ISSN - 2394-7721

American Journal of Oral Medicine and Radiology

Journal homepage: www.mcmed.us/journal/ajomr

THE DOMAINS OF REGENERATIVE ENDODONTICS IN PEDIATRIC DENTISTRY

Mitali Jain, Meenakshi Bodh, Samir Dutta, Ritu Namdev, Arun Kumar*

Department of Pedodontics & Preventive Dentistry, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India.

Article Info

Received 25/09/2015
Revised 07/10/2015
Accepted 08/11/2015

Key words:-
regeneration, scaffold, endodontic, revascularization.

ABSTRACT

Tissue engineering is emerging as a promising therapy to regenerate missing teeth and dental tissues. Dental tissue regeneration depends on the activity of progenitor cells or stem cells to be seeded within a scaffold to generate a new tissue construct. By combining different types of stem cells with different types of scaffolds and controlling the cell culture and tissue engineering conditions, the outcome of the construct can be altered to create various types of dental tissues, from teeth to salivary glands. 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.

INTRODUCTION

Revascularization is a regenerative treatment and a biologically based alternative approach to treat necrotic immature teeth that unlike apexification and artificial apical barrier techniques, allows continuation of root development[1,2] Regenerative endodontic procedures (REPs) can be defined as biologically based procedures designed to replace damaged structures including dentin and cells of the pulp-dentin complex[3]. There have been several terms used to describe the introduction of new living tissue into the canal space. These include:

1. Regeneration,
2. Revascularization,
3. Revitalization.

There has been debate as to which of these 3 terms (ie, revascularization, revitalization, or regeneration) is most appropriate to describe the outcome of procedures used to regenerate pulp tissue. The term Revascularization describes there-establishment of the vascular supply to existing pulp in immature permanent teeth. Revitalization describes the in growth of tissue that may not resemble the original lost tissue [3].

Corresponding Author

Arun Kumar
Email: - drarun922@gmail.com

History of Dental Regeneration

In 1952, BW Hermann [4] was reported to have made one of the first attempts at dental tissue regeneration. Herman used calcium hydroxide (calxyl) to promote dentin bridge formation for vital pulp therapy following partial pulp amputation. In 1971, Nygaard-Østby [5]and Hjortdal evaluated the role of the blood clot in healing. His first case series reported on nine patients aged 21 to 42 and a total of 17 teeth; there was a 13-day to 3-year follow-up prior to tooth extraction and histologic analysis. In 1974, Myers and Fountain [6] reported an increased root length and calcified material in necrotic canals of monkey canines with immature apices after disinfection with NaOCl and filling the canals with citrated whole blood or gel foam. In 1976, Nevinset al [7] showed that the disinfection of necrotic canal space in immature monkey incisors followed by the placement of a gel (containing collagen, calcium chloride, and dipotassium hydrogen phosphate) and short obturation with gutta-percha could result in ‘‘various forms of hard and soft connective tissue’’ including ‘‘cementum, bone, and reparative dentin’’ lining the walls of the root canal. Although these were encouraging findings, histologic analysis was unable to confirm the regeneration of a pulp-dentin complex. Regenerative procedures then went largely ignored until an interest in these treatments was piqued by a case report by Iwaya and colleagues.

Basic Concepts of Regenerative Endodontics

Regeneration approaches use a combination of scaffolds, stem cells, growth factors, tissue engineering, organ tissue culture, transplantation, and tissue grafting [8].

STEM CELLS

A stem cell is commonly defined as a cell that has the ability to continuously divide and produce progeny cells that differentiate into various other types of cells or tissues. Stem cells are classified as

1. Embryonic/fetal
2. Adult/postnatal cells

Mesenchymal Stem Cells (MSCs)

In 1963, hematopoietic stem cells giving rise to blood cells were identified in bone marrow. Since then, it has been established that bone marrow is also the primary source for multipotent MSCs. Bone marrow MSCs (BMMSCs) can differentiate into osteogenic, chondrogenic, adipogenic, myogenic, and neurogenic lineages.

