be cryopreserved safely, have immunosuppressive qualities, and express mesenchymal markers. Explant cultures or enzymatic digestion can be used to isolate dental mesenchymal stem cells (DMSCs). Autologous stem cells are great option since there is no chance of immunological rejection, less expensive, and they eliminate legal and ethical concerns.

Dental mesenchymal stem cells that have been isolated and grouped according to their position in the tooth are:

- Dental Pulp Stem Cells (DPSCs)
- Stem cells from Human Exfoliated Deciduous teeth (SHEDs)
- Periodontal Ligament Stem Cells (PDLSCs)
- Dental Follicle Stem Cells (DFSCs)
- Stem Cells from the dental Apical Papilla (SCAPs)

3.1 Dental pulp stem cells (DPSCs)

These are a common source of dental tissue-derived stem cells obtained from the pulp of permanent teeth. In 2000, Gronthos et al. were the first to spot MSCs in the dental pulp of teeth that are today referred to as DPSCs. He further studied the proliferation and differentiation capabilities of DPSCs and stated that they possess high plasticity and trans-differentiation potency of their population [13]. These cells possess the potential to differentiate into osteogenic, adipogenic, chondrogenic, and neural cells and show high expression of surface markers of MSCs. Due to their profound regeneration, differentiation, and proliferation capabilities, they can be induced *in vitro* to differentiate into cells of odontoblastic phenotype [14]. Dr. Irina Kerkis in 2006 reported discovery of Immature Dental Pulp Stem Cells (IDPSC), a pluripotent subpopulation of DPSC using dental pulp organ culture [15]. DPSCs can also be harvested from one cavity and applied to dentin regeneration in many teeth.

DPSCs could therefore be used as a generic allogenic source of MSCs.

3.2 MSCs from dental pulp of exfoliated deciduous teeth (SHED)

MSCs from dental pulp of exfoliated deciduous teeth (SHED) was first isolated in 2003 from pulp of human deciduous teeth [16]. *In vitro*, these cells may produce dentin, induce bone formation, and differentiate into various nondental mesenchymal cell descendants. SHED show enhanced population doublings, faster rates

of proliferation, *in vivo* osteoinductive potential, and the capacity to organize into sphere-like clusters. They cannot, however, repair whole dentin/pulp-like complexes *in vivo* like DPCSs. Dental stem cells may be useful for treating neurodegenerative illnesses and repairing damaged motor neurons because of their capacity to generate and release neurotrophic substances [17].

This multilineage potential makes SHEDs alternative source of dental stem cells.

3.3 MSCs from dental follicle (DFSCs)

The dental follicle, which encircles the developing tooth, contains a collection of dental mesenchyme stem cells that are essential for the growth of the alveolar bone, cementum, and periodontal ligament. In 2005, Morsczeck et al. isolated DFPCs from the dental follicle of human third molar teeth, and these cells were discovered to display the stem cell markers Notch and Nestin. DFSCs have the capacity to differentiate into osteoblasts, adipocytes, and nerve-like cells *in vitro* but could only produce cementum *in vivo*. A further investigation found that 4 weeks after DFSC implantation into mice, a new periodontal ligament had formed [18].

3.4 MSCs from the periodontal ligament (PDLSCS)

The periodontal ligament houses stem cells that self-renew and specialize to generate other tissues, such as cementum and alveolar bone, and it may be separated from the root of removed teeth [19]. *In vitro* differentiation of PDLSCs into adipocytes, osteoblasts, and chondrocytes is possible. Alkaline phosphatase, bone sialoprotein, osteocalcin, and TGF-receptor type I are among the cementoblastic/osteoblastic markers that are expressed by cultured PDLSCs, in addition to CD105, CD90, CD73, STRO-1, and CD146/MUC18 [20].

3.5 MSCs from apical papilla (SCAP)

In 2006, Sonoyama et al. identified apical part of dental papilla of human teeth as a unique source of MSCs, which can be considered for oral tissue regeneration. SCAP can be isolated from human third molars, thus making them easily accessible. Due to their greater ability to proliferate, they encourage the formation of roots and seem to be more effective in promoting tooth development than PDLSC. They create dentin and odontoblast-like cells. Additionally, they show adipogenic differentiation ability. Similar to DPSCs, SCAP exhibits stem cell markers (STRO-1, CD146, and CD34), but with substantially greater rates of proliferation and mineralization [21].

