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Review

Basics of dentin-pulp tissue engineering

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Abstract: Regeneration is reconstruction of a tissue with the shape and function of the original tissue including vascularization and innervations. Highly degradation of dentin-pulp complex can not be reversed by its own repair mechanisms. For decades, endodontic treatments including pulpectomy and preparation of root canals have been the first choice for these cases. However, root canal treatment method has some unsatisfying consequences like; esthetic problems as a conclusion of discoloration caused by endodontic filling materials, undermined integrity of tooth structure, postoperative fractures, coronal leakage or periapical microleakage, lost sense of environmental changes which can make the recurrent caries or apical infection unnoticeable for patient and shortened lifetime in comparison with vital teeth. Currently regeneration of dentin-pulp complex by tissue engineering approach is thought to be a more appropriate choice instead of root canal treatment according to these outcomes. In this review, we will discuss basic components as stem cells, signaling molecules and scaffolds and also methods used for dentin-pulp tissue engineering.

Keywords: dentin; growth factors; scaffolds; stem cells; regeneration; pulp; tissue engineering

1. Introduction

Dentin and pulp tissues are specialized connective tissues derived from ectomesenchymal cells, formed from the dental papilla of the tooth bud [1]. According to the interactions between these two tissues, many investigators consider them as a single tissue which is called the dentin-pulp complex. Pulp-dentin complex has important functions as supplying nutrient to keep the mechanical properties of tooth tissues, serving as a sensory organ, inducing tertiary dentinogenesis and responding immunologically to bacterial infiltration to save tooth vitality which is one of the most important factors affecting the lifetime of the teeth [2].

Inflammation, and/or injury occurred in dentin-pulp complex can evoke a response as tertiary dentinogenesis [3–5]. Tertiary dentinogenesis can be subdivided into two as reactionary and reparative dentinogenesis according to the survival and death of the primary odontoblasts respectively. While reactionary dentin is secreted by post-mitotic primary odontoblasts and Hoehl's cells which can not be renewed after their destruction; reparative dentin is secreted by odontoblast-like or osteoblast-like cells which are differentiated from multipotent or monopotent progenitors located within the pulp [4–9]. These two mechanisms are induced especially by the signaling molecules found in dentin and released by demineralization and/or injury caused by acids produced by microorganisms, acidic components of restorative materials and acidic, basic and neutral chelating agents [5,9,10]. Nowadays, dentin is accepted as a bioactive extracellular matrix by being reservoir for various types of matrix molecules, growth factors, cytokines, neuropeptides, neurotrophic factors and serum/plasma proteins [3,9,11–14]. While higher concentrations of these molecules compromise cell survival, lower concentrations can stimulate odontoblast-like and endothelial cell proliferation.

Regeneration is reconstruction of a tissue with the shape and function of the original tissue including vascularization and innervations [15]. Differentiation of progenitor cells by the signaling molecules released from dentin matrix is the first step for regeneration of dentin-pulp complex. Pulp capping applications can induce tertiary dentinogenesis by releasing these molecules from dentin matrix but may not be effective for a total regeneration of dentin-pulp complex [11]. On the other hand highly degradation of dentin-pulp complex can not be reversed by its own repair mechanisms. For decades, endodontic treatments including pulpectomy and preparation of root canals have been the first choice for these cases. However root canal treatment method has some unsatisfying consequences like; esthetic problems as a conclusion of discoloration caused by endodontic filling materials, undermined integrity of tooth structure, postoperative fractures, coronal leakage or periapical microleakage, lost sense of environmental changes which can make the recurrent caries or apical infection unnoticeable for patient and shortened lifetime in comparison with vital teeth [2,11,13,15]. Currently regeneration of dentin-pulp complex by tissue engineering approach is thought to be a more appropriate choice instead of root canal treatment according to these outcomes. In this review, we will discuss basic components and methods of dentin-pulp tissue engineering.

2. Basic components of dentin-pulp tissue engineering

Regeneration of dentin-pulp complex has mainly three components [8–11,16–19] as: (1) stem cells which have capacity to differentiate in the cells of the original tissue, (2) scaffold, which act as a platform that provides a three-dimensional substrate/temporary matrix for developing cells and mimic the natural extracellular matrix (ECM), (3) signaling molecules which can provide chemotaxis, differentiation and proliferation of stem cells.

