CHAPTER 2

REVIEW OF THE LITERATURE

Regenerative endodontics

Regenerative medicine offers a promise for the restoration of tissues and organs damaged by disease, trauma or congenital deformity. Regenerative medicine uses the combination of cells, engineering materials and proper biochemical factors to restore, maintain or improve biological functions (31, 32).

Regenerative endodontics can be defined as biologically based procedures desire to replace damaged structures, including dentin and root structures, as well as cells of the pulp- dentin complex (31). The main objective of regenerative endodontics (Figure 1) is to generate/regenerate dentin-pulp complex (31). Therefore, vital pulp tissue would be preserved or regenerated keeping the tooth in homeostasis. This concept has become an interest since conventional calcium hydroxide and MTA apexification could neither strengthen nor ensure further root development. In an immature tooth, with regenerative endodontics, root development can continue, ensuring a thicker dentinal wall that is resistant to fracture, and closure of the apex, that makes subsequent endodontic therapy, if required, more predictable (33). In additions, the importance of non-infected vital pulp is that it maintains apical periodontal health (34).

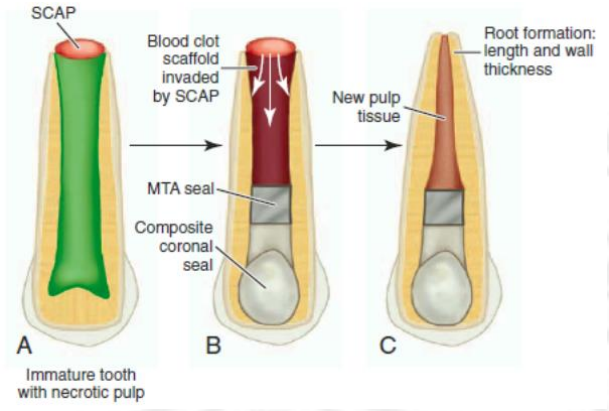

Figure 1: *The main objective of regenerative endodontics is to generate/regenerate dentin-pulp complex. In an immature tooth, a root development can continue ensuring a thicker dentinal wall that is resistant to fracture (35).*

History of Regenerative endodontics

In 1961, Nygaard-Østby evaluated whether pulp tissue can be regenerated in the root canal system. His approach was relied on the role of blood clots in the wound healing process after tearing periapical tissue of the teeth, either vital or necrotic, with endodontic files. The results were evaluated both clinically and histologically and reported that apical inflammation resolved approximately within 2 weeks and periodontal tissue healed in 1 month. The blood clot in the root canals (Figure 2) was gradually substituted by granulation tissue and fibrous connective tissue, however, mostly incomplete. Some of histological findings showed the deposition of cementum together with some evidences of resorption along the dentinal wall (16).

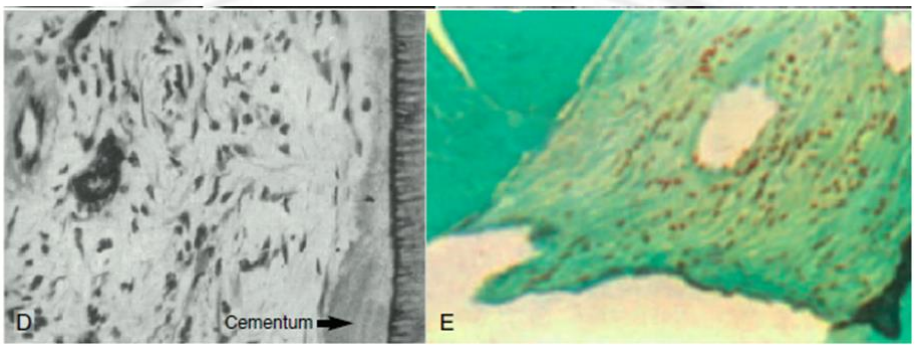

Figure 2: *The blood clot in the root canals was gradually substituted by granulation tissue and fibrous connective tissue, but, mostly incomplete. The deposition of cementum (arrow) together with some evidences of resorption along the dentinal wall (35).*

Another similar study from Nygaard-Østby and Hjortdal in 1971 revealed histologic evidence of vascularized fibrous connective tissue grew in the root canal system in 80% of teeth with vital pulp status but only 1% in the teeth with necrotic pulps. In additions, newly mineralized tissues were evidenced on some dentinal walls and appeared to be cementum not dentin (Figure 3)(36).