MSCs are found in many other tissues in the body, including umbilical cord blood, adipose tissue, adult muscle, and dental tissues; are capable of differentiating into at least 3 cell lineages: osteogenic, chondrogenic, and adipogenic; and can also differentiate into other lineages, such as odontogenic, when grown in a defined microenvironment in vitro. Definitive information on the location and distribution of MSCs is still being elucidated. Crisan and colleagues [9] demonstrated that human perivascular cells from diverse and multiple human tissues give rise to multi-lineage progenitor cells that exhibit the features of MSCs. Perivascular progenitor/stem cells can also proliferate in response to odontoblast injury by cavity preparation under ex vivo tooth culture conditions

Dental Stem Cells (DSCS**)**

A tooth develops as a result of carefully orchestrated interactions between the oral epithelial ectodermal cells that form the enamel organ (for enamel formation) and cranial neural crest derived mesenchymal cells that form the dental papilla and dental follicle. These MSCs give rise to the other components of the tooth: dentin, pulp, cementum and periodontal ligament. Beginning in 2000, several human dental stem/progenitor cells have been isolated and characterized. These cells include:

1. DPSCs (Dental Pulp Stem Cells)
2. SHED cells (Stem Cells from human exfoliated deciduous teeth)
3. SCAP cells (cells from apical papilla)
4. DFPCs (dental follicle precursor, or progenitor, cells)
5. BMMSC (bone marrow derived mesenchymal stem cells)
6. PDLSCs (periodontal ligament stem cells)

DPSCs -DPSCs were first isolated from human permanent third molars in 2000. The cells were characterized as clonogenic and highly proliferative. Dentin and pulplike tissues were generated following the transplantation of DPSCs in hydroxyapatite/tricalcium phosphate (HA/TCP) scaffolds into immunodeficient mice. A follow-up study confirmed that DPSCs fulfilled the criteria needed to be stem cells: an ability to differentiate into adipocytes and neural cells and odontoblasts (ie, multipotency) and self-renewal capabilities. Additional studies have confirmed that DPSCs can also differentiate into osteoblast-, chondrocyte-, and myoblast like cells and demonstrate axon guidance. DPSCs have also been shown to express the bacterial recognition toll-like receptors, TLR4 and TLR2, and vascular endothelial growth factor in response to lipopolysaccharide, a product of gram-negative bacteria. When compared with normal pulps, DPSCs in inflamed pulp tissues have reduced dentinogenesis activity, and an in vitro investigation has shown reduced dentinogenic potential of DPSCs exposed to a high bacterial load that can be recovered after the inhibition of the bacterial recognition toll-like receptor TLR2 [10].

SHED CELLS

SHED cells are highly proliferative stem cells isolated from exfoliated deciduous teeth capable of differentiating into a variety of cell types, including osteoblasts, neural cells, adipocytes, and odontoblasts, and inducing dentin and bone formation. Like DPSCs, SHED cells can generate dentin-pulp like tissues with distinct odontoblast like cells lining the mineralized dentin-matrix generated in HA/TCP scaffolds implanted in immunodeficient mice. However, SHED cells have a higher proliferation rate than DPSCs and BMMSCs, suggesting that they represent a more immature population of multipotent stem cells. SHED cells have shown different gene expression profiles from DPSCs and BMMSCs; genes related to cell proliferation and extracellular matrix formation, such as transforming growth factor (TGF)-b, fibroblast growth factor (FGF)2, TGF-b2, collagen (Col) I, and Col III, are more highly expressed in SHED cells compared with DPSCs [11].

SCAP CELLS

SCAP cells are found in the apical papilla located at the apices of developing teeth at the junction of the apical papilla and dental pulp. The apical papilla is essential for root development. SCAP cells were first isolated in human root apical papilla collected from extracted human thirdmolars. The cells are clonogenic and can undergo odontoblastic/ osteogenic, adipogenic, or neurogenic differentiation. Compared with DPSCs, SCAP cells show higher proliferation rates and greater expression of CD24, which is lost as SCAP cells differentiate and increase alkaline phosphate expression. SCAP cells seeded onto synthetic scaffolds consisting of

poly-D,L-lactide/glycolide inserted into tooth fragments, and transplanted into immunodeficient mice, induced a pulplike tissue with well-established vascularity, and a continuous layer of dentin like tissue was deposited onto the canal dentinal wall. Root canal irrigants used in regenerative endodontic therapy procedures should ideally support cell survival, or at least not compromise survival. An in vitro study showed that 17% EDTA used alone supported SCAP cell survival better (89% survival) than when used with either 6% NaOCl (74% survival) or 2% CHX (0% survival) [12].

