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Mini Review Open Access

Dentin-Pulp Complex Regeneration Therapy Following Pulp Amputation

Takahiko Morotomi[1]*, Yasuhiko Tabata[2] and Chiaki Kitamura[1]

[1]*Division of Endodontics and Restorative Dentistry, Department of Oral Functions, Kyushu Dental University, Japan*
[2]*Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Japan*

Abstract

It is generally accepted that dental pulp plays important roles in maintaining tooth. Pulp inflammation caused by dental caries or tooth fracture sometimes results in a severe pain, and pulpectomy to remove entire dental pulp is often performed by dentists to release a patient from the pain. After pulpectomy, the tooth without vital pulp loses its defensive ability and becomes vulnerable to exogenous stimuli. It will be valuable to establish a local regeneration therapy of dentin-pulp complex from residual dental pulp before pulpectomy to preserve abilities of dental pulp. We are trying to develop a novel therapy to induce regeneration of dentin-pulp complex following pulp amputation. In this approach, growth factors and a scaffold are exogenously supplied, while cells and blood vessels are induced from the residual dental pulp in the tooth root canal. Establishment of a newly method for pulp amputation without leading necrosis of the residual root pulp is also essential for the local regeneration therapy of dentin-pulp complex. In this mini review, we show our research strategy for local regeneration therapy of dentin-pulp complex.

Keywords: Dentin-pulp complex; Pulp amputation; Regeneration therapy

Introduction

Dental pulp, which is surrounded by hard tissue (dentin and enamel), consists of pulp cells, odontoblasts, endothelial cells, neurons, immune system cells, and the extracellular matrix, and plays key roles in maintaining the function of healthy teeth [1]. Via the apical foramen of tooth, blood vessels supply nutrients to the tooth and remove waste products, and neural network warns for harmful stimuli as pain [2]. Immune system cells including dendritic cells, macrophages, and T-lymphocytes, prevent entry of microorganisms and other foreign antigens [2]. Pulp cells and odontoblasts repair dentin that has been lost due to tooth wear or dental caries, by depositing tertiary dentin on the pulp chamber surface as a protective physical barrier in order to block exogenous stimuli [2].

Once the inflammatory response in dental pulp is triggered by stimulation such as severe infection, the internal pressure in the pulp chamber significantly elevates, resulting in severe pain for patients and pulp tissue ischemia [2]. To release patients from the pain, dentists eventually remove an entire pulp by pulpectomy [3]. If pulpectomy is not performed, ischemia develops due to impaired blood circulation, resulting in pulp necrosis [4].

Non-vital tooth becomes vulnerable to exogenous stimuli due to complete loss of perception and immune functions, and weak due to loss of metabolic capacity [3]. Further, non-vital tooth is often re-infected by bacteria. The success rate of root canal retreatment is not particularly high [5-8], and it is often necessary to repeat root canal treatment. Repetition of root canal treatment leads to cracking and/or fracture of the root. As a result, the weakening of the tooth leads to tooth extraction.

To keep sound teeth during entire life of patients, the development of novel therapies for preservation and regeneration of dental pulp are essential.

Local Regeneration of Dentin-pulp Complex Following Pulp Amputation

Problems of present pulp amputation

To avoid pulpectomy, we aim to establish local regeneration therapy of the dentin-pulp complex from residual dental pulp following "pulp amputation". Pulp amputation in the current dental treatment is as follows; after the removal of damaged coronal pulp tissue and the irrigation of the root canal orifice with chemical reagents, calcium hydroxide-based materials or mineral trioxide aggregate are applied to the root canal orifice in order to promote formation of dentin bridge for the preservation of root pulp [3,9-11] (Figure 1A). However, necrotic tissue layer remains at an interface between the residual root pulp and the dentin bridge after the pulp amputation [3]. It is also known that the newly formed dentin bridge by pulp amputation is porous hard tissue with a low degree of calcification, thus they have poor ability to protect the residual root pulp [12]. Most important issue is that pulp amputation itself never leads to the regeneration of pulp or dentin that was lost in the coronal portion.