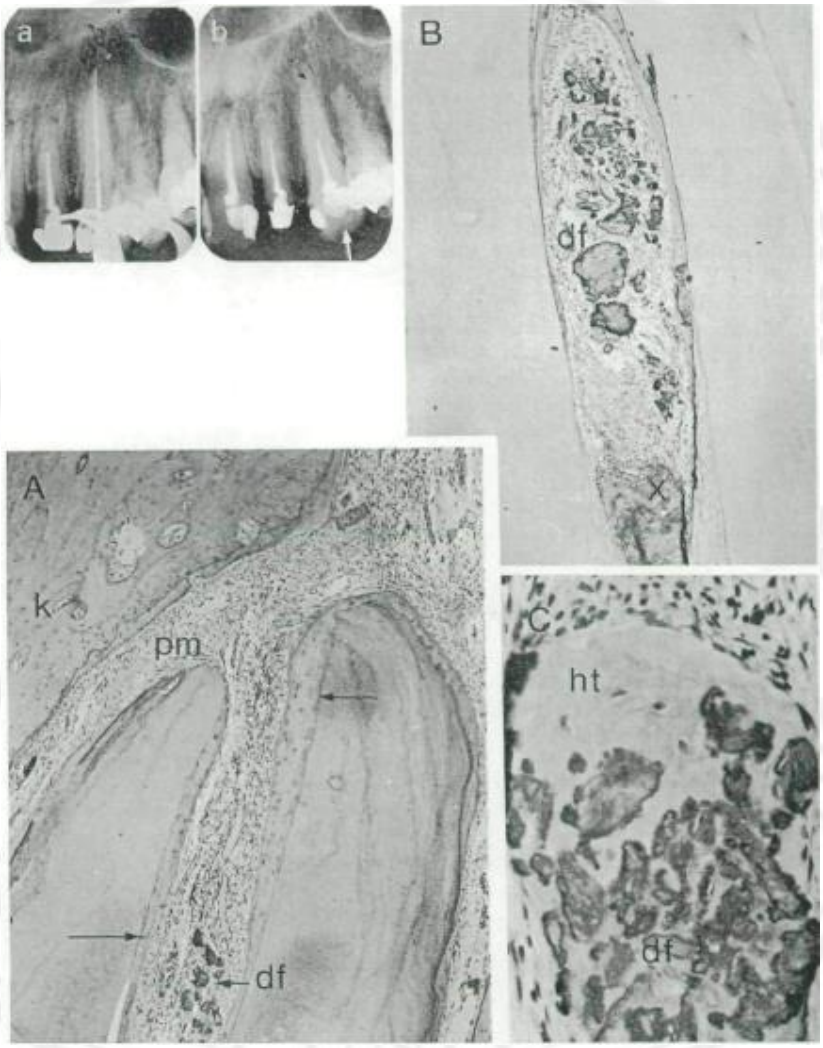

Figure 3: *Histologic evidence of vascularized fibrous connective tissue grew in the root canal system. Newly mineralized tissues were evidenced on some dentinal walls and appeared to be cementum not dentin (arrow in A) (36).*

In 1978, Hørsted and Nygaard-Østby assumed that total removal of a vital pulp, under strict asepsis, does not require a subsequent filling of the entire root canal. The empty space was replaced by fibrous connective tissue (37). Admittedly, this study was associated with clinically healthy pulp, however, a similar results also

reported when chronically inflamed pulps were treated using the same methods (16, 36).

Regeneration of a necrotic pulp was considered to be successful in avulsed immature teeth after replantation. Because of wide and short open apex, the tissue may grow into the root canal quickly. The non-infected necrotic tissue act as a scaffold which the blood vessels can grow slowly from apical to coronal direction (38). Furthermore, there is an evidence (39) which showed that if the radiographic apex is larger than 1.1 mm and tooth is replanted within 45 minutes will advocate the chance for revascularization by 18%. Doxycycline soaked over the tooth before replantation also significantly improved the incidence of successful revascularization (40, 41).

However, regenerative endodontics in the necrotic tooth with periapical pathosis might be difficult to achieve. Theoretically, if it is able to create a sterile environment as in avulsed tooth, regeneration should occur.

A paradigm shift in the management of immature teeth with necrotic pulps

A conventional method for treating immature teeth with necrotic pulps is calcium hydroxide apexification/apexogenesis. Briefly, a root canal is disinfected using various irrigants such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylene diamine tetra acetic acid (EDTA) (42) following by $Ca(OH)_2$ medication for a period of time. Calcific barrier at the root apex is often suspected after 20 months (43). The disadvantages of $Ca(OH)_2$ can be summarized as (43).

1) Long time used for the entire treatment

2) Multiple visits and inevitable clinical costs

3) Increased risk of tooth fracture as a long-term $Ca(OH)_2$ dressing (7, 8, 44)

From these drawbacks, the use of Mineral trioxide aggregate (MTA) was recently introduced showing more predictable results (4). Root canal cleaning and shaping followed by immediate MTA apical plug makes it possible to complete the root canal treatment in less treatment visits. Therefore, placement of the bonded restoration into the root canal system can be done which may prevent the tooth from fracture (43). In additions, MTA apical barrier method spends less treatment period, which improved patient compliance (45, 46).

Unfortunately, both $Ca(OH)_2$ apexification and MTA apical barrier provided a small increase in root length and width after a period of time (5, 6). Thus, alternative approaches to obtain these purposes are being interested.

In 2001, the first case report was introduced in regenerative endodontic field by Iwaya et al (10). That case presented an immature mandibular second premolar with sinus tract and large periapical radiolucency. During the treatment session, patient felt discomfort on preparing access cavity and a smooth broach insertion indicating that some of vital tissues may remain in the apical part of canal space. The root canal was mainly irrigated with NaOCl and hydrogen peroxide (H_2O_2) without mechanical cleaning and a two antibiotic mixture (metronidazole and ciprofloxacin) was used as inter-appointment intra-canal dressing. Bleeding was created. At the 30^{th} months follow up, periapical film revealed a completion of root apex and the

thickness of the canal walls. The tooth also responded positively to electronic pulp testing.

From the previous successful results (10), the same idea was continually developed by, Banch and Trope in 2004 (9). A new treatment protocol known as "revascularization" has been proposed after a successful treatment of right mandibular second premolar with large radiolucent area was presented. The clinical technique used in this study was adapted from Rule and Winter and Iwaya et al (10, 47). Briefly, a mixture of ciprofloxacin, metronidazole and minocycline, described by Hoshino et al (27), was applied after chemical disinfection. For a period of time, the medication was removed and a blood clot was created inside the root canal. After 2-year careful follow-up, the root development continued to complete with a positive response to cold test. Later, Chueh and Huang reported a case series (11) which data were collected from 1988 to 2000. All of their cases demonstrated complete healing with apical maturation. Currently, a variety of case reports have been published with different results using similar and adapted treatment protocols (12-15).

