



## TISSUE ENGINEERING IN REGENERATIVE ENDODONTICS -A REVIEW

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### ABSTRACT

Tissue engineering is mainly used to replace the impaired or damaged tissues with new tissues. The key cells involved for tissue engineering are stem cells, the morphogens or growth factors. They will rapidly multiply and differentiate and form tissues. This new technique is now most commonly used in endodontics. The aim of this study was to review about the dental pulp stem cells, which are most common growth factors, and the scaffolds used to control their differentiation. To study the clinical technique for the management of immature non-vital teeth based on this novel concept.

**KEYWORDS:** Dental pulp stem cells, morphogens, scaffolds, regenerative endodontics, pulp revascularization.

### INTRODUCTION

Now a day's root canal treatment has high level of success for several conditions. The main concept in this treatment is regenerative approach. In which replace diseased or necrotic pulp tissues with healthy pulp tissues, mainly to revitalize teeth. This type of regenerative approach if we are using in endodontics it's called as regenerative endodontics. This method provides a new range of biologically based clinical treatments in endodontic diseases.

#### Tissue Engineering

Tissue engineering is a rapidly developing field. It has the principle of engineering and life science. These principles are mainly applied for, the development of biological substitutes which can restore, maintain or improve functioning of tissue. The major elements involved for tissue engineering are stem cells, morphogens or growth factors, and an extracellular matrix scaffold. The chief tissues required for regenerative endodontics are dentin, pulp, cementum and periodontal tissues <sup>[1-3]</sup>.

#### Key Elements for Tissue Engineering

#### Stem cells

These were considered to be the most important cells in regenerative medicine.

Research on stem cells provided knowledge about, the development of an organism from a single cell, and how healthy cells can replace damaged ones in adult organisms. Stem cells have the capacity for continuous division. Either for self-replication (replicate themselves), or multilineage differentiation (to produce specialized cells which can differentiate into various other types of cells or tissues) <sup>[4]</sup>.

#### Types of stem cells

##### Early embryonic stem cells

The human development occurs with the division of newly fertilized egg or zygote. It produces a group of stem cells called an embryo. These early stem cells are called totipotent, which means these can be developed to any kind of cell in the body.

### ***Blastocyst embryonic stem cells***

After five days of fertilization, embryo develops a hollow ball-like structure which is known as blastocyst. The embryonic stem cells of the blastocyst are pluripotent, means they have the ability to become almost any kind of cell in the body. Researchers can induce these cells to create a specialized cells. But, this method is not yet fully developed. Mainly due to the source of embryonic stem cells which is controversial, and it's mainly associated with ethical and legal issues. This reduced the development of new therapies<sup>[5]</sup>.

### ***Fetal stem cells***

8 weeks after development of embryo is known as a fetus. By this period a human-like form will be developed. Fetal stem cells were responsible for the initial development of all tissues before birth. Fetal stem cells were also pluripotent.

### ***Umbilical cord stem cells***

Fetus gets the nutrients and oxygen rich blood from placenta by umbilical cord. Umbilical cord blood contains stem cells, which are genetically similar with new born. Stem cells of umbilical cord are multipotent. They can be developed to a limited range of cell types. For the purpose of medical therapy umbilical cord stem cells can be stored cryogenically.

### ***Adult stem cells***

These stem should be called as post natal stem cells. Because, these cells are also present in infants and children. These cells mainly stay on tissues which are already developed, and they maintain the growth of these tissues throughout life.

These were also multipotent. Usually adult stem cells generate the same cell types, on which they are residing. Researchers had observed plasticity with these cells. It means that stem cells from one tissue may develop cells of different tissues<sup>[6]</sup>. These particular stem cells were present on almost whole body tissues<sup>[7]</sup>, including dental tissues<sup>[8, 9]</sup>. Till now, four types of human dental stem cells have been isolated and characterized: i) Dental pulp stem cells (DPSCs)<sup>[10]</sup>, ii) Stem cells from human exfoliated deciduous teeth (SHED)<sup>[11]</sup>, iii) Stem cells from

apical papillae (SCAP)<sup>[12,13]</sup>, and iv) Periodontal ligament stem cells (PDLSCs)<sup>[14]</sup>.

All these dental stem cell were developed from permanent teeth, except SHED. Identification of these stem cell provided a better knowledge, on the regenerative property of pulp and periodontal ligament tissues after tissue damage<sup>[1]</sup>.