Novel strategy for the local regeneration of dentin-pulp complex following pulp amputation

Our strategy for the local regeneration of dentin-pulp complex is to induce outgrowth of pulp cells, capillaries, and neurons from the residual root pulp with the modification of the current therapy (Figure 1B). It is well known that three factors are considered to be essential for tissue regeneration; cell, growth factor, and scaffold [13]. For successful regeneration, it is also necessary to induce a capillary network and a closed space that will create a suitable environment [14]. In the development of the dentin-pulp complex regeneration therapy following pulp amputation, it is possible to induce dental pulp stem cells (or pulp progenitor cells), which can differentiate into odontoblasts producing newly dentin, and capillaries from the residual root pulp

***Corresponding author:** Takahiko Morotomi, Division of Endodontics and Restorative Dentistry, Department of Oral Functions, Kyushu Dental University, Japan, Tel: 81932853081; E-mail: morotomi@kyu-dent.ac.jp

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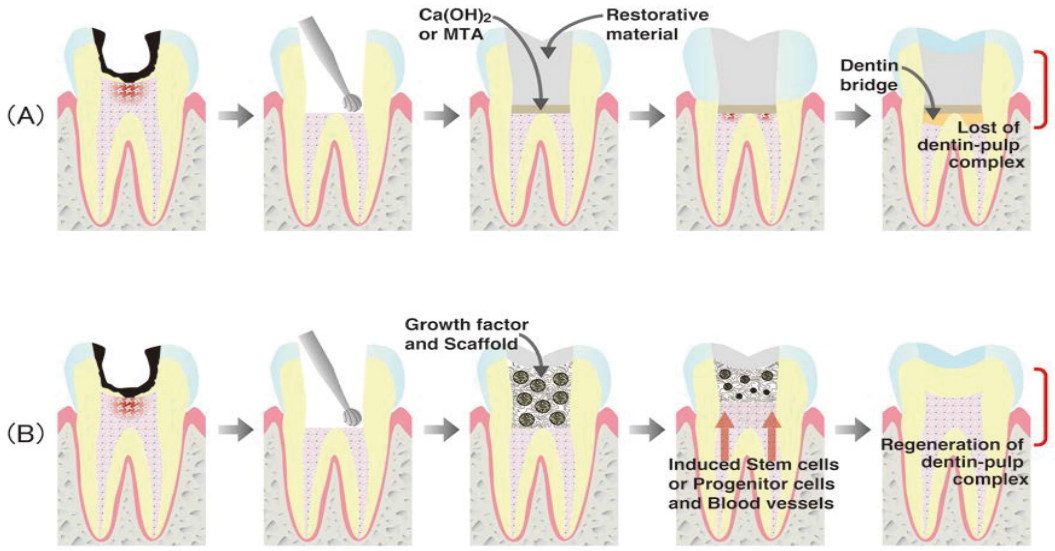

Figure 1: (A) Conventional method for vital pulpotomy. Coronal pulp and dentin are completely lost.
(B) Local regeneration of dentin-pulp complex from residual pulp following vital pulpotomy. Several growth factors and scaffold are implanted into the pulp and dentin defect area. Residual root pulp and blood vessels are induced into the defect area, and dentin-like hard tissue is formed.

tissue. Also, a closed space for tissue regeneration can be secured by application of temporary sealing with adhesive materials that are already used in the clinic. For the establishment of local regeneration of dentin-pulp complex, the choice of an appropriate growth factor, appropriate delivery system of a growth factor, and a scaffold to induce stem cells and blood vessels from the residual pulp are critical points.

Candidate growth factor for regeneration of dental pulp

Fibroblast growth factor-2 (FGF-2) plays an important role in physiologic conditions of odontogenesis [15-17], as well as pathologic conditions [18-20]. We focused on the potency of FGF-2 and chose it as the growth factor for local regeneration therapy of the dentin-pulp complex.

Delivery system of growth factors to the local tissue site is one of important factors for the regeneration. *In vivo*, growth factors usually effects for a certain period at low concentrations. If the growth factors are administered by directly injection in the pulp chamber, they rapidly lose their biological activities due to diffusion and degradation, and their effects are eliminated. To overcome this problem, we focused on a gelatin hydrogel incorporating FGF-2. It was previously demonstrated that a gradual and continual release of FGF-2 was achieved by *in vivo* biodegradation of gelatin hydrogels that incorporated FGF-2 [21-24]. Furthermore, the controlled release of FGF-2 from gelatin hydrogels induced regeneration of angiogenesis [25], bone [26-28], periodontal tissues [29], and other tissues [30-32].