Current clinical overview for revascularization (Adapted from Pathway of the pulp) (35).

- At the first appointment, the treatment alternatives, risks and benefits should be described to patients after clinical diagnosis.
- Following informed consent, the tooth is anaesthesized, isolated and gaining access.
- Scout the root canal system and determine working length with small file but minimal instrumentation.

- Slowly copious irrigated with NaOCl and CHX.
- Irrigation using closed-end needles, together with slow infusion, to reduce any irrigants passing through the open apex.
- Medicated the root canal system with triple antibiotics, discharge patient for 3-4 weeks.
- At the second appointment, the patient is evaluated for resolution of any signs of an acute infection.
- If the acute signs and symptoms have resolved, the tooth is isolated and re-established of the coronal access. Medicament is removed by small file and copious irrigation, after that, dry the canal with sterile paper points.
- A file is placed a few millimeters beyond the root apex, laceration of the apical tissue, to induce bleeding up to 3 mm from the CEJ.
- A small piece of Colla-plug may be inserted to serve as resorbable matrix to restrict the positioning of the MTA. About 3 mm of MTA is placed followed by restoration. A 12-18 months recall should be considered to evaluate continued radiographic improvement in root development.

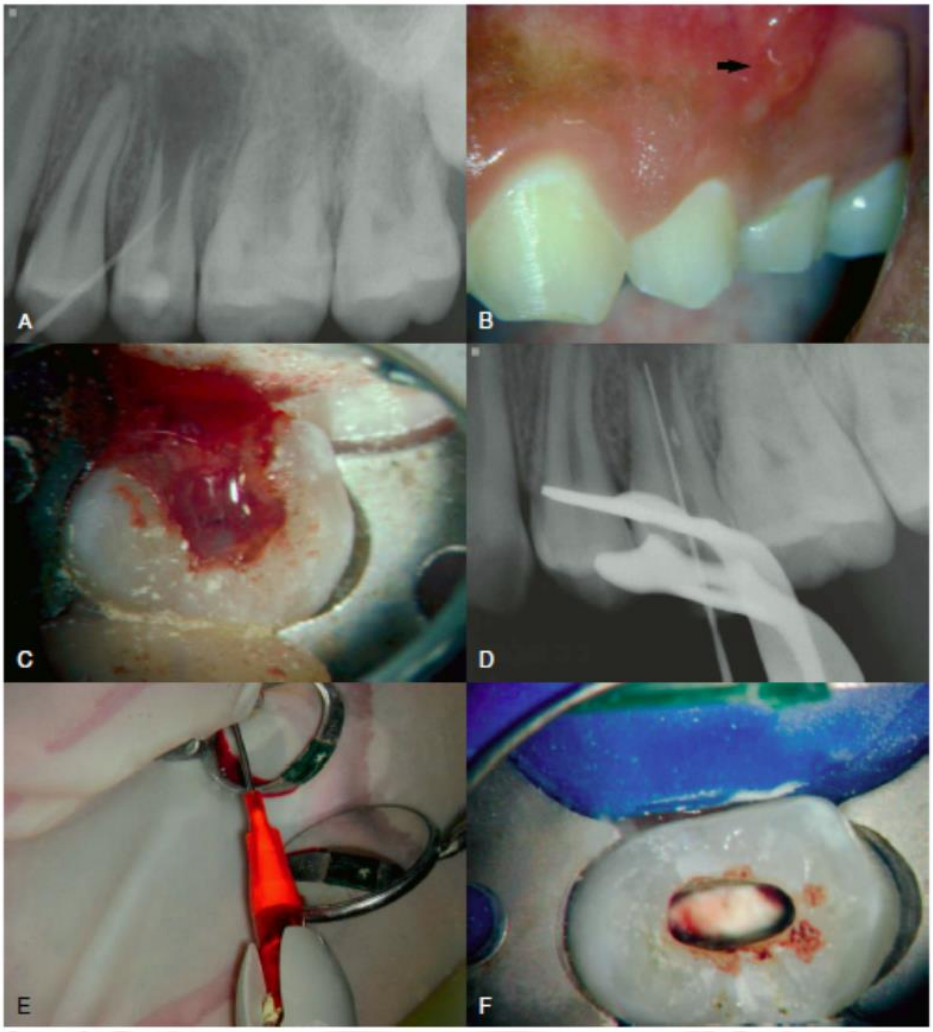

Figure 4: *Revascularization procedures (A) First appointment, clinical examination revealed a sinus tract. (B) Soft tissue swelling that required incision and drainage. (C&D) Purulent/hemorrhagic exudate upon access. (E) The working length file was inserted and the tooth was slowly irrigated with 20 ml 5.25% NaOCl and 20 ml of 0.12% chlorhexidine using a MaX-I-probe inserted into the apical third. The tooth was dried and medicated with triple antibiotics. The patient returned 1 month later. The tooth was isolated, accessed and the medication removed by slow irrigation of*

NaOCl. (F) CollaPlug was placed below the CEJ to position the MTA coronal to the blood clot (35).

