

## Tissue Engineering and Stem Cells in Periodontal Regeneration

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### Abstract

**Background and Aim:** Reconstructive treatments in dentistry aimed at achieving complete regeneration of destroyed structures both in soft and hard tissues. To date, this goal has been tried to accomplish using various bone grafts, growth factors, and barrier membranes. Stem cells are the most fascinating area of biology today and have been used clinically in the field of medicine to treat many incurable diseases.

**Materials and Methods:** The English literature indexed in the MEDLINE/PubMed database was systematically searched and original papers were critically reviewed on this subject, until the second quarter of 2011. Additional papers were obtained by manual searching reference lists of previously published review papers.

**Results:** More than fifty years ago, the first allogenic hematopoietic stem cell was transplanted in patients. However, the promise of other stem cell populations for tissue replacement and repair remains unachieved. When considering cell-based interventions for tissue regeneration, the factors influencing therapeutic success and safety are more complicated than for traditional treatments. So, it is important for dentists to have a brief understanding about the principles and clinical applications of stem cells in tissue engineering field.

**Conclusion:** This article reviews the most recent published data to regenerate dental and periodontal tissues based on scientific principles and provides fundamental information to readers about the different aspects involved in tissue engineering. Ideal combination of cells, scaffolds, and growth factors for tissue engineering may be extended over future years. The findings collected in this literature review show that we are now at a stage in which engineering a complex tissue, such as the periodontium, is no longer an unachievable goal and the next decade will certainly be an exciting time for dental and periodontal research.

**Key Words:** Periodontal regeneration – Stem cells – Literature review

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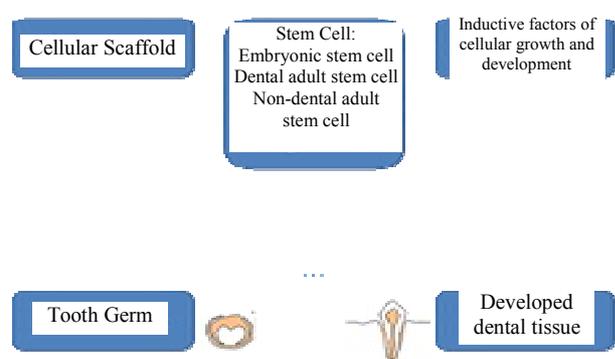
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### Introduction

The term tissue engineering was described by Vicanti and Langer in 1993 as a process through which tissues and organs are reconstructed by cellular grafts with or without a cellular scaffold. Contrary to important achievements no major progress has been made as expected in this field

for almost 20 years [1]. Tissue engineering is plausible with the use of a biocompatible scaffold, suitable growth factors and stem cells. Development of a tissue and an organ is an active and dynamic process and the resultant structures following developmental processes are maintained by the means of hemostatic and homeo-

static processes. There are similarities between reconstruction and repair mechanisms of an organ and its primitive development, therefore one should mimic these mechanisms to achieve the optimal goal of construction of a total organ [2]. The ability to widely proliferate and differentiate embryonic and adult stem cells made it possible to make use of these cells in reconstruction of injured tissues and organs [3]. In recent years more considerations have been paid to molecular and ultrastructural aspects of stem cells [4]. Tissues are derived from different types of cells that conform a network in extracellular matrix and such a structural discipline is achieved through the effects of genetic and epigenetic factors [5]. This mechanism was more complicated than previously thought. Intercellular relationships, their proliferation cycles, apoptosis processes, as well as synthesis and secretion of extracellular matrix components and molecular signals, growth factors and differentiation are seen in a developing cell-rich environment. In general, tissue reconstruction is influenced by extracellular matrix [6]. Therefore, a supporting and conducting scaffold for cells in reconstruction process is a principal requirement for tissue engineering. (fig.1)



**Figure 1:** principles of tissue engineering in brief

A large number of studies are available in this field. Searching the following key words in PubMed reveals the total published articles about stem cells as follows; 178700 articles for *stem cell* , 1618 articles for *dentistry AND stem cell*

185 articles for *stem cell AND periodontology* and 212 articles for *stem cell AND periodontal regeneration*.