2.1. Stem cells

Stem cells are clonogenic cells with a capacity of self-renewal and multi-lineage differentiation [13,19,20]. They can be classified according to their potency which is related to the stages of cell division and differentiation [19]. While the number of division increases, potency of the cell decreases. The fertilization of an egg by a sperm forms a totipotent cell, zygote, which

divides into identical totipotent cells in the first hours. Totipotent stem cells can form embryonic and extra-embryonic tissues by differentiating into more than 200 cell types [11,19–21]. At blastocyst stage, inner cell mass contains pluripotent embryonic stem cells (ESC) that can differentiate into endoderm, mesoderm or ectoderm [2,13,19,21]. Multipotent or adult/post-natal stem cells are undifferentiated cells that are able to differentiate into multiple but limited numbers of cell types related with healing mechanisms of a tissue [10,11,19,21]. While oligopotent stem cells can differentiate into a few cell types, unipotent stem cells can only differentiate into cells of their own phenotype [19,21].

Although embryonic stem cells have a high potential for regeneration, using these cells is controversial according to ethical issues, procurement methods, risks of immunological rejection and tumorigenicity [2,11,19,20,22]. By reprogramming somatic cells, induced pluripotent stem cells (iPSC) were obtained with a pluripotent nature to overcome some of these issues [19,23–25]. Somatic cells even which are already differentiated can be reprogrammed as pluripotent stem cells by the over-expression of Oct3, Oct4, Sox2, Nanog, Klf4, c-Myc and Lin 28 [6,23]. Although it seems like a good alternative to use these cells as an autologous cell source, the protocols to obtain these cells are costly and technically challenging [6]. Adult stem cells which are found almost in all body tissues like bone marrow, adipose, skeletal muscle, peripheral blood, umbilical cord and dental tissues, have capacity of differentiation which can be efficient for tissue engineering [2,11,20].

Currently mesenchymal stem cells (MSC/adult stem cells) found in dental and nondental tissues are preferred for dentin-pulp tissue engineering according to their remarkable ability of self-renewal and plasticity when exposed to foreign microenvironment [11,12,26–35]. MSCs can have capacity to differentiate into osteo/odontogenic, dentinogenic, adipogenic, neurogenic, chondrogenic and myogenic cells [11,12,16,29–35]. For dentin-pulp tissue engineering dental stem cells searched till now were dental pulp stem cells (DPSC), stem cells from human exfoliated deciduous teeth (SHED), stem cells derived from apical papilla (SCAP), dental follicle stem cells (DFSC)/dental follicle precursor cells (DFPC) and tooth germ progenitor cells (TGPC) while mainly searched nondental stem cells are bone marrow derived mesenchymal stem cells (BMMSC), adipose derived stem cells (ADSC) and human umbilical vein endothelial cells (HUVEC) [26,27,36,37]. BMMSC are considered as gold standard for stem cell therapies and obtaining stem cells from the oral cavity is simpler and less invasive.

DPSCs are the first identified dental stem cells which have subpopulations of cells with different morphologies, sizes, proliferative rates and developmental potentials [13,29,31,38]. DPSCs are capable to differentiate into specialized cell types under in vitro, in vivo and ex vivo conditions [35]. They can generate both dentin-like tissue with a mineralized matrix and vascularized pulp-like tissues by differentiating into odontoblast-like cells [19,30,35]. Additionally although inflamed pulp is routinely discarded by pulpectomy, dental pulp stem cells with a regenerative capacity were derived from inflamed pulp (DPSC-IP) which can be an alternative cell source as a sound pulp tissue [35].

SHED, originating from neural crest, are the stem cells derived from exfoliated deciduous teeth especially incisors and canines. They believed to be an immature form of DPSCs with a higher growth potential, increased cell population doublings rate and differentiation capacity according to their higher levels of TGF-β2 (transforming growth factor) and FGF2 (fibroblast growth factor) [13,22,31,35]. SHED can be differentiated into odontoblast-like cells forming small dentin-like structures, pulp-like tissue, neural cells, adipocytes and osteoblasts [13,31,39].