DFPCs

The dental follicle forms at the cap stage by ectomesenchymal progenitor cells. It is a loose vascular connective tissue that contains the developing tooth germ, and progenitors for periodontal ligament cells, cementoblasts, and osteoblasts. DFPCs were first isolated from the dental follicle of human third molars. Because DFPCs come from developing tissue, it is considered that they might exhibit a greater plasticity than other DSCs. Indeed, different cloned DFPC lines have demonstrated great heterogeneity. In addition, after transplantation in immunodeficient rodents, DFPCs differentiated into cementoblast like and osteogenic like cells, and surface markers compatible with those of fibroblasts were identified in human dental follicle tissues, suggesting the presence of immature PDL fibroblasts [13-15].

GROWTH FACTORS

Growth factors and cytokines may act as signaling molecules that modulate cell behaviour by mediating intracellular communication. Growth factors are polypeptides or proteins that bind to specific receptors on the surface of target cells. Growth factors can initiate a cascade of intracellular signaling, and act in either an autocrine or paracrine manner. Cytokines are typically referred to as immunomodulatory proteins or polypeptides. Cytokines are often used interchangeably with growth factors because many cytokines share similar actions with growth factors [16].

PLATELET-DERIVED GROWTH FACTOR

Platelet-derived growth factor (PDGF) is released by platelets, and has potency in promoting angiogenesis and cell proliferation. The chemotaxis and proliferation of mesenchymal stem/progenitor cells can be induced by PDGF in the injury site. In trauma, hemorrhage is followed by blood-clot formation in dental pulp. Platelets in the blood clot release a-granules containing PDGFs and attract neutrophils and macrophages. These cells play key roles in early wound healing by producing other signaling molecules for the formation of granulation tissues.

However, PDGFs appear to have little effect on the formation of the dentin like nodule in dental pulp cells isolated from rat lower incisors, although PDGF-AB and –BB isoforms stimulate the expression of dentin sialoprotein (DSP). PDGFs stimulate cell proliferation and dentin matrix protein synthesis, but appear to inhibit alkaline phosphatise (ALP) activity in dental pulp cells in culture. PDGFs enhance the proliferation of fibroblasts in human dental pulp. PDGF-BB may increase the expression of vascular endothelial growth factor (VEGF) in osteoblasts and promotes angiogenesis at the site of dental pulp injury. In vivo, PDGF promotes de novo formation of dental pulp–like tissues in endodontically treated human teeth that are implanted in rats [17].

TRANSFORMING GROWTH FACTOR b

The transforming growth factor b (TGFb) family comprises a group of diverse growth factors including TGFb, bone morphogenetic proteins (BMPs), growth/differentiation factors (GDFs), anti-Mullerian hormone (AMH), activin, and nodal. TGFb1 regulates a wide range of cellular activities, such as cell migration, cell proliferation, cell differentiation, and extracellular matrix synthesis. TGFb1has been shown to increase cell proliferation and production of the extracellular matrix in dental pulp tissue culture, and promotes odontoblastic differentiation of dental pulp cells. The effect of TGFb1 can be synergistically upregulated by fibroblast growth factor 2 (FGF2), as evidenced by the increased ALP activity, the formation of mineralized nodule, and the expression of DSP and dentin matrix protein 1. The dentinogenic ability of dental pulp cells in the mechanically exposed dental pulp of dog teeth is shown to be induced by exogenous TGFb1 [18-20].

BONE MORPHOGENETIC PROTEIN

BMPs comprise a subgroup of the TGFb superfamily and are involved in many biological activities including cell proliferation, differentiation, and apoptosis. BMPs have strong osteoinductive and chondrogenic effects. BMP2 was discovered by *Urist*, who showed ectopic bone formation in connective tissues by transplanted dimineralized bone. BMP2, BMP4, BMP7. and BMP11 are of clinical significance because of their role in inducing mineralization.

