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Biotecnologie Odontostomatologiche

CICLO XXVIII

Regenerative Endodontics:
a review of clinical protocols.

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Regenerative endodontics: review of clinical protocols.

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Chapter 1

1.1 Apexification with Calcium Hydroxide

Traumatic injury or a depth caries penetration, during the developing of dentition, could lead to a premature pulp necrosis of immature permanent tooth. Dental injuries are most common in young patients (8-14 years), when children are most active. In these cases endodontics treatment aimed to keep the dentition in physiologically functional state for the maintenance of oral and systemic health. Teeth with immature root development present challenges in cleaning, shaping and obturation of large canals with open apices (1-3). In these cases conventional root canal treatment can be overcome using Calcium Hydroxide $Ca(OH)_2$ apexification approach (4) or Mineral Trioxide Aggregate (MTA) apical plug technique (5). In both clinical approaches the risk of future root fracture and tooth mobility, due to a poor root-crown ratio, still remains.

Apexification is defined as ‘‘a method of inducing a calcified barrier in a root with an open apex or the continued apical development of an incompletely formed root in teeth with necrotic pulp”. Teeth treated with this material require the placement of long-term calcium hydroxide in the root canal to induce formation of an apical hard

tissue barrier. The formation of the apical barrier is necessary to allow the filling of the root canal system without risk of overfilling

The high pH and antimicrobial properties (6) of $Ca(OH)_2$ combined with the permeability of dentin (7) guided to a common and well established use of calcium hydroxide in dentistry.

Hermann introduced the use of calcium hydroxide in 1920 (8); it was often applied within the root canal system as intracanal medicament and in apexification procedure. In 1964 Kaiser (9) proposed the use of calcium hydroxide mixed with camphorate parachlorophenol to induce the formation of calcified barrier across the apex.

Frank (5), who emphasized the importance of reducing contamination within the root canal by instrumentation, medication and dressing the canal space temporarily with a reasonable paste seal, popularized this procedure.

The use of calcium hydroxide mixed with saline solution (10), sterile water (11), or distilled water (12) has been investigate with good clinical success; the mechanism of action of calcium hydroxide in induction of an apical barrier remain controversial; two different aspects could play an important role in osteogenetic potential of calcium hydroxide: mineralized and antibacterial actions. Ca(OH)2 induced a multi-layered necrosis (13) with subjacent mineralization; the necrosis generates a low grade of irritation of underlying tissue sufficient to produce a matrix that mineralized. Calcium was attracted to this area and the mineralization of newly formed collagenous matrix is initiated from the calcified foci. The apical barrier formation is more successful in the absence of microorganism, the release of hydroxyl ions cause the damage to bacterial cytoplasmic membrane, protein denaturation and damage to bacterial DNA (14-19). The hard tissue barrier formed at the end of the root has been histologically described as follow: an outer layer composed of a dense acellular cementum-like tissue and a more central mix of irregular dense fibro-collagenous connective tissue containing foreign material highly mineralized (20). By using repeated $Ca(OH)_2$ dressings, during a 3 to

6 months period, it demonstrated that it was possible not only to induce healing of the apical lesion but also to induce closure of the root apex with calcified tissue (apexification).

When Ca(OH)2 is used in apexification procedure, the therapy could extend from months to years before achieve the desired effect (21,22). In a review Sheehy and Robert reported an average duration for apical barrier formation ranging from 5 to 20 mouths (21); the high variability could be due to: presence or absence of periradicular lesion, and /or stage of root development and consequent apical width (23). Immature teeth treated with $Ca(OH)_2$ showed an high failure rate because of an unusual incidence of root fracture. This might be the direct consequence of changes in the physical properties of dentin due to the use of $Ca(OH)_2$ medicament (1, 24, 25). Cvek (1), investigated 885 luxated non-vital teeth over a period ranging from 3 to 54 months, reported that 168 teeth suffered a cervical root fracture within the follow-up period, ranged from 3.5 to 5 years. Each failed teeth showed a cervical resorptive defect near the fracture; this may be a result of a change in the organic matrix (2, 25). Calcium hydroxide dissolves pulp tissue because of denaturation and hydrolysis of dentin proteins, furthermore the high pH reduce the organic support of the dentin matrix; both effects result in a loose of connection between collagen fibrils and hydroxyapatite crystals, that could negatively influence mechanical properties of dentin (2, 25, 26, 27). The weakening of the root structure in term of on micro-tensile fracture strength of dentine tissue after prolonged used of calcium hydroxide, was supported by *in vitro* studies too (28, 29).

Outcome assessment of calcium hydroxide in apexification treatments, showed a success rates ranged from 79% to 100% (1,30-34). Based on a meta-analysis and systematic review the rate of clinical success and apical barrier formation mineral trioxide aggregate and calcium hydroxide as material used for the endodontic management of immature teeth had no perceivable discrepancy (35).

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Chapter 2

Despite the high success rate of long term calcium hydroxide apexification (mid 90%), the disadvantage of this technique (1), multiple appointment during an extended period of time, susceptibility to root fracture, risk of coronal micro-leakage during the therapy; lead to alternative treatment that might be offer a more predictable and better long-term prognosis for necrotic teeth with incomplete root development.

Artificial apical barriers with a variety of materials have been suggested as an alternative to traditional Ca(OH)2 apexification (2-7). In 1999 Torabinejad and Chivian proposed the use of a new material, Mineral Trioxide Aggregate (MTA), as artificial apical barrier for the treatment of immature necrotic teeth (8).

2.1 Mineral Trioxide Aggregate: Chemical composition and setting reaction

Mineral trioxide aggregate (MTA) was developed at Loma Linda University, in the 1990s, as a root-end filling material. It was used primarily to seal lateral root perforations (8,9) and as root-end filling material (10-13). In 1998 Mineral trioxide aggregate (MTA) received acceptance by the US Federal Drug Administration and became commercially available as ProRoot MTA (Tulsa Dental Products, Tulsa, OK, USA).