To clarify whether FGF-2-incorporated gelatin hydrogel induces dentin-pulp complex regeneration, we implanted them with collagen sponge scaffold into the coronal pulp chamber of the rat first molar after pulp amputation. We found that controlled release of FGF-2 from gelatin hydrogel induced regeneration of pulp tissue and osteo-dentin like hard tissue at the defect area, demonstrating the efficacy of FGF-2-incorpotated gelatin hydrogel [33,34].

Suitable scaffold for dentin-pulp complex regeneration therapy

In our *in vivo* study, we used collagen sponge as scaffold and it did not show negative effects on the local regeneration of dentin-pulp complex. However, no studies had confirmed which scaffold materials were suitable for dentin-pulp complex regeneration in pulp chamber. We sought a suitable scaffold for the therapy and focused on the potential of hyaluronic acid (HA). HA is one of glycosaminoglycans that are widely distributed in human body. It plays important roles in maintaining morphologic organization by preserving extracellular spaces and is reported to be well suited for tissue engineering material [35-39]. Further, HA was reported to suppress production of proinflammatory cytokines such as interleukin-1 and tumor necrosis factor-alpha by activated macrophages [40,41]. Based on these superior properties, HA is extensively used in the fields of health care and cosmetics today.

To clarify whether HA sponge is useful as a scaffold for dentin-pulp complex regeneration therapy, we carried out *in vitro* and *in vivo* studies. *In vitro* study, odontoblast-like cells (KN-3 cells) established from the incisors of 7-day-old rats were used [42,43], and found that KN-3 cells adhered to HA sponge. *In vivo* study, implantation of HA or collagen sponges in pulp and dentin defect areas following pulp amputation, showed that pulp tissue regeneration was induced into tissue defect, while inflammatory cell invasion in HA sponge implantation area was significantly less compared with collagen sponge [44]. These results suggest that HA sponge has an ideal property as a scaffold for dental pulp regeneration.

These studies suggest the feasibility of the local regeneration therapy following pulp amputation using existing agents and materials. However, for the clinical application of this therapy, we have to establish new techniques for pulp amputation without necrosis of the root pulp, as well as the combination of FGF-2 with other factors to induce regenerated dentin with proper quantity and quality.

In vitro Studies to Protect Dental Pulp from Necrosis

In a tooth targeted for pulp amputation, coronal dental pulp is in an inflammatory state because of dental caries and/or trauma. Amputation of coronal vital pulp will cause an additional severe stimulation of root pulp, resulting in necrosis of the remaining root pulp. A critical point to avoid necrosis is how to regulate heat stress and ischemia simultaneously produced by pulp amputation.

Heat stress produced by rotary cutting instruments used to remove infected dental hard tissue is known as one of the most severe exogenous stimuli for dental pulp [2,45]. It is also known that ischemia, caused by inflammation and/or local anesthetics containing a vasoconstrictor [46-48], induces hypoxia and starvation in dental pulp [49,50]. Pulp cells have abilities to resist to heat stress and ischemia [51,52]. We examined the combinatorial effects of these stimuli, and found that the effects of heat stress on dental pulp cells were significantly reinforced by starvation. These results imply that use of vasoconstrictor-free local anesthetic would be beneficial for preserving the root pulp following pulp amputation [53,54].

We have also performed studies aimed to increase the resistance ability of pulp cell to lethal heat stimuli. It is known that fever-range heat stress, the range of 40-42°C, has a beneficial role in various mammalian cells [55-57], and exogenous fever-range heat stress as hyperthermia has been widely used as a physical therapy for various diseases [58]. We hypothesized that the fever-range heat stress for several hours can increase resistance of pulp cells to stimuli. KN-3 cells were pre-treated with fever-range heat stress at 41°C for 12 hours, followed by additional lethal heat stress at 49°C for 10 minutes. We found that the cells pre-treated by heat stress survived with odontoblast-like properties after lethal heat stress, and that various heat shock proteins (HSPs) accumulated in the cells [59], and transient cell-cycle arrest was induced by the fever-range heat stress for 12 hours [60]. Accumulation of HSPs and/or cell-cycle arrest is able to induce cellular resistance to various stimuli [61-65]. We are now continuing research to identify the effective methods to induce HSPs accumulation and transient cell-cycle arrest.

In vitro Studies to Induce Regenerated Dentin with Proper Quality and Quantity

Previously we showed that newly dentin was induced on the occlusal side of the regenerated dental pulp through the controlled release of FGF-2 from gelatin hydrogels [34]. However, the induced dentin did not have an ideal structure with dentinal tubules, and its quantity was insufficient for protecting the dental pulp or withstanding bite forces. These weak points should be overcome before clinical application.