Developing dental tissues like dental follicle, dental mesenchyme and apical papilla are also described as sources for MSC-like stem cells [31]. TGPCs are stem cells identified in the dental mesenchyme of the third molar tooth germ during the late bell stage [30,31]. They have a high proliferation activity and capability to differentiate into lineages of the three germ layers [31]. They are capable to form dentin and neural cells [30,35]. DFSCs are the stem cells derived from the dental follicle (especially from the dental follicle of third molars) surrounding the developing tooth germ prior to eruption [30]. They can form both pulp-dentin complex and cementum-periodontal ligament complex due to their heterogeneous characteristics [40]. SCAP are the stem cells isolated from the apical papilla of developing permanent teeth [30,32,35]. They show higher proliferation rate and dentin matrix regeneration ability than DPSCs. They can both differentiate to odontoblast-like cells forming an organized three-dimensional dentin-like structures and primary odontoblasts especially to form root dentin [22,32]. They even can survive after inflammation of pulp and disinfection of root canal due to their higher vascularized location then pulp chamber [32].

Adult stem cells present at a low frequency indicating that isolation of real stem cells can be challenging [10,16]. The mostly preferred method for cell purification to enrich the population of a specific cell type is labelling cell lineages with fluorescent antibodies and purifying them by FACS [10]. This can be succeeded by the identification of specific cell-surface markers for a particular cell type. The surface markers defined for dental stem cells are listed in Table 1.

Table 1. Markers for dental stem cells [10,30,35,41].

Dental stem cell type	MSC surface markers (CD antigen)		Other markers
	Positive	Negative	
DPSC	CD9, CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD146, CD166, CD271	CD14, CD19, CD24, CD31, CD34, CD45, CD117, CD133	Collagen, vimentin, laminin, nestin, fibronectin, Oct3, Oct4, Nanog, STRO-1,Sox-2, DSPP, OCN, BSP
SHED	CD13, CD29, CD44, CD56, CD73, CD90, CD105, CD146, CD166	CD11b, CD14, CD19, CD34, CD43, CD45	Nestin, Oct4, Nanog, STRO-1, DSPP, SSEA-3, SSEA-4
SCAP	CD13, CD24, CD29, CD44, CD51, CD56, CD61, CD73, CD90, CD105, CD106, CD146, CD166	CD14, CD18, CD34, CD45, CD117, CD150	Oct3, Oct4, STRO-1, DSPP, OCN, BSP, Nanog, Survivin
DFSC	CD9, CD10, CD13, CD29, CD44, CD53, CD59, CD73,CD90, CD105, CD106, CD166, CD271	CD31, CD34, CD45, CD133	Notch-1, Nestin
TGPC	CD29, CD44, CD73, CD90, CD105, CD106, CD166	CD14, CD34, CD45, CD133	Oct4. Nanog, Sox-2, klf4, c-myc

2.2. Signaling molecules

Tooth morphogenesis and differentiation are regulated by the sequential and reciprocal interactions between the stomadial epithelial and cranial neural crest derived mesenchyme during

embryogenesis [12]. It is known that the regeneration process does not exactly recapitulate these mechanisms which occur during embryogenesis according to the loss of the enamel epithelium and maturation of the tissues by the alterations of the cells and matrices after the tooth eruption [12]. During the tooth development signaling molecules are fossilized within the dentin matrix [11,12,42].The release of these molecules according to injuries or treatment methods like pulp capping or application of EDTA, can stimulate the differentiation and proliferation of the stem cells as a reparative action [3,11–14]. Signaling molecule related processes (signaling pathways) can control the regulation of proliferation rate of the cells, induction of the differentiation into another cell type, or the formation of mineralizable matrices [13]. Signaling pathways are composed of cell-surface receptors, intracellular molecules and transcription factors regulating gene expression [43]. Examples of signaling pathways having a role in dental tissue formation are; Notch signaling, Wingless-integration pathways (Wnt1, Wnt/β catenin), mitogen-activated protein kinases pathways (p38 MAPK pathway, ERK MAPK, JNK MAPK), phosphatidylinositol 3 kinase (PI3K)/Akt signaling pathway, sonic hedgehog pathway, heme oxygenase 1 pathway, signaling pathways mediated by EphrinB1-4 [9,10,12,39,43–48]. According to this knowledge, signaling molecules became a part of the dentin-pulp complex regeneration to induce the formation of the desired tissue types by controlling the differentiation and proliferation of the stem cells.