#### Types of stem cells

Stem cells are comprised of two groups of embryonic and adult types. Human embryonic stem cells are pluripotent or totipotent cells, namely, they can give rise to all cellular lineages. These cells which are obtained from blastocyst stage of embryonic development are capable of giving rise to more than two hundred different cell types in human body [7]. These cells are unique to be able to survive undifferentiatedly in laboratory conditions for an unlimited time while keeping their ability to differentiate into all specialized cell types. Adult stem cells are found in most of the embryonic and adult tissues. These cells are derived from tissues that permanently regenerate such as peripheral blood and gustatory epithelium as well as tissues with reduced regeneration ability such as brain. It appears that adult stem cells act in long term preservation of the tissue or replacement of injured or lost cells. [8] Adult stem cells are mostly derived from bone marrow which consists of hematopoietic and mesenchymal stem cells. Hematopoietic stem cells were the first cells successfully used in medical treatments especially in treatment of leukemia and immune deficiency syndromes, but these cells are not able to give rise to connective tissue [9] Mesenchymal stem cells (MSCs) are able to be therapeutically used in a wide spectrum of musculoskeletal, cardiac and immune disorders. These are multipotent stem cells and are easily isolated and are of major importance in research. These cells are present in tissues of bone marrow, fat, periosteum, synovial membrane, skeletal muscle, dermis, blood, pericytes, trabecular bone, umbilical chord, lung, dental pulp and periodontal ligament. In addition to the role of these cells in natural growth, they act as parts of adult tissue repair. Nowadays, it has been proved that MSCs play roles in regeneration of cartilage, bone, fat, muscle, tendon, skin, and neural tissue [10] Two major resources of

MSCs are bone marrow and adipose tissue. Both have an equal capability to give rise to cells and tissues of mesodermal origin. The advantage of adipose MSCs is their availability. These cells are similar in their morphology, immune phenotype, isolation and colony density. Adipose-derived stem cells have a reduced ability to differentiate into bone and cartilage [11]. The majority of investigations have been performed on mesenchymal bone marrow stem cells. These cells have shown to possess the ability to produce osseous and cartilaginous tissues in vivo. [12]

#### **Types of dental stem cells**

Five different stem cell types have been isolated and defined up to now including:

- \*Dental pulp stem cells (DPSCs)
- \*Stem cells from exfoliated deciduous teeth (SHED)
- \*Periodontal ligament stem cells (PDLSCs)
- \*Stem cells from apical papilla (SCAP)
- \*Dental follicle progenitor cells (DFPCs)

Dental stem cells are isolated from tissues with an ability to give rise to dental cells and have properties similar to those of mesenchymal cells including the ability of self-renewal and the ability to give rise to similar cell lineages. Of course, this ability is different from that of bone marrow mesenchymal stem cells. (See table 1)

#### **Examples of application of stem cells in repair and regeneration of dental tissues**

Stem cells can be used for repair of damaged cells in pulp and dentin. Even tissue engineering has recently been used for enamel defects. Literature has indicated that cells isolated from third molar pulps can produce a structure similar to dentin-pulp complex in mice [13]. Cells isolated from human deciduous teeth are able to produce pulp-like tissue [14]. In a study, dentin and periodontium was regenerated after simultaneous implantation of stem cells of apical papilla and periodontium within dental sockets of guinea pigs. The results of such investigations can provide hope that stem cells be replaced by concurrent implants in regeneration of tooth roots.