Bone morphogenetic protein 2 (BMP-2) is known to induce the differentiation of dental pulp stem cells into odontoblasts [66], and *in vivo* dentin formation after pulp amputation [67]. BMP-2 is a growth factor to be approved by the US Food and Drug Administration for clinical use such as oral maxillofacial surgery [68]. Our previous study with KN-3 cells showed that BMP-2 induced the expressions of dentin matrix proteins such as dentin sialoprotein and dentin matrix protein-1, markers of the differentiation of odontoblasts [69-71], and that Smad signaling pathway involved in the induction process [72]. Furthermore, we found that platelet-rich plasma (PRP) enhances differentiation of KN-3 cells, as well as alkaline phosphatase activity [73]. PRP is an autologous blood product, and it has been used for wound healing of soft tissue and bone repair as a source of growth factors in several clinical settings such as orthopedic surgery [74-77]. These results suggest that the suitable combination of BMP-2, FGF-2 and PRP may solve the problem in local regeneration of dentin-pulp complex after pulp amputation.

Conclusion

In this mini-review, we show our strategy for the local regeneration therapy of dentin-pulp complex, and critical points to overcome before clinical application of this method with ideal efficacy and safety. We believe that our challenge for the local regeneration therapy of dentin-pulp complex would change the current dental treatment to preserve dental pulp.

References

1. Rudolph P (2006) Essentials of oral histology and embryology: a clinical approach. Mosby Elsevier, USA.
2. Hargreaves KM, Goodis HE (2002) Seltzer and Bender's dental pulp. Quintessence Publishing, USA.
3. Bergenholtz G, Hørsted-Bindslev P, Reit C (2009) Textbook of endodontology. Wiley-Blackwell, USA.
4. Heyeraas KJ, Kvinnsland I (1992) Tissue pressure and blood flow in pulpal inflammation. Proc Finn Dent Soc 88 Suppl 1: 393-401.
5. Van Nieuwenhuysen JP, Aouar M, D'Hoore W (1994) Retreatment or radiographic monitoring in endodontics. Int Endod J 27: 75-81.
6. Rotstein I, Salehrabi R, Forrest JL (2006) Endodontic treatment outcome: survey of oral health care professionals. J Endod 32: 399-403.
7. Imura N, Pinheiro ET, Gomes BP, Zaia AA, Ferraz CC, et al. (2007) The outcome of endodontic treatment: a retrospective study of 2000 cases performed by a specialist. J Endod 33: 1278-1282.
8. Ng YL, Mann V, Gulabivala K (2008) Outcome of secondary root canal treatment: a systematic review of the literature. Int Endod J 41: 1026-1046.
9. Nosrat IV, Nosrat CA (1998) Reparative hard tissue formation following calcium hydroxide application after partial pulpotomy in cariously exposed pulps of permanent teeth. Int Endod J 31: 221-226.
10. Reston EG, de Souza Costa CA (2009) Scanning electron microscopy evaluation of the hard tissue barrier after pulp capping with calcium hydroxide, mineral trioxide aggregate (MTA) or ProRoot MTA. Aust Endod J 35: 78-84.
11. Lee H, Shin Y, Kim SO, Lee HS, Choi HJ, et al. (2015) Comparative Study of Pulpal Responses to Pulpotomy with ProRoot MTA, RetroMTA, and TheraCal in Dogs' Teeth. J Endod 41: 1317-1324.
12. Leye Benoist F, Gaye Ndiaye F, Kane AW, Benoist HM, Farge P (2012) Evaluation of mineral trioxide aggregate (MTA) versus calcium hydroxide cement (Dycal®) in the formation of a dentine bridge: a randomised controlled trial. Int Dent J 62: 33-39.
13. Langer R, Vacanti JP (1993) Tissue engineering. Science 260: 920-926.
14. Discher DE, Mooney DJ, Zandstra PW (2009) Growth factors, matrices, and forces combine and control stem cells. Science 324: 1673-1677.
15. Tsuboi T, Mizutani S, Nakano M, Hirukawa K, Togari A (2003) Fgf-2 regulates enamel and dentine formation in mouse tooth germ. Calcif Tissue Int 73: 496-501.
16. Madan AK, Kramer B (2005) Immunolocalization of fibroblast growth factor-2 (FGF-2) in the developing root and supporting structures of the murine tooth. J Mol Histol 36: 171-178.
17. Tran-Hung L, Mathieu S, About I (2006) Role of human pulp fibroblasts in angiogenesis. J Dent Res 85: 819-823.
18. Annabi B, Naud E, Lee YT, Eliopoulos N, Galipeau J (2004) Vascular progenitors derived from murine bone marrow stromal cells are regulated by fibroblast growth factor and are avidly recruited by vascularizing tumors. J Cell Biochem 91: 1146-1158.
19. Etscheid M, Beer N, Kress JA, Seitz R, Dodt J (2004) Inhibition of bFGF/EGF-dependent endothelial cell proliferation by the hyaluronan-binding protease from human plasma. Eur J Cell Biol 82: 597-604.
20. Taylor KR, Rudisill JA, Gallo RL (2005) Structural and sequence motifs in dermatan sulfate for promoting fibroblast growth factor-2 (FGF-2) and FGF-7 activity. J Biol Chem 280: 5300-5306.
21. Tabata Y, Ikada Y (1999) Vascularization effect of basic fibroblast growth factor released from gelatin hydrogels with different biodegradabilities. Biomaterials 20: 2169-2175.
22. Tabata Y, Miyao M, Inamoto T, Ishii T, Hirano Y, et al. (2000) De novo formation of adipose tissue by controlled release of basic fibroblast growth factor. Tissue Eng 6: 279-289.