As a conclusion of the known mechanisms of tooth formation during embryogenesis and reparative dentin formation, signaling molecules used to induce the differentiation and the proliferation of the stem cells are as shown in Table 2.

Table 2. Signaling molecules used for dentin-pulp tissue engineering.

TGFs	NCPs	IGFs	FGFs	Wnt Proteins	Angiogenic Growth Factors	Neurotrophic Growth Factors	Other Signaling Molecules	Cytokines and Chemokines
TGF-β1	DSPP	IGF-I	Bfgf	Wnt3	VEGF	NGF	Ephrin B1	TNF-α
TGF-β3	-DSP	IGF-II	FGF-2	Wnt7b	PDGF	BDNF	Ephrin B2	IL-8
BMP2	-DPP			Wnt10a	PlGF	GDNF	Ephrin B3	IL-10
BMP4	DMP1			Wnt10b	EGF	GCSF	MMP-3	SCF
BMP6	BSP			in			β-catenin	SDF-1α
BMP7	MEPE			conjunc			Angiogenin	
Gdf11				tion			Angiopoietin-2	
				with			Leptin	
				Shh				

Signaling molecules used for dentin-pulp tissue engineering for it at Transforming growth factors (TGFs) is a large family of structurally related proteins which includes TGF-α, TGF-β, BMPs, activins, inhibitin, Müller inhibiting substance (MIS) and growth differentiated factors [3,49–51]. TGF-β is a signaling molecule affecting modulation of embryonic development, cell differentiation and proliferation, immunoregulation and healing [51]. It has shown that especially the TGF-β1 and TGF-β3 isoforms have an effect on dentin-pulp complex regeneration by up-regulation of matrix secretion and odontoblast-like cell differentiation according to the induction of collagen and alkaline phosphatase production [3,5,11,13,14,51,52]. The TGF-β participated in the serine/threonine kinase complex activate these processes by binding to TGF-β receptors 1 and 2 [13]. Bone morphogenic

proteins (BMPs) as a subgroup of TGFs, also have an important role throughout the embryonic tooth development, initiation, morphogenesis, cytodifferentiation and matrix secretion [2,20]. BMP family members which are expressed during odontoblast differentiation and dentin matrix formation are BMP2, BMP4, BMP6, BMP7 and Gdf11 (growth/differentiation factor 11) [2,11,13,46,49,50,53]. BMP signals are transduced from the plasma membrane to the nucleus through Smad proteins as receptor activated Smads (R-Smads), common mediator Smads (co-Smads) and inhibitory Smads (I-Smads) to stimulate cell differentiation and ECM secretion [2,20,46]. Smad-4 is a co-Smad and is the only shared component in signaling pathways of both TGF-β and BMP.

Dentin matrix protein 1 (DMP1) and dentin sialoprotein (DSP) and dentin phosphoprotein (DPP) obtained from dentin sialophosphoprotein (DSPP) are noncollagenous proteins (NCPs) that induce dentin mineralization and maturation [7,11,54]. These molecules are the members of small integrin-binding ligand, N-linked glycoprotein (SIBLING) family additionally to osteopontin (OPN), bone sialoprotein (BSP) and matrix extracellular phosphoglycoprotein (MEPE) [9,11,13,17]. It has been shown that DMP1 has a role as a morphogen to induce cytodifferentiation of stem cells [11,54]. Also BSP and dentonin derived from MEPE had an effect on dentin formation [6,11]. Additionally to their regulatory effects, DMP-1, BSP, DSP, DPP and DSPP serves as specific markers for odontoblasts as alkaline phosphatase [9,24,35,38,39,47,55–57].

Insulin is an important hormone for the growth and proliferation of somatic cells. Insulin like growth factors (IGFs) I and II have similar effects with insulin and they play an important role at cytodifferentiation, cell proliferation and matrix mineralization [58]. The combinations of IGFs with other growth factors like PDGF-BB can synergistically induce these processes [59]. The effect of IGF-I was described by stimulating Ephrin1 and Ephrin2 gene expressions [59]. It has been shown that although both increase regeneration capacities, higher concentrations of IGF-II are needed [58].

FGFs are signaling molecules which can also induce cell growth, differentiation, migration, survival regulation, angiogenesis and can provide maintenance of stem cells in pluripotent stage [4,13,56,57,60,61]. FGFs generally used for dentin-pulp complex regeneration are basic fibroblast growth factor (bFGF) and FGF-2 [4,62].