### **Progenitor cells**

Generally intermediate cells are known as precursor or progenitor cells. These progenitor cells are developed from stem cells. Before to the full differentiation of stem cells these cells were formed. These cells will be differentiated along a particular cellular development pathway. Till the stem cells get the property of multitissue differentiation and self renewal properties, they were considered as progenitor cells<sup>[15]</sup>.

### **Dental pulp stem cells (DPSCs)**

Gronthos et al first isolated DPSCs in the year 2000. They have capacity to regenerate dentin pulp complex similar to regeneration caused by normal human teeth. Later, same group identified<sup>[16]</sup>, that these cells have a high proliferative capacity, a selfrenewal property and a multi-lineage differentiation potential. Scientists also isolated a selected subpopulation of DPSCs called as Stromal Bone-producing Dental Pulp Stem Cells (SBP-DPSCs). These are multipotential cells, they have the ability to give a variety of cell types and tissues. They are osteoblasts, adipocytes, myoblasts, endotheliocytes, and melanocytes, as well as neural cell progenitors (neurons and glia), being of neural crest origin<sup>[17-21]</sup>.

Several studies were conducted on DPSCs<sup>[10, 16, 22-30]</sup>. These studies has shown that DPCs were multipotent stromal cells, they have extensive proliferative capacity, they can be safely cryopreserved, they have several scaffolds of applications, they posses long lifespan, immunosuppressive properties<sup>[31]</sup>, and they can form mineralized tissues which is similar to dentin<sup>[32, 33]</sup>.

Paakkonen et al. demonstrated that DPSCs got gene expression pattern which was similar to the mature native odontoblasts. This property can be helpful for in vitro studies of odontoblasts, that they can form a humanderived cell line.<sup>[34]</sup> However, no

definite proof was established for their ability to produce a dentin. R Takeda et al. isolated the hDPSCs from tooth germs at the crown-completed stage. They had observed that these cells were highly proliferative and had the potential to generate a dentin-like matrix *in vivo*.<sup>[35]</sup>

These properties are not long lasting due to changes in gene expression profile. Abe et al. isolated apical pulp derived cells (APDCs) present on human teeth. They described that these cells has the capacity to regenerate even on hard tissues.<sup>[36]</sup>

### SHED

These cells were first isolated by Miura et al.<sup>[11]</sup>. They observed that these cell have a greater capacity to regenerate to a variety of cells in comparison to DPSCs. They can regenerate to neural cells, adipocytes, osteoblast-like and odontoblast-like cells. The main function of these cells is to form mineralized tissue<sup>[18, 37, & 38]</sup>. This can be used for regeneration of orofacial bone<sup>[39]</sup>.

Because of the ethical issues associated with the use of embryonic stem cells, and limited availability of autologous postnatal stem cells with multipotentiality, SHED has become an alternate source for dental tissue engineering<sup>[11]</sup>. Compared to the stem cells from adult human teeth, SHED was more helpful for tissue engineering. They got high proliferation rate than stem cells from permanent teeth<sup>[11]</sup>. They can also be retrieved from a tissue that is disposable and readily accessible<sup>[40]</sup>. They were suitable for young patients during mixed dentition, who were already suffered with pulp necrosis immature permanent teeth because of trauma.<sup>[41]</sup>

### SCAP

Sonoyama et al discovered a new variety of population of mesenchymal stem cells (MSCs), which were residing on the apical papilla of permanent immature teeth. They were also known as stem cells from the apical papilla (SCAP)<sup>[13]</sup>. They reported that these cells express various mesenchymal stem cell markers. These cells has the capacity to form odontoblast-like cells, producing dentin *in vivo*. They were also primary source for odontoblasts formation on root dentin. It was observed that on the infected immature permanent teeth with periradicular periodontitis or abscess, apexogenesis may occur. It is mainly due to the presence of SCAP on the apical papilla. It can survive

during pulp necrosis, mainly because of its proximity to the vasculature of the periapical tissues. So, these cells has the capacity to generate primary odontoblast for complete root formation after the endodontic disinfection<sup>[13]</sup>.