Dental pulp regeneration has also been investigated and several studies have disclosed the possibility of regeneration of lost pulpal tissue. This treatment has been considered for revitalization and regeneration of extirpated infectious pulp. A pulp-like tissue with an adequate blood supply has been observed following 3-4 months of subcutaneous implantation of stem cells of apical papilla and dental pulp stem cells in mice. [15,16]

#### **Total tooth regeneration**

Tooth loss is an unfavorable problem especially when accompanied by loss of bone. Therefore, complete regeneration of bone with a favorable dental function is a big challenge in dental research. One recent study focused on use of embryonic and post-natal dental germ cells [17].

In another study murine embryonic dental mesenchymal cells were implanted in murine renal capsule and new tooth germ, although malformed, was observed after 3 weeks. Other studies have also shown that enamel-like tissues can be produced from epithelial rests of Mallassez [18, 19]

Application of stem cells in regeneration of periodontal tissue (periodontal tissue engineering) requires progenitor cells, growth factors for differentiation of the cells and also a suitable cellular scaffold.

#### **1. Cellular precursors for periodontal regeneration**

Periodontal tissue is derived from a population of neural crest cells referred to as dental follicle. Dental follicle forms a dense connective tissue sheath around the developing tooth. At the time of differentiation and root formation, the dental follicle forms alveolar bone, cementum and PDL. The second source of periodontal ligament tissue precursors are located around blood vessels of this area and have a role in its regeneration following root formation [20] In addition to the dental follicle and PDL progenitor cells, use of other progenitors such as mesenchymal bone marrow stem cells and cemental progenitor cells are reported to be successful in regeneration of PDL. Suitability of these progenitors are rooted

**Table1:** Capabilities of different stem cells in brief

Stem cell type	Regeneration capability	Regeneration experience in animal models
Dental pulp stem cells	bone/tooth/fat/ cartilage/muscle/nerve	pulpodentin complex-type structure odontoblast-like cell osseous-like tissue
Stem cells of apical papilla	tooth/fat/cartilage/ muscle/nerve	Pulp-dentin like tissue Odontoblast- like cell Cementoid tissue
Periodontal ligament stem cell	bone/cementum/fat/ cartilage/muscle/nerve	Periodontal membrane like tissue
Dental follicle progenitor cells (DFPCs)	cementum/tooth /fat/ cartilage/muscle	Formation of cementum ground substance

in their flexibility. In other words, an adult stem cell is able to transform into different target tissues using proper inductive agents [21]. Although the ability of progenitors and adult stem cells in production of variable periodontal lineages has been well established, the conducting factor(s) that lead(s) such regenerations toward formation of a true biologically functional periodontium is still unclear.

#### 1.1. Dental follicle progenitor cells (DFPCs)

Dental follicle consists of a population of immigrating neural crest cells that give rise to periodontium. Although it has a histologically uniform configuration, it contains a number of heterogenic cell populations from osteogenic lineages to undifferentiated mesenchymal cells. [22] Dental follicle contains ideal progenitors for regeneration of periodontal tissues. Implanted DFPCs form a fibrillar tissue that is able to re-assemble PDL. Although DFPCs form PDL like fibers, it is still unclear that whether these fiber are able to mineralize to give rise to cementum or alveolar bone. Certainly, some investigations have declared that osseous-like tissues are also produced [23]

Hydrogel implanted DFPCs produce mineral precipitates following a favorable culturing condition, but their osteogenic activity is less than that of bone-marrow-derived mesenchymal cells or PDLPGs.

#### 1.2. Periodontal Ligament Progenitor Cells (PDLPGs)

Osteogenic and cementogenic activity of these cells specifically following induction by growth factors and extrinsic proteins is higher in comparison with DFPCs. It is probable that at least some of the PDL progenitors are derived from migratory cells located in alveolar bone. This might be a reason for their improved responsiveness to mineralization conditions [22]. In a study on dogs, use of PDLPGs showed promising results in regeneration of alveolar bone in furcation involvements [24].