23. Tabata Y, Nagano A, Ikada Y (1999) Biodegradation of hydrogel carrier incorporating fibroblast growth factor. Tissue Eng 5: 127-138.

24. Yamamoto M, Ikada Y, Tabata Y (2001) Controlled release of growth factors based on biodegradation of gelatin hydrogel. J Biomater Sci Polym Ed 12: 77-88.

25. Marui A, Tabata Y, Kojima S, Yamamoto M, Tambara K, et al. (2007) A novel approach to therapeutic angiogenesis for patients with critical limb ischemia by sustained release of basic fibroblast growth factor using biodegradable gelatin hydrogel: an initial report of the phase I-IIa study. Circ J 71: 1181-1186.

26. Kawaguchi H, Nakamura K, Tabata Y, Ikada Y, Aoyama I, et al. (2001) Acceleration of fracture healing in nonhuman primates by fibroblast growth factor-2. J Clin Endocrinol Metab 86: 875-880.

27. Tabata Y, Yamada K, Hong L, Miyamoto S, Hashimoto N, et al. (1999) Skull bone regeneration in primates in response to basic fibroblast growth factor. J Neurosurg 91: 851-856.

28. Hayashi K, Kubo T, Doi K, Tabata Y, Akagawa Y (2007) Development of new drug delivery system for implant bone augmentation using a basic fibroblast growth factor-gelatin hydrogel complex. Dent Mater J 26: 170-177.

29. Nakahara T, Nakamura T, Kobayashi E, Inoue M, Shigeno K, et al. (2003) Novel approach to regeneration of periodontal tissues based on in situ tissue engineering: effects of controlled release of basic fibroblast growth factor from a sandwich membrane. Tissue Eng 9: 153-162.

30. Nakayama J, Fujioka H, Nagura I, Kokubu T, Makino T, et al. (2009) The effect of fibroblast growth factor-2 on autologous osteochondral transplantation. Int Orthop 33: 275-280.

31. Kimura Y, Hokugo A, Takamoto T, Tabata Y, Kurosawa H. (2008) Regeneration of anterior cruciate ligament by biodegradable scaffold combined with local controlled release of basic fibroblast growth factor and collagen wrapping. Tissue Eng Part C Methods 14: 47-57.

32. Yasuda Y, Koyama H, Tabata Y, Fujihara Y, Oba M, et al. (2008) Controlled delivery of bFGF remodeled vascular network in muscle flap and increased perfusion capacity via minor pedicle. J Surg Res 147: 132-137.

33. Kikuchi N, Kitamura C, Morotomi T, Inuyama Y, Ishimatsu H, et al. (2007) Formation of dentin-like particles in dentin defects above exposed pulp by controlled release of fibroblast growth factor 2 from gelatin hydrogels. J Endod 33: 1198-1202.

34. Ishimatsu H, Kitamura C, Morotomi T, Tabata Y, Nishihara T, et al. (2009) Formation of dentinal bridge on surface of regenerated dental pulp in dentin defects by controlled release of fibroblast growth factor-2 from gelatin hydrogels. J Endod 35: 858-865.