The literature widely documented the physical, chemical and biological characteristics of MTA; it's consist in a powder contains 50-75% calcium oxide (CaO) and 15-25% silicon dioxide (SiO2).

These two components together comprise 70-95% of the cement (11, 14, 19, 20, 21).

Scanning electron microscope (SEM) of polished sections of un-hydrated MTA embedded in resin, show distinctive cement grains and bismuth oxide particles, which are separated from one another. The mean value of the prisms was 87% calcium and 2.47% silica, the remainder being oxygen. In areas of amorphous structure, there seemed to be 33% calcium, 49% phosphate, 2% carbon, 3% chloride, and 6% silica.

The elemental composition of MTA as shown by energy dispersive spectroscopy (EDS) indicates the presence of calcium, silicon and oxygen with minor peaks for aluminium, potassium, magnesium and bismuth. The phases present in un-hydrated MTA, determined by X-ray diffraction analysis, exhibits peaks for tricalcium silicate, dicalcium silicate and bismuth oxide. Each phase has a particular pattern that can be subsequently searched and matched with data derived from the International Centre of Diffraction. MTA contains other phases such as dicalcium silicate and tricalcium aluminate in minimal quantities. In the first publication on MTA composition, calcium phosphate was the main constituent of MTA (9). Further analysis demonstrated that the former appeared as discrete crystals and the latter as an amorphous structure with no apparent crystal growth but a granular appearance.

When these raw materials are blended, they produce tricalcium silicate, dicalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite. Various methods have been used to examine MTA composition including energy dispersive analysis with x-ray (EDAX), inductively coupled plasma optical emission spectroscopy (ICP-OES), x-ray diffraction analyses (XRD), x-ray fluorescence spectrometry (XRF), energy x-ray spectrometry, and energy dispersive spectroscopy (9–18).

Mineral Trioxide Aggregate is available in two commercial form grey MTA (GMTA), the oldest formula, and white MTA (WMTA), the

newest formula. The difference between the grey and the white materials is the presence of iron in the grey material, which makes up the phase tetracalcium alumina-ferrite. This phase is absent in white MTA.

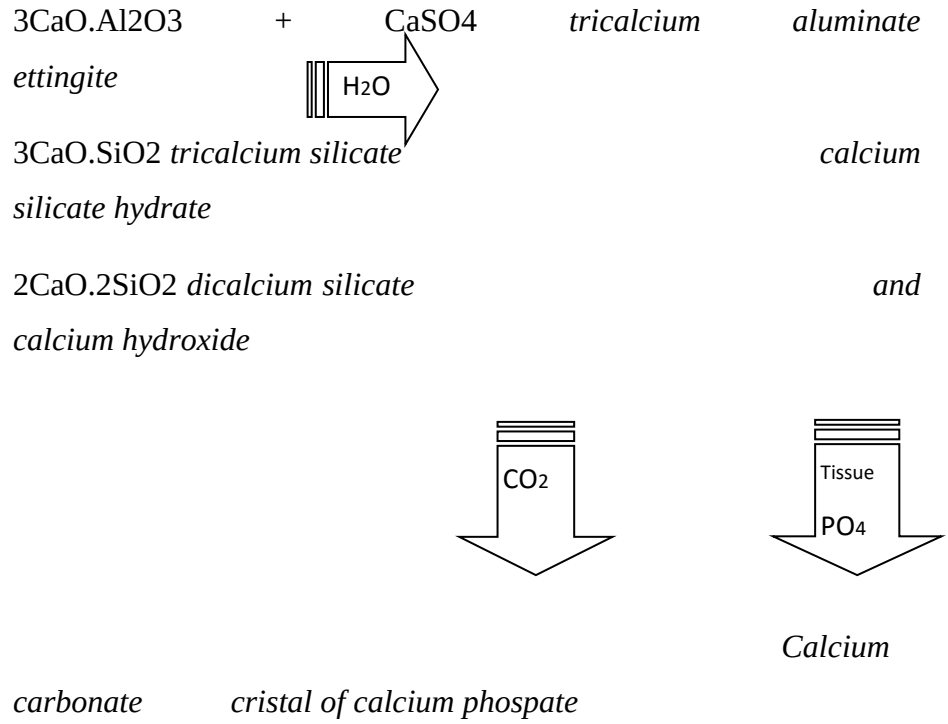
Mixing MTA powder with sterile water in a 3:1 powder-to-liquid ratio (22) we obtain a colloidal gel that solidifies into a hard structure. During the initial stages of the reaction of MTA after hydratation, calcium silicate hydrate is formed, coating the cement particles and preventing further reactions. Tricalcium aluminate dissolves and reacts with the calcium and sulfate ions present in the liquid phase to produce ettringite, which also precipitates on the cement particle surface. The initial phase is followed by a dormant period, wherein the hydrate coating on the cement grains prevents further hydration. The dormant period lasts for 1-2 hours, which is a period of relative inactivity and the cement is plastic and workable. Following the completion of the dormant period, setting of the cement proceeds to the acceleration stage, where the hydration process accelerates again. Sulfate ions are depleted and monosulfate forms from ettringite. Crystalline calcium hydroxide also precipitates from the liquid phase (20).

The hydration reaction takes several years to complete, although the cement mass would have achieved the final hardening and maximum physical and mechanical properties by 28 days.

If MTA is left exposed to the environment, the calcium hydroxide reacts with the atmospheric carbon dioxide resulting in the deposition of calcium carbonate on the cement surface. These deposits are commonly mistaken as being an integral part of the cement microstructure. When in contact with tissue fluids and synthetic tissue fluids, which contain phosphate ions, the calcium hydroxide produced, as a by-product of MTA hydration, reacts to form calcium phosphate and is deposited on the cement surface. Calcium phosphate is crystal on the material surface has been reported to be the reason for the bioactivity shown by MTA.