Angiogenesis is the formation of new capillary structures from pre-existing vasculature [42]. A well-functioning vascular system ensures gas exchange, nutrient supply, waste removal and regulation of inflammation for all organs. Angiogenesis is one of the most important steps for tissue engineering, as the transplanted cells need blood supply for nutrients to survive in order to initiate regeneration [18,63]. This process is induced by several polypeptide growth factors like vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), PlGF (placenta growth factor) and EGF (epidermal growth factor) [18,33,42,62,64]. As well as these growth factors can be applied as a single molecule (mostly preferred one is VEGF), currently developed concentrated growth factor (CSF) contains different types of signaling molecules (VEGF, PDGF, FGF, IGF, Chemokine receptor 4 [CXCR4]) which induce angiogenesis [65]. VEGF is a potent mitogen for endothelial cells, promoting endothelial cell survival and angiogenesis. Like other signaling molecules trapped in dentin matrix, during dentinogenesis, slow release of VEGF, FGF-2 and PDGF from the dentin matrix can induce the reparative processes of the dentine—pulp complex [42,64]. Resembling the hypoxic condition in dental pulp cavity activates hypoxia-inducible factor (HIF) which consists of HIF-1α and HIF-1β heterodimer [66,67]. HIF regulates the transcription of hypoxia regulated genes such as VEGF, PlGF and stromal cell-derived factor 1 (SDF-1) [66,68]. While HIF-1β constitutively expressed, HIF-1α is stabilized under hypoxic

conditions and binds to the VEGF gene promoter, recruits other transcriptional regulators, thereby enhancing VEGF gene expression [66,67]. According to regeneration mechanisms, different stem cells response the regeneration process with different pathways. For example for angiogenesis induced by VEGF, while bone marrow derived stem cells use MAPK pathway and adipose-derived stem cells use PI3K-Akt pathway, it has been shown that SHEDs use ERK pathway [69].

As a conclusion of being originated from cranial neural crest cells and also being closely associated with a neuronal network; neuropeptides and neurotrophic growth factors are also expressed in odontoblasts [9,15,51] which have a role on reparative mechanisms of dentin-pulp complex. For a functional regeneration and pulp homeostasis, neuronal nature of dentin-pulp complex is also should be obtained. The pulp nerve fibers contribute to angiogenesis, extravasation of immune cells to regulate inflammation, maintain pulp tissue, and strengthen pulp defense mechanisms [70]. This can be induced by using nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived growth factor (GDNF) and G-CSF (granulocyte-colony stimulating factor) [9,15]. According to the results of a study [71] showing the failure of ameloblast and odontoblast differentiation in GDNF knockout mice, especially GDNF; a member of TGF-β family was found to have an important role for dental tissue cytodifferentiation. Another growth factor, G-CSF, induces vascular endothelial differentiation, neurite outgrowth and highly mineralization of matrix and additionally it is approved by the Food and Drug Administration (FDA) for clinical use [15,72].

Additionally, signaling molecules like EphrinB1, Ephrin B2 and Ephrin B3, polyphosphate induced matrix metalloproteinase-3 (MMP-3), β-catenin, cytokines and chemokines play a key role for the cell migration, odontogenic differentiation and cell proliferation by mediating signaling pathways [15,24,59,73,74]. Cytokines and chemokines are small, potent signaling proteins produced in response to nuclear factor kappa B (NF-κB) signaling pathway as a conclusion of cellular stresses [4]. They have a role as chemoattraction and the homing of cells involved in both the immune and regenerative responses [4,9,15]. Angiogenin, angiopoietin 2 (ANG-2) and leptin are some of the proteins which have role on angiogenesis [64]. In the presence of VEGF and bFGF, ANG-2 induces proliferation and migration of endothelial cells and angiogenin induces neovascular formation [64]. Wnt3, Wnt7b, Wnt10a and Wnt10b are wingless integration related proteins (Wnt) proteins which are in conjunction with sonic hedgehog (Shh), regulating cell proliferation, migration and differentiation during tooth initiation and morphogenesis [6,43]. TGF-β1 has been shown to have interaction with Wnt/β catenin signaling to induce pulp repair. Tumour necrosis factor-α (TNF-α) stimulates DPSCs towards odontoblast-like cells by promoting differentiation and mineralized secretory activation [4,9]. Interleukins like IL-1α, IL-1β, IL-4, IL-6, IL-8 and IL-10 also promotes regeneration or repair activities by immunoregulation [4,9]. Stem cell factor (SCF) is a powerful chemokine capable of recruiting stem cells and have a role in dentinogenesis. It stimulates proliferation in a dose-manner, promote migration and enhance cytoskeletal reorganization and differentiation of cells [15]. Stromal cell derived factor-1α(SDF-1α) as a member of the CXC chemokine subfamily, has capability to induce migration and differentiation of stem cells and so promoting angiogenesis, neurogenesis, osteogenesis and odontogenic differentiation [66,75–77].