35. Angele P, Kujat R, Nerlich M, Yoo J, Goldberg V, et al. (1999) Engineering of osteochondral tissue with bone marrow mesenchymal progenitor cells in a derivatized hyaluronan-gelatin composite sponge. Tissue Eng 5: 545-554.

36. Ramamurthi A, Vesely I (2002) Smooth muscle cell adhesion on crosslinked hyaluronan gels. J Biomed Mater Res 60: 195-205.

37. Kim HD, Valentini RF (2002) Retention and activity of BMP-2 in hyaluronic acid-based scaffolds in vitro. J Biomed Mater Res 59: 573-584.

38. Liu Y, Shu XZ, Gray SD, Prestwich GD (2004) Disulfide-crosslinked hyaluronan-gelatin sponge: growth of fibrous tissue in vivo. J Biomed Mater Res A 68: 142-149.

39. Peattie RA, Nayate AP, Firpo MA, Shelby J, Fisher RJ, et al. (2004) Stimulation of in vivo angiogenesis by cytokine-loaded hyaluronic acid hydrogel implants. Biomaterials 25: 2789-2798.

40. Shimizu M, Yasuda T, Nakagawa T, Yamashita E, Julovi SM, et al. (2003) Hyaluronan inhibits matrix metalloproteinase-1 production by rheumatoid synovial fibroblasts stimulated by proinflammatory cytokines. J Rheumatol 30: 1164-1172.

41. Julovi SM, Ito H, Hiramitsu T, Yasuda T, Nakamura T (2008) Hyaluronan inhibits IL-1beta-stimulated collagenase production via down-regulation of phosphorylated p38 in SW-1353 human chondrosarcoma cells. Mod Rheumatol 18: 263-270.

42. Morotomi T, Kawano S, Toyono T, Kitamura C, Terashita M, et al. (2005) In vitro differentiation of dental epithelial progenitor cells through epithelial-mesenchymal interactions. Arch Oral Biol 50: 695-705.

43. Nomiyama K, Kitamura C, Tsujisawa T, Nagayoshi M, Morotomi T, et al. (2007) Effects of lipopolysaccharide on newly established rat dental pulp-derived cell line with odontoblastic properties. J Endod 33: 1187-1191.

44. Inuyama Y, Kitamura C, Nishihara T, Morotomi T, Nagayoshi M, et al. (2010) Effects of hyaluronic acid sponge as a scaffold on odontoblastic cell line and amputated dental pulp. J Biomed Mater Res B Appl Biomater 92: 120-128.

45. Zach L (1972) Pulp lability and repair; effect of restorative procedures. Oral Surg Oral Med Oral Pathol 33: 111-121.

46. Jacobsen I, Kerekes K (1977) Long-term prognosis of traumatized permanent anterior teeth showing calcifying processes in the pulp cavity. Scand J Dent Res 85: 588-598.

47. Kim S, Edwall L, Trowbridge H, Chien S (1984) Effects of local anesthetics on pulpal blood flow in dogs. J Dent Res 63: 650-652.

48. Odor TM, Pitt Ford TR, McDonald F (1994) Adrenaline in local anaesthesia: the effect of concentration on dental pulpal circulation and anaesthesia. Endod Dent Traumatol 10: 167-173.

49. Stevens A, Lowe J (2000) Pathology Mosby, London, UK.

50. Kumar V, Abbas AK, Fausto N, Mitchell R (2007) Robbins basic pathology. Elsevier Health Science, USA.

51. Kitamura C, Nishihara T, Ueno Y, Nagayoshi M, Kasugai S, et al. (2005) Thermotolerance of pulp cells and phagocytosis of apoptotic pulp cells by surviving pulp cells following heat stress. J Cell Biochem 94: 826-834.

52. Ueno Y, Kitamura C, Terashita M, Nishihara T (2006) Re-oxygenation improves hypoxia-induced pulp cell arrest. J Dent Res 85: 824-828.

53. Morotomi T, Kitamura C, Toyono T, Okinaga T, Washio A, et al. (2011) Effects of heat stress and starvation on clonal odontoblast-like cells. J Endod 37: 955-961.

54. Morotomi T, Kitamura C, Minakami M, Itaya K, Ushio S, et al. (2012) Effect of Heat Stress on Clonal Odontoblast-like Cells under Starvation Condition. J Jpn Endod Assoc 33: 105-112.