One of the characteristics of a bioactive material is its ability to form an apatite-like layer on its surface when it comes in contact with physiologic fluids (23, 24).Apatite formation is a common characteristic of calcium silicate–containing biomaterials (25,26). Hydroxyapatite can release calcium and phosphorus continuously, a process required for bone metabolism (27). In addition, this phenomenon increases the sealing ability of MTA and promotes the regeneration and remineralization of hard tissues. On the basis of these results, Sarkar et al. (27) suggested that the biocompatibility, sealing-ability, and dentinogenic activity of MTA result from the physiochemical reactions between MTA and tissue fluids during the formation of HA.

Several liquids have been used to hydrate MTA powder. The mean setting time of MTA is 165± 5 minutes, which is longer than amalgam, Super EBA, and intermediate restorative material (IRM) (9).



2.2 Biocompatibility and bioactivity of Mineral Trioxide Aggregate

The MTA cement reflect a current requirement to have materials for endodontic therapy that are able to stimulate the healing process of periapical tissues, instead of merely biocompatible or inert materials. In the past decade, the two major characteristics that justify the successful use of MTA in endodontics were the excellent seal ability and biocompatibility. In case of necrotic teeth with immature root development, MTA has been advocated as an apexification material because it permits an adequate seal of the canal preventing bacterial leakage, and a poor inflammatory reaction in periodontal tissue.

Nowadays, it's well-documented the favourable biologic response stimulated by MTA in human periapical tissue; cell from periradicular healing tissue migrate to the apex and differentiate in cells capable of secreting a cementum, osteocementum or osteodentin organic matrix under the influence of specific cellular signals (28). Bone and periodontal healing/regeneration is a complex event that involves the different stages (30), in which the migration and invasion of multipotent mesenchymal stem cells are required (31).

When MTA has been placed in direct contact with human tissues, the following reaction have been observed:

- *Releases calcium ions and facilitates cell attachment and proliferation*. The ability of MTA to moderate the migration of cells should probably be considered to be an important stage in the induction of tissue repair. Apical barrier formation can occur even in the presence of gaps between the MTA plug and the root canal walls. This observation was probably related to the ability of MTA to enhance cell migration of human bone marrow stem cells (29).

  Currently human PDL cells were used to simulate the root-end environment; these cells are responsible for the formation and

maintenance of periodontal ligament fiber attachments as well as repair, remodelling, and regeneration of the adjacent alveolar bone and cementum. In a human PDL cell culture study, cell attachment to MTA was observed; PDL fibroblasts showed proliferation on MTA and survival.

- *Creates antibacterial environment by its alkaline pH*. The bioactivity of MTA has been attribute to its ability to produce hydroxyapatite (Ca5(PO4)3(OH)) in presence of phosphate buffered saline. The hydroxyapatite is a crystal presents in form of calcium carbonate, calcium phosphate and calcium floruro. Initially an amorphous calcium phosphate phase is formed, which acts as a precursor to the secondary phase during which carbonated apatite is formed. Carbonated apatite is also known as biologic apatite and represents the mineral phase of hard tissue (bone, dentin, and cementum) (32, 33). The bioactivity of MTA could be attributed to its capacity to form carbonated apatite, which is important in formation and maintenance of the bone tissue biomaterial interface. The apatite formed by the cement-Phosfate Buffered Saline (PBF) system was deposited within collagen fibrils, promoting controlled mineral nucleation on dentin as reported by Reyes-Carmona et al (34). The possible biologic and clinical significance of their findings includes the following:

  (1) the interaction of MTA and Portland cement with dentin in a phosphate-containing fluid promotes a biomineralization process,

  (2) This process could be significant in minimizing leakage,

  (3) The formation of the interfacial layer and the intratubular mineralization process could influence the push-out bond strength, and

  (4) The formation of carbonated apatite precipitates could be responsible for the ability of the cements to stimulate repair and dentinogenesis or cementogenesis.

- *Modulates cytokine production.* Basing on *In vitro* and *in vivo* animal study, MTA seems to play a role on the production of signalling molecules. Macrophages produce different types of cytokines, signalling molecules and inflammatory products. Up regulation of various types of cytokines and biologic markers has been reported in the presence of MTA in several cell culture studies when compared with control or other tested materials. These cytokines and biologic markers include interleukin (IL)-1a (36, 37), IL-1 b (36-38), IL-2(39), IL-4(39), IL-6(36, 38, 40), IL-8 (41), IL-10 (39), IL-18 (36), osteocalcin (36, 39, 42-44), alkaline phosphatase (42, 44, 45), bone sialoprotein (44), osteopontin (44), and BMP-2 (45).
- *Encourages differentiation and migration of hard tissue producing cells.* MTA can induce osteoblastic/cementoblastic differentiation of human periodontal ligament cells, which express calcium sensing receptors (CaSR) and bone morphogenetic protein-2 (BMP-2) receptors that are potentially involved in osteogenesis. BMP induce the production of bone when injected into ectopic sites. PDL human fibroblast attached to MTA produce an osteogenic phenotype, which reflects up-regulation of the expression of alkaline phosphatase, osteonidogen, osteonectin, and osteopontin (42).
- *Forms hydroxyapatite (or carbonated apatite) on the surface of MTA and provides a biologic seal.*

2.3 Clinical use of Mineral Trioxide aggregate as apical plug

An MTA plug in the apical portion of the root forms a barrier that prevent the extrusion of the root filling material, the ensuing permanent bonded restoration increases fracture resistance of immature teeth and enhances the retention of natural dentition (46,47). Previous case series and prospective studies reported a high percentage of successful outcomes at one or two years follow-ups

when MTA was used as the apical plug in necrotic teeth with open apices (48-52). Different techniques for delivering MTA to the apical portion, time of therapy (one or more appointments), and use of intermediate medication with calcium hydroxide as intracanal dressing material have been proposed. The lack of both consensus regarding techniques and the limited follow-ups has encouraged the development of new studies (50-53). Only a few studies discuss at long time the clinical outcomes of the treatment of immature and necrotic teeth using MTA as an artificial apical barrier. The percentage of clinical and radiographic success of MTA apexification range is variable from 68.4% to 100% with a maximum median follow-up of 30.9 months (48,49,54, 55).