3.6 Gingiva-derived mesenchymal stem cells (GMSCs)

Zhang et al., in 2009, recognized human gingival tissue as a potential source of MSCs for tissue regeneration and therapy. Based on their quantity and accessibility, the GMSCs were examined. When isolated, they demonstrated greater proliferation potential than BM-MSCs [21]. *In vitro*, the GMSC successfully differentiated into mesoderm adipocytes and osteoblasts, as well as endodermal and neural ectodermal cells implying stem cell characteristics. Furthermore, the GMSCs were shown to have stem cell-specific cellular markers and a phenotype consistent with mesenchymal progenitor cells [22].

4. MSCs of nondental origin

The bone marrow microenvironment is the body's major MSC niche. MSCs are known to live in two distinct niches: endosteal and perivascular. The endosteal niche is considered to keep MSCs quiescent for a long time, whereas the perivascular niche is thought to keep MSCs proliferating and mediating circulation. BMMSCs and adipose-derived MSCs have been found in BM perivascular regions [23].

- Bone marrow mesenchymal stem cells (BMMSCs)
- Adipose tissue derived stem cells (ADSCs)

4.1 Bone marrow mesenchymal stem cells (BMMSCs)

Bone marrow mesenchymal stem cells (BMMSCs) are stem cells derived from the bone marrow. They can differentiate along a variety of mesenchymal lineages. BMMSCs have emerged as a unique option for the tissue engineering of teeth and may be used to produce both mesenchymal and epithelial cells [24]. Both BMSC and DPSC are capable of forming structures that resemble teeth or bones and have several traits in common with one another. In contrast to DPSC, BMSCs have a decreased odontogenic potential. After being implanted into damaged periodontal sites, BMSCs have the ability to develop alveolar bone, periodontal ligament, and cementum *in vivo*. In order to treat periodontal diseases, bone marrow offers an alternate source of MSC [25]. BMMSC harvesting results in poor cell numbers, discomfort, and morbidity.

4.2 Adipose-derived stromal cells (ADSCs)

ADSCs are a subset of pluripotent mesenchymal stem cells that are generated from fat. They are capable of multilineage differentiation, which includes adipogenesis, osteogenesis, and chondrogenesis. Due to their easy accessibility and efficiency in getting, through lipectomy and various esthetic and medical operations. ADSCs are the most often employed source of MSCs [26].

5. Scaffolds

The scaffold is a three-dimensional structure that serves as a template or framework for cell attachment, growth, and regeneration of new tissue. The purpose of a scaffold in tissue engineering is to provide a temporary framework for cells to attach and grow, and to mimic the natural extracellular matrix (ECM) that surrounds cells in the body [27].

The main functions of scaffolds in tissue engineering are:

- Structural support: Scaffolds provide a three-dimensional structure that can mimic the shape and mechanical properties of the target tissue. This support is critical for the survival and growth of the cells, and for the development of functional tissue.

- Cell attachment and proliferation: Scaffolds can provide a surface for cells to attach and proliferate. This attachment is vital for the formation of functional tissue, as it allows cells to interact and communicate with each other.

- Diffusion of nutrients and waste products: Scaffolds can facilitate the diffusion of nutrients and waste products throughout the developing tissue. This is important for the survival and function of the cells.

- Guided tissue regeneration: Scaffolds can be designed to promote the growth of specific types of tissue, such as bone, cartilage, or muscle. By controlling the properties of the scaffold, researchers can guide the development of the tissue to achieve a desired outcome [27].

Scaffolds can be made from a variety of materials such as natural polymers, synthetic polymers, metals, ceramics, or composites that are used to carry biologically active molecules to the site of regeneration [27, 28].

The ideal properties of scaffold are:

- It must be nontoxic

- It should be biocompatible, biodegradable, and highly cell adhesive

- It should be porous, to facilitate cell seeding

- It should have optimal physical and mechanical properties.

In tissue engineering, scaffolds have been fabricated using several natural and synthetic polymers.