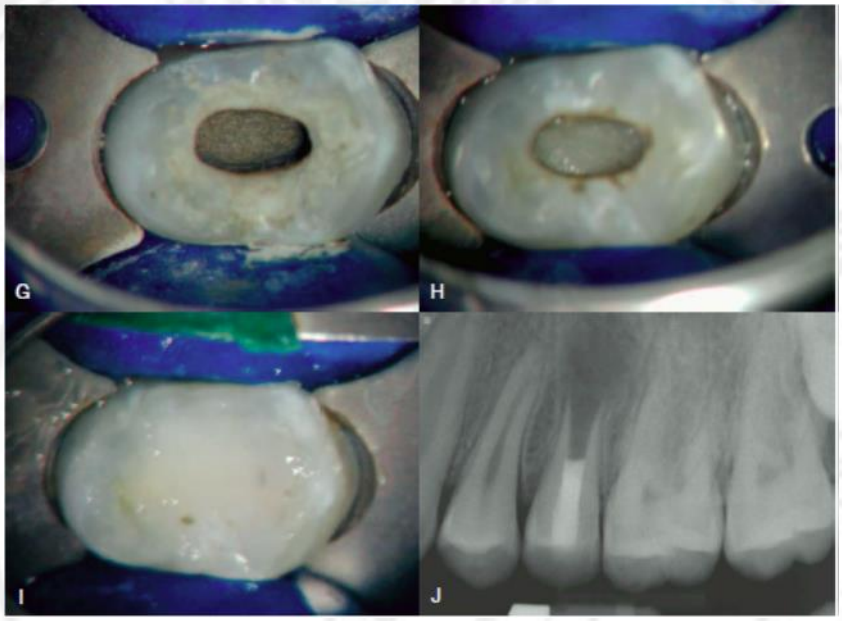

Figure 4: *cont'd (G) MTA was applied and positioned coronal to the blood clot (H-J) The tooth was sealed by Fuji II and composite resin (35).*

The hypothesis called "Lesion Sterilization and Tissue Repair (LSTR)" proposed that the complete removal of infection in the lesion by application of antibiotic mixtures may contribute to resolution of dentinal, pulpal, and periapical lesion (48). The objective of this LSTR therapy is to eradicate causative bacteria from infected site using antibiotic combination, and after the affected site had been sterilized, the lesion would be regenerated by natural recovery process.

The root canal infection is considered to be a polymicrobial infection, including both aerobic and anaerobic bacteria (49). Hence, any single antibiotics may not result in an effective sterilization of the root canal system. A combination of antibiotics would be necessary to introduce to the root canal for effective sterilization.

The triple antibiotic mixture which appears to be most promising consists of metronidazole, ciprofloxacin, and minocycline (26, 27). This drug combination was investigated in laboratory and found that it was very effective in sterilization of carious lesions, necrotic pulp tissue, and infected root dentin of deciduous teeth (26). Moreover, an in vitro study form Hoshino et al revealed that these drugs alone would not result in complete elimination of bacteria. On the other hand, in combination, these drugs potentiated to sterilize all samples (27). Another study showed that the combination of antibiotics was very effective in killing bacteria in the deep layers of root canal dentin (28).

Metronidazole is a nitroimidazole compound which exhibits a broad spectrum against protozoa and anaerobic bacteria. It has strong anti-bacterial properties to anaerobic cocci, gram negative/positive bacilli. The mechanism of metronidazole is it permeates the cell membrane of bacteria and then bind to the DNA, bursting its helical structure, and leads to a rapid cell death. Metronidazole has an excellent activity against anaerobes but has no activity against aerobes.

Minocycline, a semi-synthetic derivative of tetracycline, is a group of bacteriostatic antimicrobial agent. It has a broad spectrum against gram-positive/negative microorganisms and also is effective against most spirochaete, many anaerobes, and facultative bacteria. This drug reaches the bacterial cells by passive diffusion through the outer membrane followed by active transport to the inner membrane. Then, it inhibited protein synthesis on ribosomal surface.

Ciprofloxacin is a synthetic floroquinolone and has a bactericidal effect. It inhibits the DNA gyrase result in degradation of the DNA by exonucleases.

Ciprofloxacin has a very potent activity against gram-negative bacteria but less effective for gram-positive bacteria and most anaerobic bacteria are resistant to ciprofloxacin. It may assume that such a single antibiotics can not be properly used for the treatment of mixed infections.

The drug recipe (34) for clinical application is comprised of ciprofloxacin 200 mg, metronidazole 500 mg, and minocycline 100 mg. The sugar coating of tablet form drug was removed with surgical blade and pulverized individually with pestles and mortars. The capsule drug was opened and crushed individually in separate mortars. Pulverized each antibiotics into fine powder then combine equal amount of each drugs (1:1:1) on mixing pad. The vehicles used for this preparation are macrogol ointment and propylene glycol (MP) in equal amount (1:1). Separate small amount of 3Mix and consolidate into MP at the ratio 1:5 (MP: 3Mix) for creamy consistency or 1:7 for standard mix (smear easily but does not crumble).

Recent study showed that an application of this drug combination as a root canal medication for 2 weeks demonstrated the significant reduction of bacterial counts in immature dog teeth with apical periodontitis (50).

In 2009, a case report revealed the efficacy of triple antibiotic paste consisting of metronidazole, ciprofloxacin, and minocycline in disinfection of immature teeth with apical periodontitis. This case exhibited the complete resolution of large periapical lesion after intracanal medicated with triple antibiotic paste for 4 months and the root continuation was completed after 1 year long-term medication. These results implied that the use of triple antibiotic paste allow for regenerated vital tissue to occupy the canal space (51).

Moreover, an animal study revealed triple antibiotic paste induced moderate reaction in subcutaneous tissue at 7 and 15 days but was similar to the control group. These reactions reduced in intensity from day 30 onward. This study found that triple antibiotic was biocompatible similar to calcium hydroxide (52).