The long term outcome of this treatment was documented in a clinical study performed, in the last ten years, at department of Endodontics at University of Florence (56).

The clinical success of the apical plug technique was, in general, judge using PAI score in association to clinical signs and symptoms:

- PAI score ≤ 2 and the absence of signs and symptoms was associated to a heling case;
- PAI 3 or 4 with score improved at follow-up from immediate post-treatment radiograph without signs and symptoms was associate to an healing case;
- failure was diagnosed when signs or symptoms were present or the PAI was > 4 (7-10).

Sometimes the results basing on clinical and radiographic criteria were dichotomized as healed or disease (55).

The clinical protocol of the treatment of immature necrotic teeth could have different approach but in all cases, the necrosis of the teeth imply the presence of infected pulp. In immature teeth cleaning and shaping of the root canal system challenging because of the thin dentinal walls,

the disinfection could be achieved with calcium hydroxide and currently root canal irrigation; sodium hypoclorite and EDTA (49).

Antibacterial action of calcium hydroxide is directly proportional its strong alkalinity; Sjögren at al. (57) showed that calcium hydroxide for seven days was highly effective in killing root canal flora, but for long time it may denature the carboxylate and phosphate groups leading to a collapse in the dentine structure. Until Cvek in 1992 underlined as long-term apexification with calcium hydroxide reduction the root strength make the teeth more susceptibility to the fracture (58). Andreasen et al. reported that immature roots that had Ca(OH)2 placed within the root canal for 100 days showed a significant reduction in fracture resistance versus control; but up to 4 weeks of calcium hydroxide did not adversely affect the fracture resistant (59). The dentinal strength is determined by the link between hydroxyapatite and collagenous fibrils. The high alkalinity of calcium hydroxide may denature the carboxylate and phosphate groups leading to a collapse in the dentin structure. The pre-treatment use of calcium hydroxide before the application of apical plug of MTA could adversely influenced the formation of apical barrier. In *in vivo animal* study, Felippe et al. showed no significant differences in the formation of apical tissue barrier, bone and root resorption, and the presence of microorganisms between the two experimental groups: teeth treated with CH pretreatment and apical plug with MTA and teeth treated without CH pretreatment. In addition, their findings determined that placing MTA alone results in more complete apical barrier formation compared and they further demonstrated that the amount of MTA extrusion was significantly higher in samples pretreated with CH compared with those without CH pretreatment (60).

The main clinical drawbacks of MTA, when used as apical plug, include a difficult handling characteristics, long setting time, an absence of a known solvent for this material, and the difficulty of its removal after curing. The long setting time of MTA is one of the reasons that MTA should not be applied in 1 visit. This has been cited

as one of the shortcomings of this material. There is no known solvent for set MTA; presumably, MTA cannot be removed from the root canal when it is used as an apical barrier or root canal filling material. An investigation using both rotary file and ultrasonic devices for retreating root canals filled with WMTA as a root canal filling material demonstrated the inability of these devices to completely remove set MTA. Finally handling of MTA is not simple for some of its clinical applications and requires practice; in particular in case of long immature root where the carrier can not arrive in the apical portion; for a correct adaptation of MTA to the canal walls the use of microscopic device is usually required.

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Chapter 3

3.1 Dental stem cell; apexogenesis and apexification

Necrosis of dental pulp in immature permanent teeth before complete root development poses a clinical challenge. As previously described apexification with calcium hydroxide and/or MTA apical plug technique enable the formation of a calcified barrier or an artificial barrier, respectively, at the root apex of canal system. Both technique is able to stimulate regeneration of pulp tissue, and continued root development, so the risk of fracture in a tooth with thin dentine walls remains. Cervical root fracture was markedly higher in endodontic treated immature teeth than in mature teeth and the range of incidence recorded is from 27% to 77% basing to the stage of root development (1). Postnatal stem cells with the capacity to self-replicate and differentiate into specialized tissue types have been identified in dental tissue. The tooth with immature apices, by regenerating tissue, could restore the physiologically functional dentition.

Regeneration of dentin relies on having vital pulps; however, regeneration of pulp tissue has been difficult as the tissue is encased in dentin without collateral blood supply except from the root apical end. Attempts to regenerate pulp tissue have been a long quest. With the advent of modern tissue engineering concept and the discovery of dental stem cells, regeneration of pulp and dentin has been tested. Moony and Rutherford conduct the first team that initiated the testing of pulp tissue engineering (36-38). This attempt arrested due to the lack of isolation and characterization of pulp stem cells that potentially may differentiate into odontoblasts. Regenerated pulp tissue should be functionally competent, e.g., capable of forming dentin to repair lost structure.

Gronthos et al. demonstrated *in vivo* the ability of pulp cells to generate dentin, human pulp/dentin complex can be formed ectopically in immunocompromised mice (39). This discovery has promoted the investigation on the stem cell-based regenerating pulp/dentin for clinical applications.

In the early 1960s, Nygard Ostby (2) showed that new vascularized tissue could be induced in the apical third of the root canal of endodontically treated mature teeth with necrotic pulps and apical lesions. This was accomplished by the creation of a blood clot in the apical third of a cleaned and disinfected root canal by using an apically extended root canal file just before root canal filling. He proposed that through formation of a clot (scaffold), a vasculature could be established to support growth of new tissue into the unfilled portion of the root canal. Teeth that had been treated following this procedure provide provided histologic evidence in support of his concept. In order to validate this hypothesis Myers & Fountain, in 1974, reported an increased root length and calcified material in necrotic canals of monkey canines with immature apices after disinfection with NaOCl and filled the canals with citrated whole blood or gel foam (3). The hard deposition of hard tissue in the root canals has also been shown in reimplanted teeth after traumatically avulsion (4,5).

It appeared that the non-vital pulp acted as a matrix into which the new blood vessels and tissue could grow (5,6).