#### 1.3. Cemental progenitor cells

It is not clear whether the origin of cellular and acellular cementum producing cells is similar. However, the difference exists in their gene expression and mechanisms of cementum deposition. After formation of cemental matrix, cementoblasts remain within the cellular cementum to change into cementocytes. On the other hand cementoblasts in acellular cementum lie down on the cementum surface. Biochemical composition of these two types of cementum are probably different and require their specific cells and factors for their formation [22]. Some cementum-derived cells have shown an ability to produce mineral tissue after implantation in mouse [25]. Cementum-derived cells and cementoblasts are very good examples for evaluation of the effects of cementum-specific mineralization agents during cementogenesis. Surely,

selective stimulation is a very complicated process for mineralization of cementum.

## **2.Required inductive agents for periodontal regeneration**

Agents present in extracellular matrix environment around progenitor cells have a central control on their lineage, cellular behavior, and fate. Platelet-derived growth factor (PDGF) is one of the initial growth factors that is naturally produced in repair of periodontal injuries. It is abundantly found in alveolar bone and during cemental regeneration, showing its considerable potential in reconstruction of lost periodontium. [20] Fibroblast growth factor (FGF) is an important chemical mediator in cellular migration and proliferation. Two mechanisms have been depicted for the positive role of FGF in periodontal regeneration: 1) angiogenesis and initiation of wound healing procedure and 2) its role in growth of immature PDL cells. Connective tissue growth factor (CTGF) plays an integral part in development of dental organ, periodontal tissue regeneration, mesenchymal tissue regeneration and wound healing. CTGF belongs to a superfamily that initiates fibroblast proliferation, migration and attachment as well as extracellular matrix formation. Transforming growth factor beta (TGF- $\beta$ ) induced formation of PDL-like fibers in class II furcation defects eight weeks after implantation in non-human samples [22]. Certain types of bone morphogenetic protein (BMP) cause induction of new hard tissue formation. Certain types of BMP are capable of inducing new cementum and alveolar bone formation [26], although one of the adverse effects of BMP-2 treatments is ankylosis [22].

### **1.Periodontal regeneration scaffold**

PDL is surrounded by two mineralized tissues i.e., cementum and alveolar bone. This structure stabilizes PDL network and is composed of collagen and hydroxyl-appatite. Varying types of scaffolds have been utilized in simulation of these tissues [22]. The most common types of these scaffolds are made of collagen, natural extracellular matrix, calcium phosphate, resorbable syn-

thetic polymers and peptides. Regarding abundance of papers, some examples of application of stem cells in periodontal regeneration are separately considered in laboratory, animal and human models.

### **a.Application of stem cells in periodontal regeneration in vitro**

PDL progenitor and stem cells are reported to produce osteogenic, adipose, myofibroblastic (mesodermal derivatives), and neural crest like cells [27], as well as chondrocytes [28], cementocytes, collagen and PDL producing cells [29] after being placed in proper culture medium and use of inductive agents. It is theorized that PDL stem cells can be used for differentiation of PDL producing cells in treatment of periodontal diseases [27] Osteoid and cementoid structures have been produced following implantation of these cells in mice [30] Differentiations pertinent to cementogenesis were observed following influencing non-collagenous proteins of dentin on human PDL stem cells [31]. Cementoblast and osteoblast were produced in a favorable culture medium by PDL stem cells of dog [32].

Cementoblast, PDL cells, and osteoblast were produced under the influence of BMP and EMD by human stem cells of dental follicle in vitro. Osteo- and cementoblast-like cells were also produced following the application of insulin and dexamethasone [33,34]. Hydrogel-implanted progenitor cells of dental follicle lay down mineral precipitates in a favorable culturing environment but indicate reduced osteogenic activity with respect to bone marrow stem cells and PDL progenitor cells [22].