Human recombinant BMP2 stimulates the differentiation of dental pulp cells into odontoblasts, inducing mRNA expression of dentin sialophosphoproteins (DSPPs) and higher ALP activity on BMP2 application, but has no effect on cell proliferation. DSPP expression and odontoblastic differentiation are regulated likely via BMP2-induced signaling by nuclear transcription factor Y. BMP2 also stimulates the differentiation of dental pulp stem/progenitor cells into odontoblasts in vivo and in vitro. Human recombinant BMP2 or BMP4 induces dentin formation when used in capping materials over amputated canine pulp. Osteodentin formation occurs in amputated canine pulps treated with BMPs in collagen matrix. Bovine dental pulp cells treated with BMP2 and BMP4 differentiate into preodontoblasts. BMP7, also known as osteogenic protein

1, promotes dentin formation when placed over amputated dental pulp in macaque teeth. The dentinogenic effect of BMP7 on amputated dental pulp has been shown in several animal models including rats, ferrets, and miniature swine [21-23].

Vascular Endothelial Growth Factor

VEGF is a heparin-binding protein with specific affinity to endothelial cells, and plays a key role in angiogenesis. The functions of VEGF involve the proliferation of endothelial cells and their enhanced survival, stimulating neovascularization in the area of injury. VEGF increases micro vessel density of the dental pulp when tooth slices containing severed dental pulp were treated with VEGF and implanted into subcutaneous tissues of severely combined immunodeficiency (SCID) mice. VEGF appears to induce the differentiation of human dental pulp cells into endothelial cells [24].

Fibroblast Growth Factor

Fibroblast growth factor (FGF) plays key roles in cell migration, proliferation, and differentiation during embryonic development and wound healing. FGF2 regulates tooth morphogenesis by controlling cell proliferation and differentiation. FGF2 is a potent angiogenic factor that stimulates formation of new blood vessels in the dental pulp along with PDGF and VEGF. Given its role in cell proliferation and angiogenesis, FGF2 acts as an early stimulating factor in formation of granulation tissue during wound healing.FGF2 induces the migration of dental pulp cells..The FGF2 on exposed dental pulp in rat molars induces vascular invasion and cell proliferation early in wound healing. Also, FGF2 stimulates reparative dentin formation or dentin particles in the exposed pulp [25].

INSULIN-LIKE GROWTH FACTOR

Insulin-like growth factors (IGFs) are single-chain polypeptides that have high sequence similarity to proinsulin. IGFs, comprising IGF-1 and IGF-2, contribute to odontogenesis and dental tissue repair by cell proliferation and differentiation. IGF-1 induces proliferation and differentiation of dog dental pulp cells into odontoblast-like cells in serum-free medium. IGF-1 with PDGF-BB has a synergistic effect on the proliferation of dental pulp cells in vitro [28].

NERVE GROWTH FACTOR

Nerve growth factors (NGFs), also known as neutrophins, promote the survival and maintenance of sympathetic and sensory neurons. NGFs play a role in regulating tooth morphogenesis and tooth innervations in rat tooth development. NGFs induce the differentiation of immortalized dental papilla cells into odontoblasts in vitro, suggesting that NGF acts as a stimulant for mineralization [29].

SCAFFOLDS IN DENTAL PULP TISSUE ENGINEERING [30-32]

Synthetic Scaffolds

The most extensively studied scaffold system for dental tooth regeneration is the use of biodegradable PGA scaffolds. The first reported studies maintained human adult dental pulp on a PGA scaffold for more than 60 days in culture. Follow-up studies used PGA scaffolds with human dental pulp and found upregulation of type I collagen, fibronectin, and several BMPs and their receptors, suggesting the capacity of this scaffold to maintain cell vitality and support the differentiation of human dental pulp cells. More recently, mixtures of PGA with both synthetic copolymers and other macromolecules were used for dental tissue engineering.