In 2001, Iwaya et al. (7) described a procedure, which they termed *revascularization* used in a necrotic immature mandibular second premolar with a chronic apical abscess. After 30 months they observed thickening of the root canal walls by mineralized tissue and continued root development. Subsequent even Banchs and Trope reported a successful case of revascularization procedure for the treatment of a necrotic teeth with large apical lesion (8).

3.2 Dental pulp stem cells

The first element of tissue engineering is a source of cells capable of differentiating into the desired tissue component. Interestingly, stem cells are found in dental pulp (17, 18), in the apical papilla (19, 20), and even in the inflamed periapical tissue collected during endodontic surgical procedures (inflamed periapical progenitor cells) (21). These findings suggest an opportunity for harvesting stem cells during clinical procedures. Indeed, the evoked bleeding during endodontic regenerative procedures conducted on immature teeth with pulpal necrosis reveals a massive influx of mesenchymal stem cells into the root canal space (22).

The dental pulp is soft tissue of ectomesenchymal and mesenchymal origin that develops from the dental papilla. Mesenchymal Stem Cells (MSCs) are defined by the International Society for Cellular Therapy as cells that express the molecular markers CD73, CD90, and CD105 and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules (14). It has been shown that stem cells are a heterogeneous population of cells, and their molecular profile is very dynamic because of their ability to express a plethora of other markers depending on their differentiation, activation, or passage (Tab. 1). Dental-tissue-derived MSC-like populations are:

- dental pulp stem cells (DPSCs),
- stem cells from human exfoliated teeth (SHED),
- stem cells of the apical papilla (SCAP),
- periodontal ligament stem cells (PDLSCs), and
- dental follicle progenitor stem cells (DFPSc),

Cell Type	*In Vitro Activity*
DPSCs	Osteo/Dentinogenic
	Adipogenic
	Chondrogenic
	Myogenic
	Neurogenic
SHED	Dentinogenic
	Adipogenic
	Chondrogenic
	Myogenic
	Neurogenic
	Osteo-inductive
SCAP	Dentinogenic
	Adipogenic
	Chondrogenic
	Myogenic
	Neurogenic
PDLS	Osteo/Cementogenic
	Adipogenic
	Chondrogenic
	Myogenic
	Neurogenic
DFPC	Cementogenic
	Odontogenic
	Adipogenic-
	Chondrogenic
	Myogenic
	Neurogenic

Table 1. Multiple differentiation property of Human Dental Stem Cells.

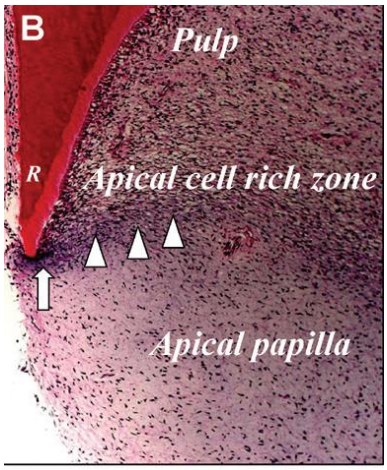

Figure 1. Histologic section of human apical papilla and dental pulp.

Huang GTJ et al. Mesenchymal Stem Cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 2009;88:792-806.