2.3. Scaffolds

Scaffold is an important part of tissue engineering which acts as a platform to provide a three-dimensional substrate/temporary matrix for developing cells [11,17,64]. An ideal scaffold is expected to; mimic the natural ECM, be biocompatible and injectable considering the anatomy of tooth, undergo biodegradation at a harmonically rate with remodeling of new tissue, provide encapsulation or surface adhesion of cells, help in spatial organization of cells and eventual replacement by appropriate tissues, ability to modify mechanical, physical and biological properties to suit specific applications [11,17,65]. Scaffolds used for dentin-pulp complex engineering are listed in Table 3.

Table 3. Scaffolds used for dentin-pulp complex tissue engineering [17,36,40,57,78–87].

Polymers		Hydrogels		Bioceramics
NATURAL	SYNTHETIC	NATURAL	SYNTHETIC	
Decellularized matrices (ECM, dentin matrix)	Polyester Poly(lactic) acid (PLA)	Alginate Chitosan	Gelatin methacrylate (Ge1MA)	Bioactive glasses Biphasic calcium phosphates
Alginate	Poly(glycolic) acid (PGA)	Collagen	PEG	Bredigite
Dextran	Copolymer of PLA and PGA (PLGA)	Fibrin Hydroxyapatite	Self-assembling peptides (SAP)	Calcium phosphates Hydroxyapatite
Chitosan	Poly(ε-caprolactone) (PCL)	Proteoglycans decellularized and demineralized bovine bone (bECM)	Emdogain	Tricalcium phosphate
Cellulose	Poly(urethanes)			
Polysaccharides	Poly(ether ester)			
Starch	Poly(ethylene glycol) (PEG)			
Hyluronic Acid (HA)	Poly(butylene terephthalate) (PBT)			
	Polyhydroxybutyrate (PHB)			
	Magnetic nanofiber scaffolds (MNS)			

Natural polymers can be obtained from many sources like plants, animals, algae, bacteria and tissues of host [17,40,82]. Currently decellularized ECM mainly derived from animals or the tissues of host and xenogeneic ECM scaffolds are used for tissue engineering [36,82]. Natural polymers provide a favorable environment for tissue regeneration and temporary controlled release of signaling molecules [17,36,40]. Although they can be processed into many forms, the procedures to process and sterilize are challenging. They can also cause undesirable immunoresponse and pathogen transmission [17,83].

Synthetic polymers can easily processed into any shape, have functional groups to attract cells or bind signaling molecules [17]. They have tailorable mechanical properties, degradation rate, wettability and protein adsorption. Although the procedure is more easy and cheaper than other types, solid walled ones can not exactly duplicate the structure of ECM and there can be accumulation of acidic degradation products which can prevent regeneration. Synthetic polymer scaffolds with a nanofibrous architecture have been found more effective on induction of attachment,

proliferation and differentiation of stem cells by mimicking the architecture of natural ECM as well as the mineralization than solid walled ones [83,85,88]. Additionally in a research it was observed that magnetic nanofiber scaffolds promoted angiogenesis too [78].

Bioceramics are biocompatible, biodegradable and osteoconductive. Although they can induce mineralization well, their brittleness and poor processability to form porous structures are limiting their applications [17,79]. Bioceramics are mainly classified as phosphate based and silicate based bioceramics [81]. Mostly used phosphate based bioceramics were tricalcium phosphates and biphasic calcium phosphate as the combination of hydroxyapatite and tricalcium phosphate. On the other hand bredigite is a silicate-based bioceramic. A study [81] showed that it up-regulated the expression of pluripotency genes without additional cytokine release better than bFGF, where tricalcium phosphate lacked this property.