Histological characterization after pulp revascularization

To date, several histological observations have been reported with controversy (17-22). The first histological evidence in dog models revealed the outcome of regenerative endodontic treatment that the root canal walls were deposited by hard tissue (43.9%), formation of vital tissue (29.3%) and apical closure (54.9%)(18). Later in 2010, a histological study in infected immature dog's teeth found that revitalization approach allows ingrowth of vital tissue, which mainly resembled cementum, PDL and bone (Figure 5). Only 1 case from 48 teeth in all experimental groups revealed partially survived pulp tissue with the evidence of odontoblast lining in the canal. All the rest of the cases in this study appeared no typical pulp tissue or new dentin regenerated in the canal spaces. Another study by Da Silva has also reported a similar result in an experimental dog model (22). Moreover, a recent case report by Martin (2012), who used platelet-rich plasma in revascularization technique to treat a necrotic immature tooth, revealed the tissues regenerated in the root canals were mineralized tissue resemble cementoid/osteoid and some fibrous connective tissue. No odontoblast-like cells, which are indicators of pulp-like tissue, was observed lining at the dentin wall (Figure 6)(21).

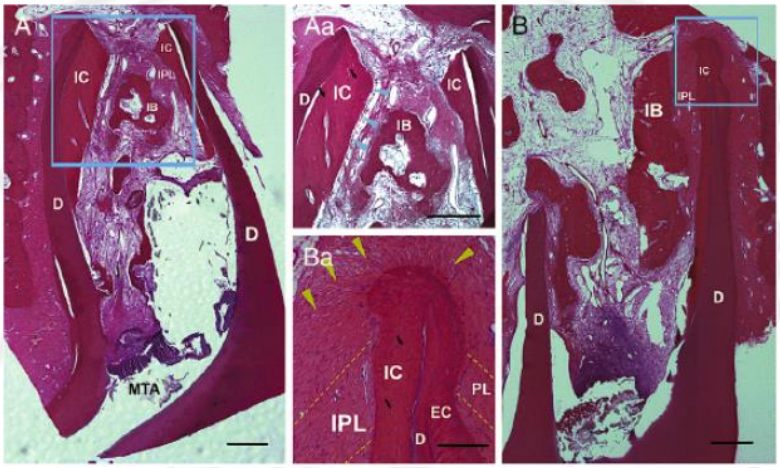

Figure 5: *Histological study of infected immature dog's teeth. Thicken root resulting from the deposition of intracanal cementum (IC) onto dentin. Intracanal bone-liked tissues (IB) scattered in the root canal space togethered with intracanal PDL-liked (IPL) tissues (17).*

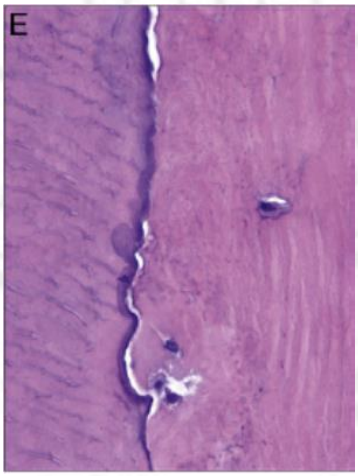

Figure 6: *Regenerated tissue in the root canals. Few cells housed in lacunae can be seen in the newly formed mineralized tissue, resembling cementocytes/osteocytes(21).*

On the other hand, a few studies reported an interesting histological outcome. For example, a case report which revascularization was completed for 14 months. The

intra-canal tissue was collected because of pain on the day of examination. Histologically, the tissue exhibited vital pulp-like structure with no evidence of bone in the specimen (Figure 7)(19). More recently, another report that non-infected traumatized immature tooth was treated with revascularization technique using calcium hydroxide revealed the histological appearance of pulp-like tissue inside the canal (Figure 8)(20).

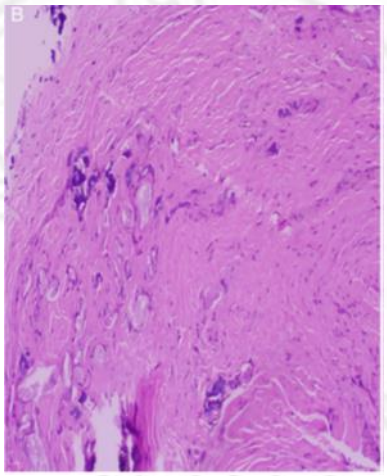

Figure 7: *Pulp-like connective tissue generated in a human tooth after treatment with PRP (19).*

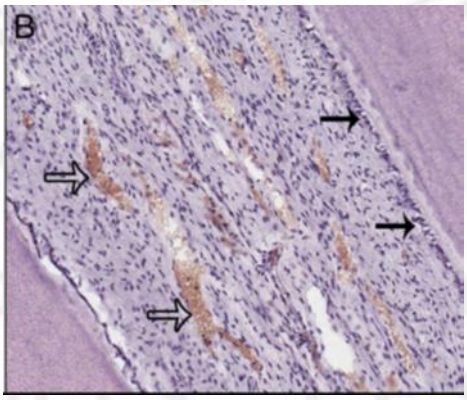

Figure 8: *Flatten odontoblast-like cells lined along the predentin (20).*

Since the results after regenerative treatment are controversy, a long-term outcome may reveal more consequences. However, some investigators have stated the possible situations which may affect the outcome of the treatment as followed (17).