### **b.Practical results from application of stem cells in periodontal regeneration in animals**

#### **1.Mesenchymal stem cells**

In an investigation alveolar bone was removed from above first molar root in mice to produce periodontal defects. Gel scaffolds accompanied by bone marrow-derived mesenchymal stem cells (MSCs) were placed in the defects. Control mice received only gels without scaffolds. New

bone, cementum and PDL formation was observed in all mice but more bone and functionally better periodontal fibers were produced in those who received stem cells [35]. Bone marrow stem cells of iliac crest have also been utilized to treat class II furcation involvement defects in dogs, with the final result of new bone, cementum and PDL formation in the area [36]. It has been depicted that bone marrow and other mesenchymal stem cells retained their capability to produce cementum, PDL and alveolar bone following implantation in immunodeficient dogs [37]

### **2.PDL stem cells**

PDL stem cells have been used in treating periodontitis in pigs with promising results in periodontal regeneration [38]

With the use of a novel technique called three-dimensional culturing cementum and PDL formation was achieved following implantation of human PDL stem cells in mice- a technique called tissue engineering of cementum-PDL complex. Reconstruction of alveolar bone was accomplished following use of PDL progenitor cells in furcation defects of dogs [24] PDL stem cells were also used in treatment of surgically induced periodontal defects in immunodeficient mice. Ability of only 61% of these cells to produce cementum- and PDL-like structures indicated that these cells were within varying differentiation stages prevailing the whole stem cell population to be differentiated into PDL-forming cells [40]. Murine PDL progenitor cells were used to produce PDL around titanium implants. At the site of tooth loss, periodontium was formed surrounding the inserted implant [41] Human PDL progenitor cells were grafted to the murine first molar following placement in a proper culture medium with eventual formation of PDL fibers and a tissue resembling acellular cementum [37] Autologous grafts of dental stem cells were used to treat advanced periodontitis in hounds. In this study three groups of dental stem cells namely dental pulp stem cells, dental follicle progenitor cells, and PDL stem cells were

used. PDL stem cells had a superior efficacy in regeneration of periodontal fibers, alveolar bone and cementum [42].

### **3.Dental follicle stem cells**

Implantation of dental follicle stem cells have been carried out with the aim of PDL formation in mice [23]. This technique resulted in scarce bone and cementum formation. However, these cells possess better flexibility and differentiation in comparison with other dental stem cells [42]. Stimulated human stem cells accompanied with EMD gel caused more cementum, new alveolar bone, and PDL formation in comparison with control group after implantation in mice [43].

### **c.Application of stem cells in regeneration of human periodontium**

In a study on three patients, re-implantation of the patients' autologous PDL progenitor cells was used in order to regenerate intrabony periodontal defects. The subjects included two 25-year-old and another 42-year old men. Their periodontal pocket depths were recorded to be 4.8 to 10 mm. The subjects were observed for 32 through 72 months for their probing depths, gingival recession, and attachment gain. Use of PDL progenitor cells eventually lead to surprising therapeutic results. Within the observation period, no complications were reported. Parallel with this experiment, PDL stem cells were implanted in mice following placement in an appropriate culture medium. The authors concluded that PDL progenitor and stem cell are similar in terms of their proliferation ability, expression of mesenchymal surface molecules, and ability of multiple differentiations [44].

### **Conclusion**

Dental involvement is frequently seen in a major part of population. Therefore, its repair and regeneration has been at the center of attraction for a large number of investigators. Novel cellular treatments can provided hope to provide regeneration and replacement of injured tissues in near future. Definitely, this method is confronted with a considerable challenge casting doubt on its

feasibility. Availability of stem cells with a high proliferation ability and ease of isolation from lost or extracted teeth are considered contributing factors in the widespread propensity to such investigations. Evaluation of the available literature indicates that there is a long distance between tissue engineering and clinical experience. Differentiation of human cells is time consuming and it is very difficult to provide a controlled culturing condition for a long time. Any molecular change in the environment causes formation and differentiation of other types of cells that might have been contradictory with tissue regeneration purposes. It is unclear whether use of stem cells can completely provide tissue regeneration. Neoplastic changes of these cells due to mutations and genetic changes are also possible. The possibility of transplant rejection also should not be overlooked. In addition embryonic stem cells are able to produce teratoma.

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