Hydrogels are highly biocompatible and viscoelastic similar to soft tissue [17]. Their elasticity permits the injection methods but due to low mechanical stiffness they are not suitable for certain applications. They provide uniform cell encapsulation and efficient transport of nutrients and waste.

Scaffolds are classified according to their material type, but they can also be produced as a combination of different types of materials or incorporated with pulp capping agents or signaling molecules to eliminate their disadvantages and to obtain more qualified structures for desired tissue formation. These are called hybrid scaffolds.

Although scaffold is an important component of tissue engineering, to induce angiogenesis with dental tissue formation, scaffold-free prevascularized microtissue spheroids were also designed, searched and found successful for dentin-pulp complex regeneration according to technological challenges of placing 2 or more different cell types inside a 3D scaffold [37,89].

3. Methods for regeneration of dentin-pulp complex

Mainly there are two methods for regeneration of dentin-pulp complex: (1) cell transplantation, and (2) cell homing.

3.1. Cell transplantation

In this method, exogenous stem cells are directly injected to the needed anatomic site (cell injecting) of the host or applied by being loaded onto scaffolds (cells seeded scaffolds) either incorporated with signaling molecules or not [15,20,21,90]. For cell injecting method, main problems at directly injection were difficulty for adequate localisation and direct contact with the immune system which prevents efficacy of the therapy [21]. It has been observed that cells encapsulated into a delivery vehicle were able to proliferate and differentiate. According to this observation, using a delivery vehicle to carry and deliver the material thought as a solution to increase the efficacy of the therapy [21,91]. Examples for the materials used for encapsulation of stem cells at dentin-pulp complex regeneration can be listed as enzyme-cleavable, customized self-assembled peptide hydrogels, PEGylated fibrin hydrogels or biodegradable lactide and glycolide [21]. Additionally, in an in vivo study [92], developed injectable hierarchical microsphere system, which is composed of VEGF encapsulated into heparin-conjugated gelatin (HG) nanospheres and which are further immobilized in the nanofibrous biodegradable poly (L-lacticacid) (PLLA) microspheres (MSs), showed the successful regeneration of pulp-like tissues that fulfilled the whole

apical and middle third root space and reached the coronal third of the canal. In addition, regeneration of a large number of blood vessels were also observed. Another option for cell transplantation can be the cells seeded scaffolds. Unlike cell injecting, scaffolds can allow superior control for stem cell delivery, saturation with time-release signaling molecules, modulation of stiffness, pore size and cell-substrate interaction. For example, in a study [93], DPSC and SCAP isolated from the human third molars were seeded onto a poly-D, L-lactide/glycolide scaffold and inserted into the canal space of root fragments, followed by subcutaneous transplantation into SCID mice. Subsequent histological analysis of the tooth fragments 3–4 months after surgery indicated that the root canal space was completely filled with pulp-like tissue with well-established vascularization.

For the cell transplantation method, the transplanted cells can be either collected from the host (autologous) and/or from other individuals (allogenic) or can be processed and grown in cultures to increase the numbers [15,90]. The stem cells can also be changed genetically by delivering specific genetic information for the expression of needed signaling molecules [20]. Since the gene not a degradable protein is being delivered, higher and more constant production levels of signaling molecules can be obtained. The secretion of multiple gene products that have synergistic effects on tissue regeneration can be accomplished by delivering multiple genes or introducing a gene that activates synthesis of multiple signaling molecules [20]. Vectors for gene delivery can be viral or non-viral [20,21]. The most preferred viral vector is adenovirus. The major disadvantage of viral vectors is the immune response that occurs from expression of viral proteins. To eliminate this disadvantage; lipofection reagents, polymers, plasmids and physical methods as non-viral constructs can be preferred but they give rise to lower expression levels [20]. While directly introducing the delivery vector to the anatomic site is in vivo application of gene therapy, transferring back the genetically modified cells harvested from the patient is the ex vivo application of gene therapy. In vivo gene therapy is simpler but limited by non-specific targeting and inefficient gene delivery. Ex vivo applications are more specific, allows both autocrine and paracrine effects and avoid safety risks of in vivo application but involves an extra step and carry the risk of contamination and development of tumorigenesis [20].