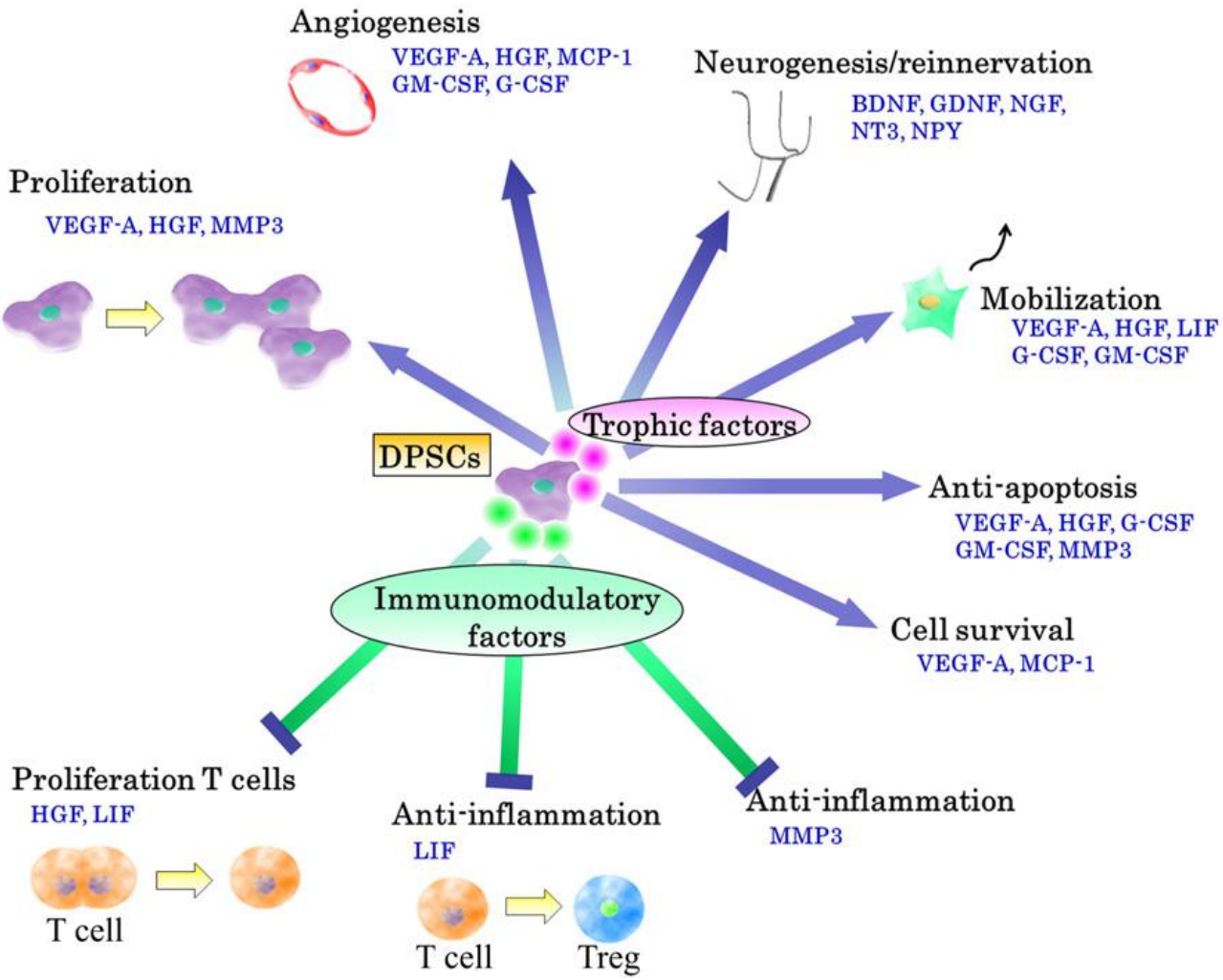

Figure 2. Potential for secretion of trophic and immunomodulatory factors and from stem cells/progenitors.

Nakashima M. et al Dental pulp stem cells and regeneration. Endod Topics 2013;28:38–50.

During the development of root and the dental pulp, the dental papilla, located apically, demonstrated the capacity to developing pulp.

SCAP are a population of dental stem cells that hold great potential in regenerative endodontics (20). These cells are believed to be the main source of undifferentiated cells in the process of root development (20), have greater proliferation rates than dental pulp stem cells (15, 20), and have been previously differentiated in odontoblast-like cells resulting in the *de novo* production of dentin *in vivo* (16). Apical papilla tissue is clinically gelatinous and easily detached from the apex. Histologically, the apical papilla is distinctive from the pulp by a cell-rich zone and for a minor contents of cellular and vascular components. SCAP stem cells are present in a quiescent stage when present in their niche; once activated or released from their niche, these cells undergo significant changes, adopting a molecular profile dictated by environmental cues.

The dental follicle surrounding the developing tooth root contains progenitor cell for the developing of periodontum (cementum, alveolar bone and PDL); whilst the inner and outer enamel epithelia fuse form the Hertwig epithelial root sheath (HERS).

Postnatal population of human dental pulp stem cells has been identified and isolated, they have the ability to differentiate into odontoblast-like cell which express the early odontoblast cell marker, dentine sialophosfoprotein, and can form a dentin complex (17).

DPSCs were capable to regenerate a dental-pulp-like complex composed of soft-fibrous tissue, mineralized matrix and odontoblast-like layer able to deposit reparative dentin-like structure on the surface of human dentin (30-32). A stem cell-based approach in regenerative Endodontics needs to full fill the requirement of regenerating dental pulp in the whole three-dimensional geometry of root canals. In case

of necrotic and immature teeth the perivascular stem cell in niches located in the apical papilla, suspected of giving to new hard tissue and root formation (33, 34), moreover other authors suggest the possibility that stem and progenitor cell could came from the periodontal ligament and when bleeding occur could be enter in the root canal (35).

Laceration of the apical papilla in patients triggers an in flow of blood into the root canal space that has a concentration of mesenchymal stem cell markers (CD73 and CD105) from 400-fold to 600-fold greater as compared with concentrations of these cells circulating in the patient's systemic blood. Thus, several local sources of stem cells are available for clinical dental procedures, and stem cells can be delivered into the root canal system of patients. When bleeding occurs, mesenchymal stem cells from the bone marrow and periodontal ligament may transplanted into the root canal (20). After bleeding a blood clot that forms inside the root canal represents a rich source of growth factors that may play an important role in the regeneration process: differentiation, growth and maturation of fibroblast, odontoblast and cementoblast.

Infact the second element of tissue engineering focuses on growth factors or other tissue-inducing mediators. Stem cells have the capacity to differentiate into a number of cell phenotypes depending on their lineage and exposure to environmental stimuli such as growth factors, extracellular matrix, hypoxia, or other conditions (18,20-26). Thus, the environment is a critical factor in regulating tissue differentiation. When lacerating the apical papilla appended a high local concentration of stem cells into the root canal space may not be sufficient to guide their differentiation into cells of the pulp-dentin complex. Instead, growth factors should be considered as important adjuncts. This is an important concept to remember when interpreting histologic studies after regenerative procedures.

Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation. Many growth

factors are quite versatile, stimulating cellular division in numerous cell types, while others are more cell specific. Growth factors play a role in signalling many events in pulp-dentine regeneration. Two important families of growth factor that play a vital role are transforming growth factor (TGF) and bone morphogenetic protein (BMP). TGF-β1 and β3 are important in cellular signalling for odontoblast differentiation and stimulation of dentin matrix secretion. These growth factors are secreted by odontoblasts and are deposited within the dentin matrix, where they remain protected in an active form through interaction with other components of the dentin matrix.

The addition of purified dentin protein fractions stimulates an increase in tertiary dentin matrix secretion suggesting that TGF-β1 is involved in injury signalling and tooth-healing reaction.

BMPs induce higher quantity and more homogeneous reparatory dentin with the presence of many tubes with defined odontoblastic process as compared to that with calcium hydroxide.

BMP-2, BMP-4 and BMP-7 have been shown to direct stem cell differentiation into odontoblasts and result in dentin formation making the BMP family the most likely candidate as growth factors.

The third element of tissue engineering is a scaffold. A scaffold is much more important than simply forming a three-dimensional tissue structure. In addition, scaffolds play a key role in regulating stem cell differentiation by local release of growth factors or by the signalling cascade triggered when stem cells bind to the extracellular matrix and to each other in a three-dimensional environment (40,41).