1) The infection into the pulp chamber caused severe tissue damage

2) Vital tissue cannot survive when the infection is heavy and spread into the periapical area

3) The use of highly concentrated triple antibiotic paste may have a toxicity to live tissue

Cells related to pulp revascularization procedure

The tooth originates as a band of epithelial cells, the dental lamina, in developing jaws. Downgrowths from this band will ultimately form the teeth. There are 3 stages of tooth formation, bud, cap and bell stage, which are determined by the shapes of these downgrownths. The tissue within invagination will become a dental pulp, which is known as the dental papilla, derived from ectomysenchymal cells. Apical portion of dental papilla during the stage of root development termed as tissue apical papilla (53).

Dental pulp is an integral part of the tooth. The primary pulp function is formative; it gives rise to odontoblasts to form dentin and also interact with dental epithelium to initiate enamel formation in early tooth development. Various types of cells in dental pulp can be found such as odontoblasts, fibroblast, cells of immune system and stem cells.

Dental pulp stem cells (DPSCs) and progenitor cells have been discovered in dental pulp. These cells can differentiate into odontoblast-like cells which are able to form new dentin (54). In addition, the ability of pulp to respond on external stimuli by forming tertiary dentin has been stated (55). Dental pulp stem cells express some genes during early dentinogenesis and are encoded by odontoblast-specific gene named dentin sialophosphoprotein (DSPP). An example of these genes is Dentin matrix proteins (DMPs) such as dentin sialoprotein (DSP) and dentin phosphoprotein (DPP)(56). DMP-1, a non-collagenous acidic matrix protein, is a bone and tooth-specific protein identified from mineralized dentin matrix. DMP-1 is a key regulator of odontoblast differentiation, the formation of dentin tubular system, mineralization and maintenance of dentin tubular space (57, 58). It has been suggested that DMP-1 might be involved in the maintenance of the mineralized tissue microenvironment (59).

Tissue apical papilla (Figure 9) can be found during the stage of root development. This tissue is attached at the apex of developing root, locating apical to epithelial diaphragm and can be removed easily with a pair of tissue forceps. There are rich source of stem cells but they have a kind of different characteristics from dental pulp stem cells (DPSCs) due to beneficial by its collateral blood supply, which enables to endure during the necrotic process (53). In culture, stem cells from apical papilla (SCAP) exhibited greater numbers of population doublings, numbers of STRO-1-positive cells and regenerating capacity when compared to DPSCs (60).

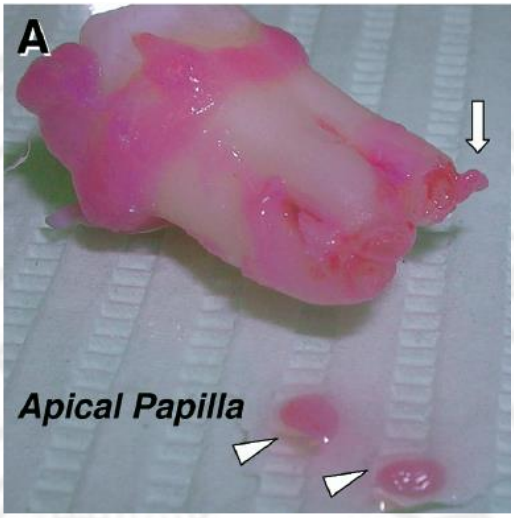

Figure 9: *Anatomy of an apical papilla. An extracted immature human molar with two pieces of apical papilla being removed from their apices (61).*

An open apex contributed a good communication between pulp space and periapical tissue. Thus, it might assume that stem cells in the pulp tissue and apical papilla may have survived the infection (Figure 10)(61). Multipotent dental pulp stem cells might differentiate into odontoblasts and deposit tertiary or atubular dentin (62). Moreover, these cells may proliferate into the created matrix and differentiate under the organization of Hertwig's epithelial root sheath cells (HERS). Blood clot, created inside the root canal, could play an important role for regeneration since it is a rich source of many growth factors. The examples growth factors are platelet-derived growth factor, vascular endothelial growth factor (VEGF), platelet-derived epithelial growth factor, and tissue growth factor. These could stimulate a differentiation and growth of stem cells in newly formed tissue matrix.

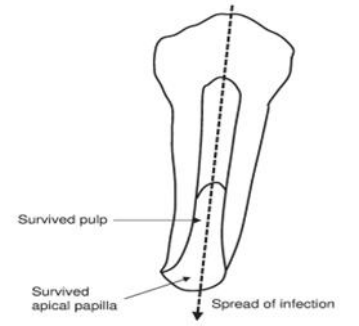

Figure 10: *The possible pathway of infection (61).*

Typical, stem cells are defined by two crucial features (61). First, the self-renewal ability. Second, some daughter cells give rise either to cells that maintain the stem cell character or differentiated cells. Recent studies (53, 61), which may explain why a continued tooth development can occur in these teeth, is the recovery and isolation of mesenchymal stem cells (MSCs) residing in apical papilla of incompletely developed teeth (53).

Tooth formation is by reason of the sequential epithelial-ectomesenchymal interaction involving many growth factors and extracellular matrix components (63). The extracellular matrix contains non-collagenous protein which mainly consist of osteopontin (OPN), bone sialoprotein (BSP), dentin sialophosphoprotein (DSPP), matrix extracellular phosphoglycoprotein (MEPE) and dentin matrix protein 1 (DMP1)(64, 65).

DMP-1, a non-collagenous acidic matrix protein, is a bone and tooth-specific protein identified from mineralized dentin matrix. As a result of its highly acidic property and capability of calcium ions binding, it plays an important role in nucleation and modulation of mineral phase morphology of the hard tissue formation (66).