### Periodontal ligament stem cells (PDLSCs)

Seo et al fist described the presence of multipotent postnatal stem cells in the human PDL (PDLSCs). They used same methodology which was used for isolation of MSCs from deciduous and adult pulp for these cells also<sup>[42]</sup>. They described that PDLSCs can differentiated into cementoblast-like cells, adipocytes, and collagen-forming cells under definite cell cultures. In immunocompromised rodents, PDLSCs generated a cementum/PDL-like structure and promoted the healing of periodontal tissue repair. Trubiani et al also supported the presence of MSCs in the periodontal ligament<sup>[43]</sup>. They isolated and characterized a population of MSCs from the periodontal ligament which expressed a variety of stromal cell markers. Shi et al.<sup>[44]</sup>, demonstrated that generation of cementum-like structures associated with PDL-like connective tissue after transplanting PDLSCs with hydroxyapatite/tricalcium phosphate particles to immunocompromised mice. These cells got several clinical applications, mainly because even after isolation from cryopreserved periodontal ligaments they maintained its stem cell properties. Which were the expression of MSC surface markers, multipotential differentiation, single-colonystrain generation and cementum/periodontal-ligament-like tissue regeneration. These properties made them an instant source for MSCs<sup>[45]</sup>. By using a minipig model, autologous SCAP and PDLSCs were loaded onto hydroxyapatite/tricalcium phosphate and gelfoam scaffolds. Then they were implanted on sockets of the lower jaw, there they formed a bioroot encircled with periodontal ligament tissue in a natural relationship with the surrounding bone<sup>[46]</sup>. Trubiani et al. observed the regenerative property of PDLSCs when they were cultivated on a threedimensional biocompatible scaffold. This property was useful in making of graft biomaterials, for bone tissue engineering in regenerative dentistry.<sup>[47]</sup> Li et al. Observed that, PDLSCs when seeded on bioengineering produced cementum and periodontal ligament-like tissue formation.<sup>[48]</sup>

### Stem cells culturing

The growth and maintenance of cells in a controlled environment outside an organism was referred as cell culture. Ideal goals of stem cell culture are to keep the cells healthy, dividing, and unspecialized.

Dental pulp stem cells were mainly cultured by two methods; the first method is enzyme-digestion method [10,11,13,49]. In this, using sterile conditions pulp tissue was collected. Then it was digested with suitable enzymes. The formed cell suspensions were cultured on dishes with a special medium. They were provided with necessary additives and properly incubated. The resulting colonies were sub cultured before confluence and then cells were stimulated for differentiation.

The explants outgrowth method was the second method of isolation of dental pulp stem cells [50-53]. In this method, initially the extruded pulp tissues were cut into 2-mm<sup>3</sup> cubes. Then they were anchored via microcarriers onto a suitable substrate, and they were directly incubated in culture dishes which contain the essential medium with supplements. It takes around 2 weeks of time for sufficient number of cells to grow and come out of the tissues. Haung et al. compared both these methods and concluded that, cells isolated by enzyme-digestion has high proliferation rate than isolated by outgrowth method. [54]

### Stem cells differentiation

Cell differentiation is the process in which specialized cells were generated from unspecialized stem cells. It is mainly triggered by signals from both inside and outside of the cells. The internal signals were controlled by the genes of cells. The genetic information will be carried across DNA, leading generation of coded instructions for the structural maintenance and functioning of a cell. Whereas the external signals for cell differentiation may include several factors. Which were chemicals secreted by neighboring cells, physical contact with other cells and presence of certain molecules in the microenvironment. Depending upon the contents of media cultured dental pulp stem cells can be stimulated to differentiate to more than one cell type. Osteo/dentinogenic medium mainly contains dexamethasone, glycerophosphate, ascorbate

phosphate and 1,25 dihydroxy vitamin D with other basic elements [10]. Adipogenic medium contains dexamethasone, insulin and isobutyl methylxanthine [55]. For neurogenic induction of cells they were cultured in the presence of B27 supplement, basic fibroblast growth factor and epidermal growth factor. [11]

### Cell lines

The first step involved in making cell lines is to cultivate the stem cells. For research and development purpose genetically identical cells should be collected and cultivated. After attaining a stable stem cell line, it can be stimulated to differentiate into specialized cell types. Generally odontoblasts cannot be induced for further differentiation, because they were postmitotic terminally differentiated cells. Odontoblast after their full differentiation produces various proteins. One is type I collagen, it forms the scaffold for mineral deposition and provides strength for mineralizing dentin. The two other major noncollagenous proteins (NCPs) had mineralization-regulatory capacities [56]. These proteins are dentin phosphophoryn (DPP; or DMP-2) and dentin sialoprotein (DSP)<sup>[57]</sup>. These two proteins were encoded by a single gene. The phenotypic characteristics of dentin were explained by DSPP or DMP-3<sup>[58-61]</sup>. One more important non collagenous protein is dentin matrix protein-1 (DMP-1). It was found primarily in dentin and bone. It mainly helps for regulating mineralization [62-64]. While DPSCs differentiation DMP-1 acts as a growth factor [65, 66].