5.1 Naturally derived scaffold materials

Natural polymers, including collagen, gelatin, chitosan, alginate, and hyaluronic acid, are used in tissue engineering applications [28].

- Fibrin: a crucial element of blood clots, is combined with thrombin to create an *in-situ* forming gel that serves as a framework for carrying different physiologically active molecules.

- Collagen: one of the most used scaffolding materials is collagen. Typically, gelatin and animal tissues are the sources of type I collagen.

- Chitosan: a cationic polymer generated from chitin is chitosan. Its scaffold creates an osteo-conductive hydrophilic surface, pointing to its potential utility in bone tissue creation. Brown algae are the source of the anionic polysaccharide known as alginate. When combined with divalent cations such as Ca.

- Hyaluronic acid: is a glycosaminoglycan made up of repeating disaccharide units that are nonsulfated. It contributes significantly to connective tissue and creates cross-linkable hydrogels with different modifications.

5.2 Synthetically derived scaffold materials

The commonly used chemical compounds to fabricate synthetic scaffolds include poly(a-hydroxyester) s, polyanhydrides, and polyorthoesters. Among these polymers, poly(a-hydroxy- ester)s such as polylactide (PLA), polyglycolide (PGA), and its copolymers are extensively used. These polymers are biocompatible, biodegradable, bioresorbable, and can be easily processed to form various 3-D structural Poly Lactic-co-Glycolic acid (PLGA) copolymers with controlled degradation matrices [29].

The behavior and mechanical characteristics of poly (lactide-co-glycolide) materials can be altered to meet specific needs. In order to accomplish effective tissue growth, they can be utilized to create nanofibrous scaffolds. The major drawback of these polymers is that when they break down, acidic byproducts may interfere with the regeneration process.

The extracellular matrix is composed of a complex meshwork of proteins and polysaccharides, which are produced by the resident cells in the tissue/organ. The extracellular matrix is generally composed of three categories of molecules: fibrous proteins (e.g., collagen, elastin, fibrillin, and fibulin), adhesive glycoproteins (e.g., laminin, fibronectin, tenasin, thrombospondin, and integrin), and glycosaminoglycans. More importantly, emerging studies suggest that the extracellular matrix can itself function as an inductive scaffold or modify a biomaterial-based scaffold for tissue and/or organ regeneration. This makes the extracellular matrix a critical element in the field of tissue engineering and regenerative medicine [28]. They are generally fabricated through decellularisation and other manufacturing processes. Extracellular matrix scaffold maintains initial geometry and flexibility, possesses a certain degree of mechanical strength, and comprises the main physical advantage of artificial scaffolds. Extracellular matrix scaffold, by obtaining an intact three-dimensional structure, also overcomes the drawback of synthetic scaffold [30].

Scaffolds can be fabricated using a variety of techniques, such as electrospinning, 3D printing, lyophilizing, phase separating, foaming, rapid prototyping, and microfabrication, and can be tailored to match the specific mechanical, chemical, and biological properties of the target tissue. For example, scaffolds for bone tissue engineering may be designed to have high stiffness and strength, while scaffolds for cartilage tissue engineering may be designed to have high flexibility and elasticity [31]. Therefore scaffold design is a critical aspect of tissue engineering, as the properties of the scaffold can significantly affect the behavior of the cells and the development of the tissue. Scaffold-based tissue engineering shows a great promise in a variety of applications, including bone and cartilage regeneration, skin tissue engineering, and organ transplantation. However, there are still many challenges to overcome in order to fully realize the potential of scaffold-based tissue engineering, including optimizing the design and fabrication of scaffolds, improving cell seeding and growth, and ensuring biocompatibility.

6. Signaling molecules

Tissue engineering involves the use of various signaling molecules to promote tissue regeneration and repair. These signaling molecules can be categorized into several groups based on their functions and roles in tissue regeneration.