A study of DMP-1 in human developing teeth (67) revealed a varying expression pattern in various stage of tooth development. In the dental lamina, a positive immunostaining for DMP-1 was seen in peripheral cells facing the developing tooth while the opposite site was negative. During the bell stage, DMP-1 was obviously seen in cytoplasm and nucleus of cells in the outer enamel epithelium, stellate reticulum and stratum intermedium whereas it was not seen in internal dental epithelium and cervical loop area.

Once dentinogenesis and amelogenesis occurred, DMP-1 was strongly observed in ameloblast layer and also expressed intensely in the nucleus and cytoplasm of odontoblasts in both coronal and root regions. At the dentinal tubules of coronal dentin has also been seen but was not found in the enamel. In the dental papilla, DMP-1 was seen in the nucleus and cytoplasm of some cells and extracellular matrix. DMP-1 is degraded after its secretion in dentinal tubules and was degraded by some proteases such as matrix metalloproteinase 8 (68).

Alkaline phosphatase (ALP) is the metalloenzyme which is attached on the outer surface of plasma membrane via phosphatidyl inositol-glycophospholipid (GPI) anchor covalently bonded to the C-terminus of the enzyme. ALP has many functions in many organelles. Human have 4 ALP genes on intestine, placenta, placental-like and tissue nonspecific (TNAP).

ALP is important for the hard tissue formation (Figure 11) and expressed early in development (69). This enzyme can be found on the cell surface and in matrix vesicles. The later in stage of mineralization, other genes e.g. osteocalcin are upregurated, but ALP expression declines.

Mineralization promoters and mineralization inhibitors are the central roles in regulating the onset of mineralization. Ankylosis protein (ANK) and nucleotide pyrophosphate phosphodiesterase (NPP1) function to suppress mineralization by elevating the extracellular concentration of pyrophosphate (ePPi). On the contrary, TNAP promotes the mineralization by decreasing the ePPi concentration and inorganic phosphate (Pi). ePPi is formed from extracellular nucleoside triphosphates (eNTP) by NPP1 and exported from cells by ANK.

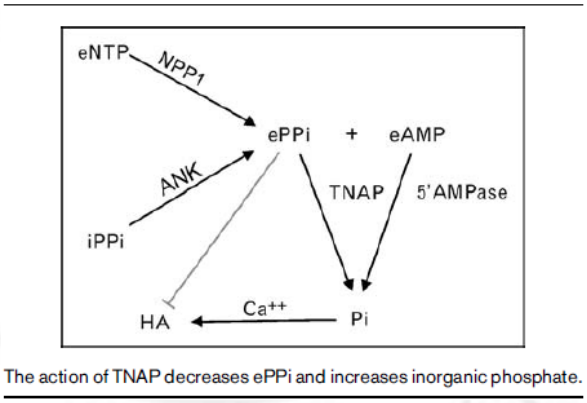

Figure 11: *The role of ALP in mineralization (69).*

Matrix vesicles also are important role in initial mineralization process. They are believed that they arise from budding of plasma membrane of hard tissue forming cells. Matrix vesicles have similar composition with plasma membrane but difference in proportion. They are enriched in acidic phospholipids (phosphatidylserine and phosphatidylinositol) and have higher TNAP activity. Membranes enriched in

phosphatidylserine attract annexins and allow to form calcium channels which Ca^{2+} can easily penetrate into vesicle to form crystal.

Bone sialoprotein (BSP) is an acidic non-collagenous glycoprotein in the SIBLING family (small, integrin-binding ligand N-linked glycoprotein). The examples of SIMBLING protein family are osteopontin (OPN), secreted phosphoprotein-1 (SPP-1), bone sialoprotein (BSP), integrin-binding sialoprotein (IBSP), dentin siaophosphoprotein, dentin matrix protein-1 (DMP-1) and matrix extracellular glycophosphoprotein (MEPE). These genes are categorized as bone gene cluster on chromosome 4 in human (70). The SIBLINGs interact with cells via integrin and bone mineral implying that they are a master key in regulating bone development, remodeling and repair (71). BSP is expressed in several cells type associated with mineralized tissue such as bone and dentin but expressed especially in osteoblasts, hypertrophic chondrocytes and osteoclasts. Their precise functional roles are still not fully understood. Nevertheless, several factors implied that BSP may have a multifaceted role in mineralization. The spatiotemporal expression of BSP at the sites of new mineralization has indicated that BSP is related to the onset of mineralization.

Cytotoxicity of three-antibiotic mixture

The mixture of three-antibiotic has been introduced as an intracanal medication due to the presence of microorganisms resistant to conventional endodontic procedure (72). Several studies (26-28, 48, 50, 51, 73) have focused on its

antibacterial efficacy. However, the study which determined the cytotoxicity of three-antibiotic mixture is deficient.

Ferreira et al (2010) determined the cytotoxicity of ciprofloxacin, clindamycin, and metronidazole at the concentrations of 5, 50, 150, and 300 mg/L for 24, 48, 72, and 96 hours on human gingival fibroblast cells. This study reported that all tested antibiotics revealed a dose-dependent manner. Cell viability at 24 hours was greater than in the other experimental times irrespectively the type of antibiotics. The concentrations at 5 and 50 mg/L of all tested antibiotics provided viable gingival fibroblast cells in all studied times (Figure 12). This study suggested the critical analysis of the use of antibiotics for intracanal dressing (72).