55. Park HG, Han SI, Oh SY, Kang HS (2005) Cellular responses to mild heat stress. Cell Mol Life Sci 62: 10-23.

56. Griffin RJ, Dings RP, Jamshidi-Parsian A, Song CW (2010) Mild temperature hyperthermia and radiation therapy: role of tumour vascular thermotolerance and relevant physiological factors. Int J Hyperthermia 26: 256-263.

57. Bull JM, Lees DE, Schuette WH, Smith R, Glatstein E, et al. (1982) DeVita VT Jr. Immunological and physiological responses to whole-body hyperthermia. Natl Cancer Inst Monogr 61: 177-181.

58. Yamaguchi T, Suzuki T, Arai H, Tanabe S, Atomi Y (2010) Continuous mild heat stress induces differentiation of mammalian myoblasts, shifting fiber type from fast to slow. Am J Physiol Cell Physiol 298: C140-148.

59. Lanneau D, de Thonel A, Maurel S, Didelot C, Garrido C (2007) Apoptosis versus cell differentiation: role of heat shock proteins HSP90, HSP70 and HSP27. Prion 1: 53-60.

60. Morotomi T, Kitamura C, Okinaga T, Nishihara T, Sakagami R, et al. (2014) Continuous fever-range heat stress induces thermotolerance in odontoblast-lineage cells. Arch Oral Biol 59: 741-748.

61. Weinert TA, Hartwell LH (1988) The RAD9 gene controls the cell cycle response to DNA damage in Saccharomyces cerevisiae. Science 241: 317-322.

62. Siede W, Friedberg AS, Friedberg EC (1993) RAD9-dependent G1 arrest defines a second checkpoint for damaged DNA in the cell cycle of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 90: 7985-7989.

63. Craig EA, Weissman JS, Horwich AL (1994) Heat shock proteins and molecular chaperones: mediators of protein conformation and turnover in the cell. Cell 78: 365-372.

64. Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381: 571-579.

65. Arya R, Mallik M, Lakhotia SC (2007) Heat shock genes - integrating cell survival and death. J Biosci 32: 595-610.

66. Iohara K, Nakashima M, Ito M, Ishikawa M, Nakasima A, et al. (2004) Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 2. J Dent Res 83: 590-595.

67. Nakashima M (1994) Induction of dentine in amputated pulp of dogs by

recombinant human bone morphogenetic proteins-2 and -4 with collagen matrix. Arch Oral Biol 39: 1085-1089.

68. McKay WF, Peckham SM, Badura JM (2007) A comprehensive clinical review of recombinant human bone morphogenetic protein-2 (INFUSE Bone Graft). Int Orthop 31: 729-734.

69. D'Souza RN, Cavender A, Sunaval G, Alvarez J, Ohshima T et al. (1997) Gene expression patterns of murine dentin matrix protein 1 (Dmp1) and dentin sialophosphoprotein (DSPP) suggest distinct development functions in vivo. J Bone Miner Res 12: 2040-2049.

70. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, et al. (2002) Stem cell properties of human dental pulp stem cells. J Dent Res 81: 531-535.

71. Qin C, D'Souza R, Feng JQ (2007) Dentin matrix protein 1 (DMP1): new and important roles for biomineralization and phosphate homeostasis. J Dent Res 86: 1134-1141.

72. Washio A, Kitamura C, Morotomi T, Terashita M, Nishihara T (2012) Possible involvement of Smad signaling pathways in induction of odontoblastic properties in KN-3 cells by bone morphogenetic protein-2, a growth factor to induce dentin regeneration. Int J Dent 2012: 1-6.

73. Yeom KH, Ariyoshi W, Okinaga T, Washio A, Morotomi T, et al. (2015) Platelet-rich plasma enhances the differentiation of dental pulp progenitor cells into odontoblasts. Int Endod J .

74. Eppley BL, Woodell JE, Higgins J (2004) Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. Plast Reconstr Surg 114: 1502-1508.

75. Jenis LG, Banco RJ, Kwon B (2006) A prospective study of Autologous Growth Factors (AGF) in lumbar interbody fusion. Spine J 6: 14-20.

76. Mehta S, Watson JT (2008) Platelet rich concentrate: basic science and current clinical applications. J Orthop Trauma 22: 432-438.

77. Alsousou J, Thompson M, Hulley P, Noble A, Willett K (2009) The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. J Bone Joint Surg Br 91: 987-996.