6.1 Growth factors

Growth factors are a class of signaling molecules that plays a critical role in tissue engineering. They are naturally occurring proteins that are produced by cells and act on neighboring cells to stimulate or inhibit their activity. Growth factors can be incorporated into the scaffold material or delivered to the target site through a variety of methods, such as direct injection or controlled release from a biomaterial carrier to stimulate the growth and proliferation of stem cells, which are the building blocks of tissues. They can also promote the differentiation of stem cells into specific cell types, such as bone, cartilage, or muscle. By controlling the activity of stem cells, growth factors can help to regenerate damaged or diseased tissues.

There are many different types of growth factors that have been identified, in tissue engineering, among them are the bone morphogenetic proteins (BMP); fibroblast growth factor (FGF); interleukins; hedgehog proteins (HHS); tumor necrosis factor (TNF); and vascular endothelial growth factor (VEGF). Among these signaling molecules, the bone morphogenetic proteins (BMPs) are known for their ability to induce the formation of bone and cartilage and have been extensively studied and applied in dental regeneration (**Table 1**) [32–34].

6.2 Dental pulp-derived factors

Smith concluded from his findings that dentin matrix may be regarded as a powerful cocktail of bioactive molecules when released following tissue injury has

Name	Origin	Function
Bone morphogenetic proteins (BMPs) [32]	Demineralized bone matrix, MMSCs, and osteoblasts endothelial cells chondrocytes	Stimulates differentiation of mesenchymal stem cells into bone-forming cells, and promotes formation of bone and cartilage. BMP-2 and BMP-7 are commonly used.
Platelet-derived growth factor (PDGF)	Platelets, macrophages, keratinocytes, and endothelial cells	Potent mitogen and chemoattractant promote wound healing, and stimulate proliferation and migration of various cell types.
Transforming growth factor-α (TGF- α)	Platelets, macrophages, keratinocytes, and brain cells	Activates neutrophils, fibroblast mitogen, stimulates angiogenesis
Transforming growth factor-β (TGF- β)	Dentine matrix, platelets, osteoblasts, chondrocytes endothelial cells, fibroblasts	signal odontoblast differentiation, promotes wound healing, inhibits macrophage, and lymphocyte proliferation
Fibroblast growth factor (FGF)	MMSCs, osteoblasts, chondrocytes, endothelial cells	Bone and cartilage regeneration, nerve growth, proliferation, and angiogenesis.
Vascular endothelial growth factor (VEGF):	Platelets osteoblasts chondrocytes endothelial cells	Endothelial cell proliferation, angiogenesis
Insulin-like growth factor (IGF)	Osteoblasts, chondrocytes, endothelial cells	Osteoblasts proliferation, osteoclasts proliferation, and ECM synthesis

Table 1.
Growth factors in tissue engineering.

the potential to dramatically influence cellular events in the dentin-pulp complexes such as the group of angiogenic growth factors or any cytokines sequestered in the dentin matrix will be released due to caries demineralization and might contribute to the overall repair process. Dentin matrix proteins such as dentin sialoprotein (DSP) and dentin matrix protein 1 (DMP-1) are known to regulate odontoblast differentiation and dentin formation [35]. The research in tissue engineering is ongoing and new signaling molecules and strategies are continually being explored to improve the regeneration and repair of tissues.

7. Applications of tissue engineering

MSCs generated from dental tissue have demonstrated their multilineage differentiation capabilities and excellent therapeutic promise in oral and systemic disorders. Together with growth factors and/or scaffolds, dental MSCs make a highly effective interaction, which is of utmost importance in tissue engineering. It has shown great potential in the regeneration and restoration of damaged tissues or organs (**Figure 3**).

7.1 Application of tissue engineering in regenerative medicine

DPSCs have been found to have therapeutic benefits for myocardial infarction [36], cerebral ischemia [37], muscular dystrophy [38], and corneal reconstruction [39] due to their adaptability. These cells were discovered to exhibit