Odontoblast cell line is useful to explore the pulp wound-healing mechanism and also to develop therapeutic strategy for pulp regeneration. odontogenic differentiation was not fully understood mainly because of two limitations.

The first is the paucity of differentiation markers, it is now overcome by the characterization of odontoblastspecific markers (DMP-1, DMP-2, and DMP-3) that can indicate the presence of a true odontoblastic cell line [61, 67 & 68]. The second limitation is the limited life span of the primary cells [69]. It's been addressed by several methodological trials including cell cloning and immortalization [61, 70-74].

## Growth factors

Morphogenesis or organogenesis is mainly mediated by signals produced by growth factors. The specialization or division of stem cells to the required cell type was mainly regulated stem cells. They mediate several key cellular events during tissue regeneration, which includes cell proliferation, chemotaxis, differentiation, and matrix synthesis [75]. Growth factors are of two types, one type is quite versatile, and it stimulates cellular division in numerous cell types, while the other one is more cell-specific. Growth factors are used for various purposes. 1) Certain growth factors were mainly used to increase stem cell numbers. Likewise platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) [76], insulin-like growth factor (IGF), colony-stimulating factor (CSF) and epidermal growth factor (EGF). 2) While few growth factors modifies the humoral and cellular immunity (interleukins 1- 13). 3) Whereas few growth factors regulate angiogenesis, e.g. vascular endothelial growth factor (VEGF) [77, 78]. Another e.g. for growth factor, transforming growth factor is helpful in wound healing and tissue regeneration/ engineering [75, 79, & 80]. 4) Growth factor such as bone morphogenic proteins (BMPs) was helpful in tooth development [81, 82] and regeneration [3].

## Bone morphogenetic proteins (BMPs)

These are multi-functional growth factors, they belongs to the family of transforming growth factor [83]. The BMPs were first identified by their ability to form ectopic bone formation, while implanted under the skin of rodents [84]. Till now, around 20 BMP family members have been identified and characterized. They have unique biological activities *in vivo*, it's mainly because of their differences in profiles of expression, affinities for receptors binding [85]. BMPs play a major role in teeth formation. Their dictation leads to initiation, morphogenesis, cytodifferentiation, and matrix secretion will occur. BMP family of growth factors is important in forming the enamel knot of teeth. Without these BMPs there won't be teeth formation [86]. BMPs [87-90] and growth factors [91] together were used directly in capping pulp. This phenomenon of adding of growth factors to stem cells was helpful in tissue engineering and to replace the diseased tooth tissues.

BMPs are used for two types of therapies for dentin regeneration. The first is the *in vivo* therapy, in which BMPs or BMP genes are directly applied to the exposed or amputated pulp. The second is *ex vivo* therapy, first DPSCs was isolated. Further they will be differentiated to odontoblasts with recombinant BMPs or BMP genes. Last step is their autogenous transplantation to regenerate dentin [86]. BMP-2 plays a crucial role in dentin regeneration [92]. Recombinant human BMP-2 increases the odontoblast like stem cells by promoting the differentiation of adult pulp stem cells in culture [53, 93 & 94]. It promotes the expression of dentin sialophosphoprotein (DSPP) gene *in vitro* mainly by promoting their alkaline phosphatase activity. [53] It also increases the hard tissue formation *in vivo* [95]. Dentin formation can be promoted by autogenous transplantation of BMP-2-treated pellet culture onto amputated pulp [96]. Even BMP-7 also showed same findings. It also known as osteogenic protein-1. It promoted the reparative dentinogenesis and pulp mineralization in several animal models [97-103]. Lin et al. [104] generated a BMP-7-expressing adenoviral vector that induced the expression of BMP-7 in primarily cultured human dental pulp cells. This expression led to a significant increase of alkaline phosphatase activity and induced the expression of DSPP, suggesting that BMP-7 can promote the differentiation of human pulp cells into odontoblast-like cells and promote mineralization *in vitro*. However, a novel role has been suggested for BMP-4, which is secreted by mesenchymal cells, in the regulation of Hertwig's epithelial root sheath (HERS) during root development by preventing elongation and maintaining cellular proliferation. Therefore it has been utilized as an agent for regulating root formation in a variety of tissue engineering applications [105].