Scaffolds may be endogenous (eg, collagen, dentin, PRP, PRF) or synthetic substances (eg, hydrogels, MTA, or other compounds) (77, 78). This principle may play a very important role in interpreting clinical regenerative studies. For example, instrumentation of dentin cylinders that was followed by irrigation with 5.25% NaOCl and extensive washing led to a dentin surface that promoted differentiation of cells into clastic-like cells capable of resorbing dentin (71). In contrast, irrigation of dentin cylinders with 17% EDTA either alone or

after NaOCl treatment produced a dentin surface that promoted cell differentiation into cells expressing an appropriate marker for a mineralizing phenotype (eg, dentin sialoprotein) (41). Accordingly, the selection of irrigants and their sequence (EDTA last) may play critical roles in conditioning dentin into a surface capable of supporting differentiation of a desired cell phenotype.

3.3 Regenerative endodontic procedure: revascularization, revitalization or maturogenesis?

The American Association of Endodontics define the term Regenerative Endodontic Procedure (REP) as follow:

"*Regenerative endodontic procedures are biologically based procedures designed to physiologically replace damage tooth structure including dentin and root structure as well as cell of the pulp dentin complex*." (Glossary of Endodontic Terms).

REP are all the procedure aimed to restore damage pulp by stimulation of existing stem and progenitor cell present in the root canal and/or the introduction and stimulation of new stem and pulp progenitor cell into the root canal under condition that are favourable to their differentiation and reestablishment of function.

The literature reports high number of studies with heterogenic term, the newest in general report "regenerative endodontics" as key word or in the title, but before it was not unusual find title with the term revitalization or revasculation.

Considering that the nature of the tissue formed posttreatment was unpredictable and that the only certainty was the presence of blood Trope chosen the term *revascularization* (9). Huang and Lin (10) supported the use of revascularization only in case of traumatized teeth (10); in case of no traumatic necrotic teeth the term *induced or guided tissue generation and regeneration* has been suggested. Subsequently Lenzi & Trope suggest the term *revitalization* and

Weisleder & Benitez *maturogeneis* (11, 12); the last definition was in accord to Hargreaves who explained the importance to describe continued root development in contrast to apexogenesis (13).

Regenerative endodontic protocols, also referred to as revascularization processes in infected, immature teeth with necrotic pulps contemplate the continuation of full root development and thickening of the root walls in immature permanent teeth with pulp necrosis (51).

The successful revascularization of immature teeth with apical periodontitis is mainly dependent upon:

- Canal disinfection:

  The development of an endodontic infection plays a critical role in treatment considerations and the success of regenerative procedures. The knowledge of bacterial biofilm, the microbial virulence, adhesion characteristics, and the antibiotic sensitivity of the organisms involved would assist in identifying the best antibacterial strategies. For most of the research performed in the 20th century, culturing of root canal microflora was the state of the art, and clinical decisions were frequently based on cultivation results. Nowadays several generations of molecular technologies have led to a dramatic improvement in knowledge of endodontic microbiology. Older molecular studies merely investigated the presence of organisms that had been identified by culturing or used the inefficient and expensive cloning and sequencing methodologies (52).

  Three endodontic microbiome in three different locations could play an important role in the endodontic infection: microflora in normal oral cavity, in necrotic root canal space, and in apical abscess. For example it can be seen from the differential abundance of microbiota how the proportion of streptococci and Veillonella spp., which are very abundant in the oral cavity, decrease in endodontic infections and the abundance of gram-negative anaerobes such as Fusobacterium

spp., Prevotella spp., and Porphyromonas spp. and the gram-positive Parvimonas spp. increase.

There should be some selectivity in the choice of antimicrobial agents, given what is known about the nature of endodontic infections.

In case of treatment of teeth with immature apex a very thin dentinal wall, minimal mechanical instrumentation is advocated so as not to further weaken the tooth structure. However, it is important to note that without the frictional force applied by a file to dentinal wall, bacterial biofilms remain intact and are much more resistant to antimicrobial agents than if they were rendered planktonic by this mechanical disruption. Therefore, a small amount of filing is performed, the intent of which is not to shape the root canal (such in mature teeth) but rather to create inroads through the biofilm to allow maximum permeation by the antimicrobials.

Moreover although maximum antimicrobial efficacy is needed to prevent bacterial irritation of the revascularized/regenerated tissue, minimal toxicity of these antimicrobials on the soft and hard tissues surrounding this newly formed tissue is critical. For example, it is known that 2.5%–5.25% sodium hypochlorite and 2% chlorhexidine are among the most effective antimicrobials in nonsurgical endodontic treatment of teeth with mature apex. However, in vitro and animal model studies have shown that these materials at these concentrations may be toxic to stem cells of the apical papilla (33), may prevent adhesion of stem cells to dentin (53), and may abrogate the bioactivity of growth factors sequestered in dentin (52). Therefore, of these agents, current clinical guidelines advocate only the use of 1.25% sodium hypochlorite at the first clinical appointment.

The use of antibiotics becomes the obvious next choice because of their selectivity, their relatively reduced toxicity, and their potential residual effect while the tissue is growing. Several different antibiotics and antibiotic combinations have been proposed. The most widely used is triple antibiotic paste (ciprofloxacin, metronidazole, and minocycline), which was historically introduced after trials on root

canal cultivable microflora (54-56). Triple antibiotic paste has been found in an animal in situ study to disinfect 70% of root canals compared with only 10% disinfected by 1% sodium hypochlorite (57). However, because of the staining effect of minocycline, it was replaced with cefaclor (51) or eliminated altogether (7). Augmentin (GlaxoSmithKline, Philadelphia, PA) was used in a recent report (58) because it has been shown to be most effective against root canal flora (59, 60), it has the clavulanic acid that inactivates beta-lactamases that are prevalent in endodontic infections (59), and Augmentin does not discolour teeth.

A creamy mix of antibiotics in a powder form with water or another sterile fluid, as is commonly advocated, results in high concentrations of the antibiotics. These high concentrations have been recently found to be toxic to the stem cells of the apical papilla (61). Therefore, lower concentrations of the antibiotics need to be used, and work is currently underway to determine the concentrations that would achieve effective disinfection with the least toxicity to the apical regenerative tissue.