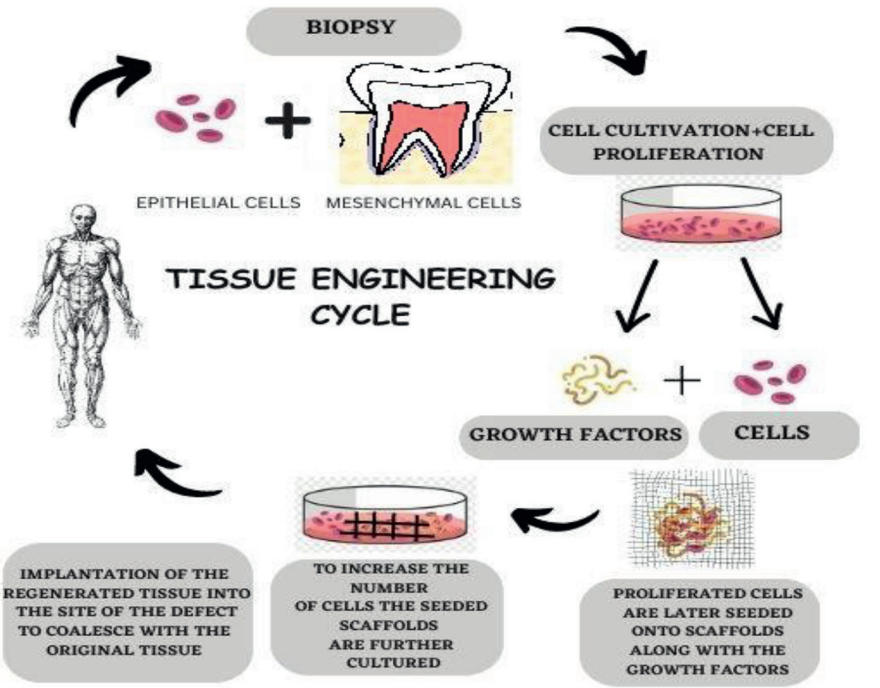


Figure 3.
Schematic presentation of tissue engineering.

neuron-specific markers in the damaged cortex, indicating that engrafted DPSC-derived cells may integrate into the host brain and may serve as a helpful source of neuro and gliogenesis *in vivo*. Furthermore, DPSC transplantation boosted neurogenesis and vasculogenesis in rats, showing that DPSCs might be a therapeutic option [40].

7.2 Application of tissue engineering in dentistry

Tissue engineering has the potential to revolutionize the way dental treatments are performed by providing new and improved approaches for repairing and replacing damaged or missing teeth. It is becoming a rapidly advancing field in the development of biomaterials that can mimic the natural structure and function of dental tissues. Thus, can benefit major dental branches like endodontics, oral surgery, and periodontics (**Figure 4**).

Potential applications in:

- Endodontics
- Periodontology
- Oral and maxillofacial

7.2.1 Endodontics

Tissue engineering can be applied in endodontics to regenerate dental pulp tissue, dentin, and other tissues that may have been damaged due to injury or disease. Here are some specific examples of how tissue engineering is being used in endodontics.

Regenerative endodontics [41] is a new treatment approach that aims at:

- Regeneration or restoration of lost or diseased dentinal tissue
- Revascularization of necrotic dental pulp

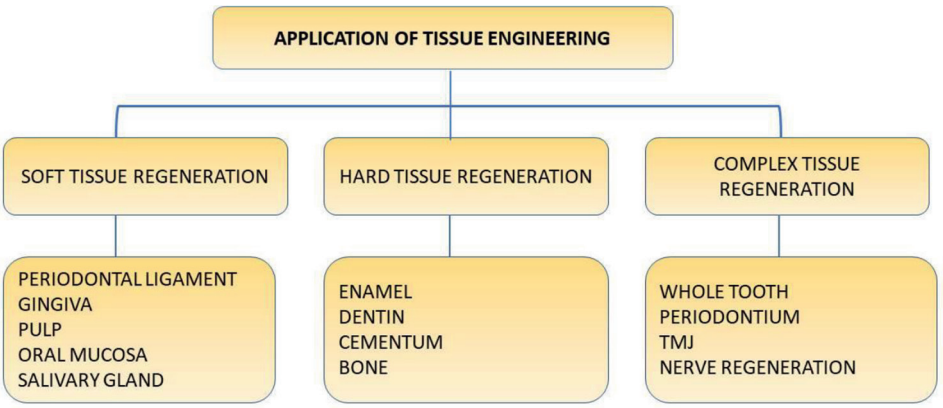


Figure 4.
Application of tissue engineering in dentistry.