## Scaffolds

Scaffolds are used to provide a physicochemical and biological three-dimensional microenvironment or tissue construct for cell growth and differentiation. It can be implanted singly or in combination with growth factors and stem cells. [66,106 - 108]

## Ideal requirements of a scaffold [66,109 -112]

(a) Should be porous to allow placement of cells and growth factors.

- (b) Should allow effective transport of nutrients, oxygen, and waste.
- (c) Should be biodegradable, leaving no toxic byproducts.
- (d) Should be replaced by regenerative tissue while retaining the shape and form of the final tissue structure.
- (e) Should be biocompatible.
- (f) Should have adequate physical and mechanical strength.

### Types of scaffold

#### a) Biological/natural scaffolds

These consist of natural polymers such as collagen and glycosaminoglycan. It offers good biocompatibility and bioactivity. The tensile strength of tissues is mainly increased by collagen. Collagen is present mainly on extracellular matrix. As a scaffold, collagen allows easy placement of cells and growth factors and allows replacement with natural tissues after undergoing degradation<sup>[113-115]</sup>. It is observed that pulp cells in collagen matrices undergo marked contraction, this might affect the pulp tissue regeneration<sup>[54,116]</sup>.

#### b) Artificial scaffolds

These are synthetic polymers. They have controlled physicochemical features such as degradation rate, microstructure, and mechanical strength<sup>[112]</sup>, for example:

- Polylactic acid (PLA), polyglycolic acid (PGA), and their copolymers, poly lactic-co-glycolic acid (PLGA).
- Synthetic hydrogels include polyethylene glycol (PEG)- based polymers.
- Scaffolds modified with cell surface adhesion peptides, such as arginine, glycine, and aspartic acid (RGD) to improve cell adhesion and matrix synthesis within the three-dimensional network [117].
- Scaffolds containing inorganic compounds such as hydroxyapatite (HA), tricalcium phosphate (TCP)

and calcium polyphosphate (CPP), which are used to enhance bone conductivity<sup>[118]</sup>, and have proved to be very effective for tissue engineering of DPSCs<sup>[119,120]</sup>.

- Micro-cavity-filled scaffolds to enhance cell adhesion<sup>[121,122]</sup>.

### Scaffolds for tissue engineering

Various researchers have showed that pulp cells can be isolated, multiplied in culture, and can be seeded onto a matrix scaffold. The new tissue formed by cultured cells was similar to that of the native pulp.<sup>[10, 22, 41, 66, 111, 123-127]</sup> These results were helpful to generate pulp and dentin in pulpless canals. Blood flow is necessary for vitality of the implanted cells. While implanting cells into root canals, vascularization should be increased. Because, they will have blood only from apical end. To promote the action, growth factors such as VEGF and/or platelet-derived growth factor or endothelial cells can be added.<sup>[46]</sup>

### Tissue engineering concept: Clinical applications

Several clinical studies showed that teeth apexification can be treated with apexogenesis<sup>[128-137]</sup>. We can also use biological based treatment, it promotes dentin of the root as well root tip formation<sup>[138]</sup>. Iwaya et al.<sup>[137]</sup> and Banchs and Trope<sup>[135]</sup> used the term 'revascularization' to describe this phenomenon, there is physiological tissue formation and regeneration. It is also possible that the radiographic presentation of increased dentinal wall thickness might be due to in growth of cementum, bone, or a dentin-like material<sup>[38,139-145]</sup>. There diversity in cellular response depending on growth factors or media to where it is cultured. For e.g. DPSCs can develop odontogenic/osteogenic, chondrogenic, or adipogenic phenotypes, depending on their exposure to growth factors and morphogens<sup>[146]</sup>.

### CONCLUSION

The clinical success rates of endodontic treatments can exceed 80%-90%. However, many teeth are not given the opportunity to be saved by endodontic treatment and instead they are extracted, with placement of an artificial prosthesis, such as an implant. Regenerative endodontic methods have the potential for regenerating both pulp and dentin tissues and therefore may

offer an alternative method to save teeth that may have compromised structural integrity

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