PLGA scaffolds have 2 different pore sizes: 150 to 180 mm and 180 to 300 mm. These scaffolds were evaluated in rabbits using autologous DPSCs and were shown to induce osteodentin formation after subcutaneous implantation for 2 and 6 weeks. PGA/PLLA and PLGA scaffolds were used in pioneering work in which scaffolds were formed in tooth molds, seeded with porcine third molar dissociated tooth buds, and allowed to grow in the omenta of athymic rats. After 20, 25, and 30 weeks, tooth-like structures containing pulp, dentin, and enamel were observed, with surrounding cells expressing BSP and amelogenin. Similar results were obtained by seeding rat tooth bud cells on both PGA and PLGA scaffolds for 12 weeks in the omentum or rat jaw [26,27].

NATURALLY DERIVED MATERIALS

Alginate has been used in dental engineering to deliver cells and/or growth factors. The alginate hydrogel with either transforming growth factor (TGF)-b1 or acid treatment was applied to slices of human teeth with vital dentin-pulp complex tissues and maintained in culture. Hydrogel with TGF-b1 or acid treatment, but not the untreated control hydrogel, induced dentin matrix secretion and formation of new odontoblast-like cells in the human tooth slices [33].

Collagens, particularly type I collagen, are major constituents of dentin and have been used to provide a 3D culture environment for various types of cells, including stem cells from the dental pulp. Compared with other natural scaffold products including gelatin and chitosan, the dental pulp cells cultured in the type I and III collagen gel exhibited a higher degree of odontoblastic differentiation as shown by alkaline phosphatase activity and expression of osteocalcin, dentin sialophosphoprotein (DSPP), and dentin matrix protein 1 (DMP1). Collagen gel can be used alone or in combination with growth factors (eg, TGF-b1, BMP4, FGF2 and other scaffold materials such as chitosan.

Chitosan/HA blend (polyelectrolyte complex) was used for compatibility studies with mesenchymal stem cells

[34]. In a 2:1 blend (HA/chitosan), cells were viable for 72 hours and no cytotoxicity was apparent. The same group used chitosan/pectin scaffolds for bone regeneration with similarly positive results. Chitosan/collagen scaffolds adsorbed with BMP7 were seeded with human adult dental pulp cells and stained positive for dentin matrix proteins DSPP and DMP1, whereas scaffolds without BMP7 were negative.

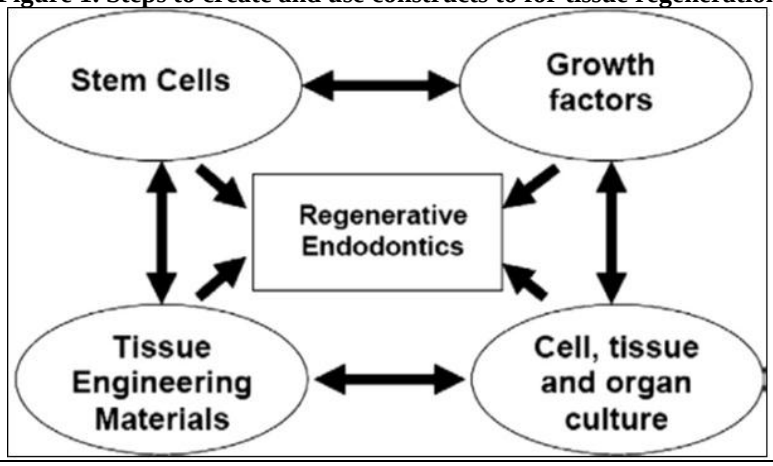
Hyaluronic acid sponges were used as 3D scaffolds for the regeneration of dental pulp. In comparison with the collagen sponge, the hyaluronic acid sponge can support cell growth in culture and in vivo from the amputated dental pulp of rat molars, with fewer immunologic reactions as shown by expression of inflammatory cytokines tumor necrosis factor (TNF)-a and interleukin-6, as well as leukocyte infiltration. However, when used as an injectable hyaluronic acid gel for soft-tissue augmentation, adverse hypersensitivity reactions were reported, due to impurities and bacterial contamination.