7.2.1.1 Dentin pulp regeneration

One of the most promising applications of tissue engineering is the regeneration of dental pulp. Dental pulp is the soft tissue inside the tooth that contains nerves and blood vessels. Dental pulp is damaged due to decay or trauma, which can lead to infection and eventually tooth loss. Initially, for the regeneration of the dentin-pulp complex, various pulp capping materials (e.g., calcium hydroxide, mineral trioxide aggregates Biodentine) were used, which stimulates the pulp progenitor cells to differentiate into odontoblast-like cells or the secretion of TGF-b131, which plays an essential in angiogenesis, the recruitment of progenitor cells, cell differentiation, and ultimately mineralization of the damaged area. Tissue engineering techniques can be used to create a scaffold that mimics the structure of dental pulp and promotes the growth of new tissue. This approach has the potential to restore the function and vitality of damaged teeth [42].

Because of the size and confinement of the pulp within the root canal(s), cell treatment and/or injectable hydrogels were the most commonly used strategy for engineering the dentin-pulp complex. Encapsulated stem cells were also used for dentin-pulp regeneration such as Gelfoam-encapsulated dental stem cells encouraged dentin-pulp complex development in pulpless root canals of juvenile permanent incisors in beagles [43].

7.2.1.2 Revascularization of necrotic dental pulp

The term "revascularization" describes the occurrence of physiological tissue creation and regeneration that really took place [44, 45]. This might be explained by SCAPs continuing to function after the infection and causing this occurrence. It is also possible that the radiographic appearance of increased dentinal wall thickness is attributable to ingrowth of cementum, bone, or a dentin-like substance. This variation in cellular response is not surprising given that DPSCs can acquire odontogenic/osteogenic, chondrogenic, or adipogenic phenotypes based on their exposure to various combinations of growth factors and morphogens.

7.2.2 Oral and maxillofacial surgery

Oral and maxillofacial surgery is a surgical specialty that involve the diagnosis and treatment of diseases, injuries, and defects in the face, jaws, and oral cavity. Here are some of the potential applications of tissue engineering in oral and maxillofacial surgery:

7.2.2.1 Maxillary or mandibular reconstruction

The main causes of aberrant maxillofacial bone tissue in the maxillofacial region are the increasing loss of jaw bone tissue and periodontal tissue inflammation. By polarizing M2 macrophages, SHEDs may lessen periodontal inflammation. Encourage the regeneration of periodontal tissue [46]. Tissue engineering can be used to reconstruct large mandibular defects that result from trauma, cancer, or congenital malformations. This approach involves the use of biomaterial scaffolds that are seeded with bone-forming cells (DPSCs/SHEDs) and growth factors that promote bone regeneration. There have been a few clinical examples reported wherein large defects like mandibulectomy, treated with a titanium mesh cage, has been used to support the

HA-coated with signal proteins and in a case of segmental jaw deformity was repaired utilizing composite scaffolds (collagen-HA-tricalcium phosphate) and bone morphogenetic protein 2 (BMP2) [47]. The results of bone tissue engineering are encouraging and might help overcome the limitations of bone autografts and allografts.

7.2.2.2 Temporomandibular joint (TMJ) reconstruction

Tissue engineering can be very useful in repairing or regenerating a bony condyle or a fibrocartilagenous disc. Thomas et al. described the first *in vitro* creation of TMJ cartilage more than two decades ago, type I meshes were employed to culture chondrocyte-like cells *in vitro*. DPSCs have been utilized to differentiate chondrocytes to restore the cartilage [48]. Puelacher et al. attempted to tissue engineer the TMJ disc a few years later. Chondrocytes (Bovine articular) were planted on disc-shaped degradable and fibrous PGA and PLA scaffolds. After seven days, naked mice were implanted with chondrocyte-loaded scaffolds. Within three months, the creation of new cartilage and organic matrix revealed the prospect of TMJ disc tissue engineering [49].

7.2.2.3 Cleft palate repair

MSCs can differentiate into osteoblasts and are considered the best potential option for the repair of alveolar cleft palates, which are congenital defects that result from incomplete fusion of the palatal shelves during embryonic development. This approach involves the use of biomaterial scaffolds that are seeded with cells that can differentiate into the various tissues that make up the palate, as well as growth factors that promote tissue regeneration. BMP-2-aided bone regeneration has been reported for the reconstruction of the alveolar cleft [50].