	24 h			48h			72h			96h		
	CP	CL	M	CP	CL	M	CP	CL	M	CP	CL	M
5 mg/L	78.61	70.06	71.01	55.84	66.16	68.15	83.80	77.32	71.34	69.88	81.68	73.95
50 mg/L	63.31	65.37	68.0	35.32	58.96	61.33	51.29	72.09	62.97	53.07	71.31	67.68
150 mg/L	37.56	45.35	62.75	26.87	36.16	56.97	33.39	42.74	56.33	29.31	30.50	62.90
300 mg/L	27.22	15.71	53.38	9.36	5.86	55.05	17.10	13.83	53.25	10.79	0.83	57.66

CP: Ciprofloxacin; CL: clyndamicin; M: metronidazole

Figure 12: *Cytotoxicity of ciprofloxacin, clindamycin, and metronidazole at the tested concentrations and experimental times (72).*

Later in 2012, Ruparel et al reported that the mixture of cipofloxacin, metronidazole, and minocycline had a detrimental effect on the viability of stem cells from the apical papilla (SCAPs) in a concentration-dependent manner. This study used a trypan blue dye automated method to count the viable cells. The results have shown that 1 mg/ml of 3Mix caused 50% cell death (LC50), whereas the lower concentrations of 0.1 and 0.01 mg/ml had no detectable effect on SCAP viability (Figure 13). This study suggested that the typical clinical paste or slurry-like

consistency appeared at 1000 mg/ml. This could reasonably assume that the paste is a saturating concentration of each antibiotic, which leads to more detrimental effects on SCAPs (29).

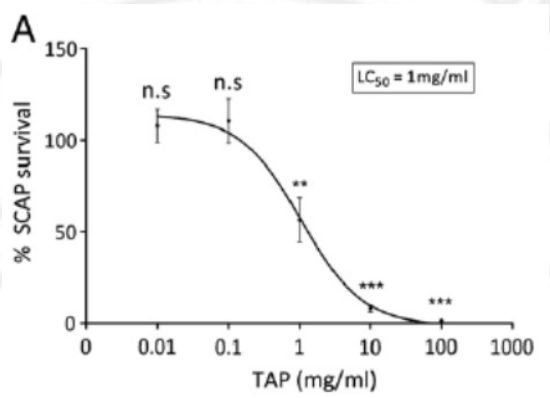

Figure 13: *Detrimental effect of Three-antibiotic combinations on SCAPs(29).*

Furthermore, Chuensombat et al (2013) reported that cytotoxicity of 3Mix was induced when concentration and time increased. The concentration of 3Mix at 0.39 µg/mL was the best candidate for use since it produced less cytotoxicity to human dental pulp cells (DPCs) and apical papilla cells (APCs) than higher concentrations (Figure 14, 15), while it was able to significantly reduce bacteria isolated from teeth with necrotic pulp (30).

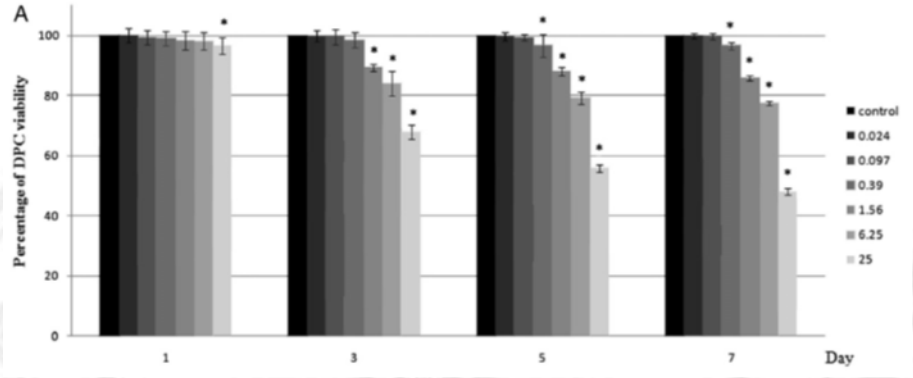

Figure 14: *DPC viability after being treated 3Mix at different concentration. 3Mix at the concentration of 0.39 µg/ml of 3Mix had less cytotoxicity on DPCs (30).*

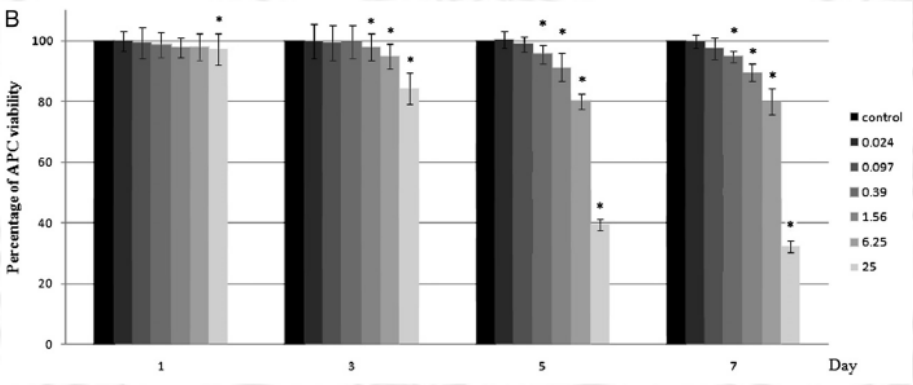

Figure 15: *APC viability after being treated 3Mix at different concentration. 3Mix at the concentration of 0.39 µg/ml of 3Mix had less cytotoxicity on APCs (30).*

The main objective of regenerative endodontic procedure is to regenerate pulp-like tissue, ideally, pulp dentin complex. However, some clinical cases were reported an unfavorable outcome (21, 22). Triple antibiotic mixture was addressed to be possible etiologic factor (17, 23, 24). Since the non-cytotoxic dose of 3Mix has already been clarified, this study would further investigate the effect of the non-cytotoxic dose on proliferation capacity, and mineralization potential of human DPCs/APCs.