Fibrin consists of the blood proteins fibrinogen and thrombin, which are produced naturally in the body after injury to establish hemostasis and enhance wound healing. Because of these properties, fibrin glue, fibrin sealant, and fibrin in other forms were produced to aid bleeding control, speed wound healing, cover holes instead of sutures, and provide slow-release delivery of antibiotics or other drugs [35]. Because of their biocompatibility, biodegradability, simple preparation, and manipulation, fibrin scaffolds have been used for multiple purposes (eg, filling in bone cavities, vascular graft, and repairing injuries to urinary tract, liver, and lung) and are also available as mixtures with other polymers such as fibrin-PEG blend. Fibrin hydrogel allows the incorporation of growth factors and bioactive molecules via a heparin-binding delivery system, cell seeding through inkjet printing, and self-assembly through a magnetically influenced technique.

Blood clots have been used as natural scaffolds for bone healing in the tooth-extraction socket as well as for dental pulp regeneration/revascularization in immature necrotic teeth.

Fibrin glue and platelet-rich fibrin can be prepared from whole blood before surgery. The mixture of these 2 components was used as a scaffold for reassembly of porcine tooth bud cells implanted in the extraction socket. After 36 weeks, these implants developed into a complete tooth or an unerupted tooth crown. The mixtures of fibrin and other polymers such as PEGylated fibrin scaffold aid in handling the material. The PEGylated fibrin scaffold is injectable, tunable, degradable, and compatible with dental stem cells. It induces osteoblastic and odontoblastic differentiation as well as the formation of dentin-like collagenous matrix and vascularized pulp-like structure after transplantation in vivo.

Figure 1. Steps to create and use constructs to for tissue regeneration



CONCLUSION

Millions of teeth are saved each year by root canal therapy. Although current treatment modalities offer high levels of success for many conditions, an ideal form of therapy might consist of regenerative approaches in which diseased or necrotic pulp tissues are removed and replaced with healthy pulp tissue to revitalize teeth. The pulp tissue repair/regeneration recapitulates tooth development. Despite the impressive progress in tissue engineering approaches to regenerative pulp therapy, numerous challenges remain.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