7.2.2.4 Nerve regeneration

Tissue engineering can be used to regenerate nerves in the facial region, which is often necessary after facial trauma or surgery. Apart from their substantial neural differentiation potential, DPSCs produced from distinct cranial neural crest cell lineages express many factors that promote nerve and axon regeneration. DPSCs have been reported to express the neural crest cell markers CD271 and SOX10, which might be utilized to induce the development of Schwann cells, which are important in peripheral nerve repair [51].

7.2.2.5 Salivary gland regeneration

In Salivary gland bioengineering, the principal role of DPSCs is to regenerate the salivary stroma or mesenchymal-derived compartment, and these cells are one of the best choices for that purpose because the tooth mesenchyme and the Salivary gland mesenchyme share a common neural crest embryonic origin. The construction of three-dimensional salivary glandular tissue was attempted using biodegradable polymer scaffolds. Human salivary epithelial cells were seeded on scaffold and implanted into the mouse models. The constructed tissue can be implanted under the oral mucosa and secrete saliva directly into the oral cavity using engineered ducts. It has been proposed that implanted functioning salivary gland tissues may be a better therapy option for xerostomic patients due to their capacity to discharge saliva at a continual rate in more physiological ways [52].

7.2.3 Periodontics

Periodontics is a branch of dentistry concerned with the prevention, diagnosis, and treatment of diseases affecting tooth's supporting structures. Periodontium is a complex structure that includes two hard and two soft tissues: periodontal ligament, Gingiva, cementum, and bone.

7.2.3.1 Periodontal regeneration

Complete regeneration of the periodontium has always been challenging due to its complex structure. MSCs have the ability to repair new cementum, alveolar bone, and periodontal ligament. *In vitro* grown periodontal ligament cells were also effectively reimplanted into periodontal defects. PDLSCs and DFSCs have emerged as an additional cell source for periodontal regeneration treatment.

Masako Miura et al. in 2004 studied the possibility that human PDL includes stem cells capable of regenerating periodontal tissue. PDLSCs are isolated using single colony selection and other methods, then implanted into immunocompromised mice to test their ability to regenerate and repair tissue. PDLSCs developed into adipocytes, cementoblast-like cells, and collagen-forming cells under certain culture conditions [53]. In dog experiments, periodontal ligament cell sheets were employed to stimulate periodontal regeneration [54, 55]. Clinical experiments employing BM-MSCs and platelet-rich plasma (PRP) have shown successful periodontal regeneration [56].

7.2.3.2 Oral mucosa

The similarities between skin and oral mucosa resulted in the development of engineered oral mucosa, which followed the same protocol, starting with the development of an epithelial sheet. Oral keratinocytes were seeded on decellularised cadaveric human dermis (AlloDerm) [57] or a three-dimensional cell-seeded scaffold to create a composite oral mucosal equivalent. Thus, skin and mucosal replacements can be utilized interchangeably.

A review of recent developments in synthetic oral mucosa noted that the basic structure of the connective tissue component and the reconstituted basement membrane in such biomimetic models only allows for a simplistic representation of the native stromal microenvironment. As a result, the majority of researchers in the field of oral tissue engineering are shifting from the use of biomimetic models to more realistic approaches [58].

7.2.3.3 Gingival augmentation

Gingival augmentation involves the use of soft tissue grafts to increase the volume and thickness of the keratinized tissue around the teeth. Gingival epithelial sheets made from autologous gingival tissues were created and utilized to treat chronic desquamative gingivitis [59]. The results demonstrated that human-cultivated gingival epithelial sheets promoted gingival augmentation. GINTUIT, an allogeneic cellular product, was recently introduced. This product contains allogeneic cultivated keratinocytes and fibroblasts in bovine collagen. McGuire et al. in 2011 determined that the product was a safe and effective treatment for enhancing the keratinized gingival zone [60].