Cell transplantation method can be too complex according to the procedure patterns as tooth extraction, pulp extirpation, in vitro cell culture, selection of stem cell populations, ex vivo cell expansion, storage and shipping. Also there is a risk for contamination, development of tumorigenesis during ex vivo cell manipulation, immunological rejection and the ability of injected cells to maintain their own phenotype [21,94].

3.2. Cell homing/Cell-free approach

As a conclusion of risks for contamination, tumorigenesis, immune rejection and not being clinically viable according to complexity and high costs of cell transplantation, cell homing/cell-free approach can be a better alternative for dentin-pulp complex regeneration [15,90,95]. Commercialized cell homing products that are approved by the FDA already exist in the market and there is no requirement of special training for the delivery of these products [96].

In cell homing method main purpose is to induce regeneration by the chemotaxis, proliferation and differentiation of host endogenous stem cells to injured tissue via biological signaling molecules loaded onto scaffolds [15,95,96]. Signaling molecules used for this method should promote angiogenesis, migration of endogenous stem cells and mineralization [90]. Cell types responding to

the signaling molecules during cell homing, are found as DPSCs, SCAP and BMMSCs [15,97,98]. It has been shown that DPSCs of inflamed pulp which reside in root canals have similar MSC properties to the DPSCs of normal pulp [99]. Another possibility is SCAP according to the apical cell-rich zone lying between the apical papilla and the pulp with a collateral circulation which gives chance for survival of these cells during pulp necrosis [98]. Additionally to these sources BMMSCs in communication with dentin-pulp complex to maintain pulp homeostasis can also be thought as possible cell source during cell homing [97,98].

According to cell homing methods there are two ways to attract the host's stem cells. One of them is to apply signaling molecules and scaffolds into the root canals. Firstly a study by Kim et al. [100], concluded by the formation of new vascular, odontoblastic-like and neural components by using a collagen scaffold including VEGF, bFGF, PDGF, NGF and BMP7. Additionally to these signaling molecules and scaffolds, following studies tried to regenerate dentin-pulp complex by using SDF1, SDF-1α, SCF, G-CSF and scaffolds composed of neutralized type-1 collagen solution or PLC and HA [96,98]. Platelet-rich plasma (PRP) application can also be thought as a cell-free approach which can be considered as a combination of signaling molecules but it has disadvantages like drawing blood from the patient and additional centrifuge and purification processes [95,96,101].

Other method for cell homing is to induce transport of both signaling molecules and stem cells into the root canals. This method is consist of the disinfection of the root canals without mechanical preparation, application of antibiotics and blood clotting which is a simple technique of revascularization [15,90,94]. An appropriate disinfection of root canals without mechanical preparation can give a chance for some vital DPSCs reside in the root canal system [102]. On the other hand, blood clot acts as a scaffold, bleeding of apical area can directly transport the signaling molecules and stem cells or inflammation caused by this trauma can induce migration of the stem cells through the root canal [90]. While this method is used for dentin-pulp complex regeneration of immature permanent teeth in the clinic, the outcome of the treatment is inconsistent. One of the disadvantages is that the origin of the limited endogenous stem cells and the nature of the tissues recolonize in the pulp space can not be determined according to the reduced periapical cell numbers by necrosis and periapical lesions [15,90]. Other disadvantages are that the teeth with apical sizes less than 1.0 mm have been shown that do not allow pulp revascularization and less than 1.5 mm healing rate decreases [90]. On the other hand it has been shown that pulp can regenerate in canals with 0.7 mm apical sizes by cell transplantation [90].

4. Conclusion

For decades endodontic root canal treatments have been the first choice for highly degraded dentin-pulp complexes. According to disadvantageous outcomes of this method like not saving the vitality of tooth, researchers started to work on regeneration of dentin-pulp complex for reconstruction of the dental tissues with the shape and function of the original tissue including vascularization and innervations. Regeneration approach is mainly composed of three components as stem cells, signaling molecules and scaffolds. Most of the researchers investigated different combinations of these components. Although there are find outs showing successful dental tissue formation, vascularization, innervation and matrix mineralization, studies must be carried on to clearly reveal pathways affecting dental tissue formation and to find out the most appropriate method which can be applied in the clinics easily for the exact regeneration of dentin-pulp complex.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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