1. Garcia-Godoy F, Murray P. (2010). Regenerative dentistry: translating advancements in basic science research to the dental practice. *J Tenn Dent Assoc*, 90, 12–8.
2. Murray PE, Garcia-Godoy F, Hargreaves KM. (2007). Regenerative endodontics: a review of current status and a call for action. *J Endod*, 33, 377–90.
3. Andreasen JO, Andreasen FM. (1994). Textbook and Color Atlas of Traumatic Injuries to the Teeth. Copenhagen, Denmark: *Munksgaard*.
4. Herman BW. (1952). On the reaction of the dental pulp to vital amputation and calxyl capping. *Dtsch Zahnarztl Z*, 7, 1446–1447.
5. Nygaard-Ostby B, Hjortdal O. (1971). Tissue formation in the root canal following pulp removal. *Scand J Dent Res*, 79, 333–349.
6. Myers MC, Fountain SB. (1974). Dental pulp regeneration aided by blood and blood substitutes after experimentally induced periapical infection. *Oral Surg Oral Med Oral Pathol*, 37, 441–450.
7. Nevins A, Finkelstein F, Borden B. (1976). Revitalization of pulpless open apex teeth in rhesus monkeys, using collagen-calcium phosphate gel. *J Endod*, 2, 159–165.
8. Becker AJ, Mc CE, Till JE. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature, 452–454.
9. Crisan M, Yap S, Casteilla L. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*, 3, 301–313.
10. Gronthos S, Mankani M, Brahim J. (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci*, 97, 13625–13630.
11. Miura M, Gronthos S, Zhao M. (2003). SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci*, 100, 5807–5812.
12. Huang GT, Yamaza T, Shea LD (2010). Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. *Tissue Eng Part A*, 16, 605–615.
13. Trevino EG, Patwardhan AN, Henry MA. (2011). Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. *J Endod*, 37, 1109–1115.
14. Morsczeck C, Gotz W, Schierholz J. (2005). Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol*, 24, 155–165.
15. Lazar-Molnar E, Hegyesi H, Toth S. (2000). Autocrine and paracrine regulation by Cytokines and growth factors in melanoma. *Cytokine*, 12(6), 547–554.
16. Deuel TF, Senior RM, Huang JS. (1982). Chemotaxis of monocytes and neutrophils to platelet-derived growth factor. *J Clin Invest*, 69(4), 1046–1049.
17. Yokose S, Kadokura H, Tajima N. (2004). Platelet-derived growth factor exerts disparate effects on odontoblast differentiation depending on the dimers in rat dental pulp cells. *Cell Tissue Res*, 315(3), 375–384.
18. Wahl SM. (1992). Transforming growth factor beta (TGF-beta) in inflammation: a cause and a cure. *J Clin Immunol*, 12(2), 61–74.
19. Tziafas D, Papadimitriou S. (1998). Role of exogenous TGF-beta in induction of reparative dentinogenesis in vivo. *Eur J Oral Sci*, 106(Suppl 1), 192–196.
20. Urist MR. (1965). Bone: formation by autoinduction. *Science*, 150(3698), 893–899.
21. Saito T, Ogawa M, Hata Y. (2004). Acceleration effect of human recombinant bone morphogenetic protein-2 on differentiation of human pulp cells into odontoblasts. *J Endod*, 30(4), 205–208.
22. Rutherford RB, Spangberg L, Tucker M. (1994). The time-course of the induction of reparative dentine formation in monkeys by recombinant human osteogenic protein-1. *Arch Oral Biol*, 39(10), 833–838.
23. Jepsen S, Albers HK, Fleiner B. (1997). Recombinant human osteogenic protein-1 induces dentin formation: an experimental study in miniature swine. *J Endod*, 23(6), 378–382.
24. Leung DW, Cachianes G, Kuang WJ. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*, 246(4935):1306–1309.
25. Marchionni C, Bonsi L, Alviano F. (2009). Angiogenic potential of human dental pulp stromal (stem) cells. *Int J Immunopathol Pharmacol*, 22(3), 699–706.
26. Tran-Hung L, Mathieu S, About I. (2006). Role of human pulp fibroblasts in angiogenesis. *J Dent Res*, 85(9), 819–823.
27. Kitamura C, Nishihara T, Terashita M. (2012) Local regeneration of dentin-pulp complex using controlled release of fgf-2 and naturally derived sponge-like scaffolds. *Int J Dent*, 2012, 190-196.
28. Joseph BK, Savage NW, Young WG. (1993). Expression and regulation of insulinlike growth factor-I in the rat incisor. *Growth Factors*, 8(4), 267–275.
29. Arany S, Koyota S, Sugiyama T. (2009). Nerve growth factor promotes differentiation of odontoblast-like cells. *J Cell Biochem*, 106(4), 539–545.

30. Mooney D J, Powell C, Piana J. (1996). Engineering dental pulp like tissue in vitro. *Biotechnol Prog*, 12, 865-868.
31. EL-Backly RM, Massoud AG, EL- Badry AM. (2008). Regeneration of dentin/pulp-like tissue using a dental pulp stem cell/polly(lactic-co-glycolic) acid scaffold construct in New Zealand white rabbits. *Aust Endod J*, 34, 52-67.
32. Young CS, Terada S, Vacanti JP. (2002). Tissue engineering of complex tooth structures on biodegradable polymer scaffolds *J Dent Res*, 81:695-700.
33. Dobie K, Smith G, Sloan AJ. (2002). Effects of alginate hydrogels and TGF-beta 1 on human dental pulp repair in vitro. *Connect Tissue Res*, 43, 387-390.
34. Coimbra P, Alves P, Valente TA. (2011). Sodium hyaluronate/chitson polyelectrolyte complex scaffolds for dental pulp regeneration:synthesis and characterization. *Int J Biol Macromol*, 49, 573-579.
35. Yang KC, Wang CH, Chang HH. (2011). Fibrin glue mixed with platelet-rich fibrin as a scaffold seeded with dental bud cells for tooth regenation. *J Tissue Eng Regen Med*, 6(10), 777-785.