7.2.3.4 Bone regeneration

For bone augmentation, autogenous or allogeneic bones, as well as artificial materials, are used. However, there are several complications with these grafts, including donor site injury, the likelihood of infection or absorption, and ethical concerns. MSCs can be employed as an alternativeto autogenous bone transplants. DPSCs are an important source of osteoprogenitor cells, which play an important role in bone tissue plasticity processes. SHED and DPSC have osteoinductive properties, which allow them to develop into osteoblasts and stimulate bone formation [61]. The study was conducted to regenerate bone in a significant osseous defect with minimum invasiveness and good flexibility. To boost osteogenesis, they used platelet-rich plasma as an autologous scaffold with MSCs. Newly developed bone was discovered at 8 weeks [56].

8. Recent advancements in tissue engineering

8.1 Whole tooth regeneration

One of the most prevalent disorders is tooth loss, which can be caused by periodontal disease, caries, or trauma. Currently, dental implants are considered the best option for restoring the missing teeth and have achieved long-term success.

There have been several ways proposed for constructing complete biological teeth. Cell-tissue recombination and dental tissue engineering are the two main techniques utilized for tooth regeneration. Dental cell-tissue recombination techniques focus on mimicking natural tooth formation processes, in which, cultivated progenitor stem cell-tissue constructions are directly implanted in the defect site [62]. Young et al. claimed to have successfully regenerated the first-ever tooth structure with dentin and enamel using tooth buds from porcine third molars. Tooth bud cells were implanted in rats after being seeded onto biodegradable scaffolds. Within five to seven months, visible tooth structures (mature enamel with enamel organ, dentin with odontoblasts and pulp chamber, Hertwig's root sheet with cementoblasts) were regenerated. However, smaller tissues (4 mm2) were created, and tissue-engineered teeth did not conform to scaffold [63]. Duailibi et al. demonstrated that cultured 4-day post-natal rat tooth bud cells seeded onto PGA/PLLA and PLGA scaffolds, implanted, and grown in the mandible, could form organized bioengineered dental tissues such as dentin, enamel, pulp, and PDL. Bioengineered mandibular implant tooth tissues produced enamel and dentin proteins and exhibited morphological and histological similarities to naturally formed dentin and enamel. It could be concluded that *in vivo* whole tooth regeneration is achievable, but it is currently difficult to overcome translational barriers and test these methods on humans [64].

8.2 The bio-root regeneration

A bio-root was created by implanting preshaped root-like scaffolds along with mesenchymal stem cells into the alveolar bone to form a functional root with root-like structure, biomechanical properties, and elements similar to natural teeth, periodontal ligament-like tissue, and dentin-like matrix structure, and the ability to support post-crown prostheses [65].

SCAP and PDLSC are now being widely researched for bioroot engineering. Only dentin structure renewal was detected. As a result, regeneration of the whole tooth

structure was not completed in many cases. Instead of attempting to build a whole tooth, Sonoyama et al. in 2006 revealed that by combining SCAP with PDLSCs, they were able to generate a bioroot with periodontal ligament tissues. The autologous SCAP and PDLSCs were placed onto HA/TCP and gel foam scaffolds, respectively, then reimplanted into the sockets using a small swine model. Three months later, the bioroot established in the porcine jaw. The bioroot structure was made up of dentin that was randomly produced by the SCAP. The bioroot was surrounded by periodontal ligament tissue and appeared to have normal relationship with surrounding bone. However, the presence of residual HA in the newly regenerated dentin formed a structure different from that of normal dentin. This leads to a reduced mechanical strength of the bioroot, nearly two-thirds of a natural tooth [21].

9. Conclusion

MSCs have shown great potential for tissue engineering applications due to their ability to differentiate into various cell types, including osteoblasts, chondrocytes, and adipocytes. This capacity allows them to regenerate and repair damaged or lost tissues.

The use of MSCs in dentistry is still an active area of research, and, further research and clinical trials are needed to optimize their effectiveness and long-term outcomes. Regulatory approval and standardization of protocols are needed before widespread clinical application. Nonetheless, the combination of MSCs and tissue engineering approaches represents an exciting avenue for advancing dental treatments and regenerative therapies in the future by providing patient-specific therapy options that optimize function, esthetics, and patient care quality.

Conflict of interest

The authors declare no conflict of interest.

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