Interestingly, calcium hydroxide was not found to be toxic in the same study (61). This medicament has been found to provide clinically acceptable results in many case reports and case series (62, 63), and so it provides an important alternative to be considered.

- Scaffold placement in the canal for the growing tissues.

  Once canal disinfection has been completed, the apex is mechanically irritated to induce clot formation, which will serve as a scaffold for tissue generation (8, 51). In any tissue engineering procedure, the cell growth and differentiation are related to an apposite scaffold (75-78).

  Extracellular matrix molecules (79) control the differentiation of stem cells. In this regard, it is anticipated that a suitable scaffold that contains growth factors might be promising tool to enrich the rate of

tissue differentiation as it would selectively bind and localize cells and undergo biodegradation over time (13).

Intracanal blood (vs circulating blood) obtained from the laceration of apical tissue have high levels of stem cell markers (22). In addition, the blood clot may serve as a matrix for the growth of new tissue (8, 64) as well as a source of growth and differentiation factors (65–67). Alternatives to a blood clot include platelet rich plasma (PRP) (7) and autologous fibrin matrix (AFM) (68). PRP and AFM contain growth factors that, along with other beneficial actions, initiate vascular ingrowth, induce cell differentiation, and improve soft- and hard tissue wound healing (69–72).

The platelets release growth factors that are trapped inside the fibrin matrix following activation. These are considered to be the stimulant for response in the periosteum and are responsible for bone repair during normal wound healing. Nevertheless, there is still concern linked to the procedures for production of autologous fibrin adhesives. Besides, legal restrictions on blood handling with concentrated platelet rich plasma have coexisted. In an effort to overcome these problems, it was contemplated to develop a new family of platelet concentrates, which came to be recognized as the platelet rich fibrin (PRF). PRF consists of an intimate assembly of cytokines, glycan chains, structural glycoproteins enmeshed within a slowly polymerized fibrin network.17 These biochemical components have well known synergistic effects on healing processes (80) Fibrin is the natural guide of angiogenesis. Fibrin constitutes a natural support to immunity (81).

Keswani et al. reported that PRF might serve as a potentially ideal scaffold in revascularization of immature permanent teeth with necrotic pulps as it is rich in growth factors, enhances cellular proliferation and differentiation, and acts as a matrix for tissue ingrowth (82).

Shivashankar et al. reported a case of revitalization of tooth with necrotic pulp and open apex using PRF (83). They described evidence

of continued thickening of the dentinal walls, root lengthening, regression of the periapical lesion and apical closure with use of PRF. The authors considered PRF to be an excellent biomaterial for pulpdentin complex regeneration. Analogously, Rudagi et al. also reported a case demonstrating the successful healing and apexification with combined use of MTA as an apical barrier, and autologus platelet rich fibrin membrane as an internal matrix (84).

A potential disadvantage of using either PRP or AFM is that they require a blood draw, which may be intimidating to practitioners and patients. It is worth mentioning that some authors have reported continued root growth in cases in which they were not able to achieve a blood clot in the canal space (74,75). This suggests that although a blood clot may increase the likelihood of favourable outcomes, it may not be necessary.

- Bacteria-tight sealing of the access aperture:

  The access cavity is restored using a material that seals it against bacteria. In most studies, the materials of choice are ProRoot mineral trioxide aggregate (MTA) glass–ionomer resin. MTA has been shown to prevent coronal bacterial filtration, is biocompatible with the adjacent pulp tissue, induces the proliferation of pulp cells, raises the pH during prolonged periods of time and allows exceptional marginal adaptation, finally it can set in the presence of blood and, once set, is highly resistant to penetration by bacteria (85).

  However other materials has been used for access sealing, such as glass–ionomer or silver amalgam and recently calcium-enriched mixture (CEM) cement, placed over the blood clot instead of MTA.

3.4 Nature of tissues present in the canals of these teeth treated with regenerative endodontics

Root development consists of 3 parts: an increase in root wall thickness, an increase in root length, and the narrowing of the canal apically leading to the formation of the root apex. Vital pulp tissue inside the root canal is presumably necessary for an increase in root wall thickness because the canal becomes thinner. An increase in root length and the formation of the apex are functions of the apical papilla and Hertwig epithelial root sheath.

Based on these guidelines, many success *in vitro* and *in vivo* studies have been reported in literature (43, 44). Recently, Torabinejad and Faras (45) presented clinical, radiographic, and histologic findings showing "pulp-like vital connective tissue" from a tooth after regenerative endodontic treatment done using platelet rich plasma (PRP) as a scaffold. Examinations of hematoxylin-eosin–stained sections revealed the presence of a mildly cellular fibrous connective tissue, fibroblasts, and blood vessels. A few lymphocytes were observed in the specimens, and there was no evidence of odontoblasts in the sections examined. The specimens contained some small scattered round to irregular-shaped granular basophilic material partially surrounded by a few flattened multinucleated foreign body–type giant cells.

Examination of the soft tissue removed from the canal showed the absence of any signs of severe pathology. The presence of a few inflammatory cells in the periphery of the specimens and scattered small calcific materials could be because of the reaction of the pulp-like tissue to the external irritants.

The removal of the soft tissue without its surrounding hard tissues such as dentin does not allow good orientation and the identification of cells that had thickened the root of this tooth after regenerative endodontic procedures. Cells (such as odontoblasts or odontoblasts-like cells) that had thickened the root after regenerative endodontic

procedures could have been left on the surfaces of the hard tissue during tissue extirpation using a barbed broach. Animal studies are needed to confirm these speculations.

These findings indicated that these types of tissues are not of pulpal origin and the whole revitalization process is not tissue regeneration but tissue repair.

Similar histological report was presented by Shimizu et al. from a tooth extracted after the completion of regenerative endodontic treatment in which more than one half of the canal was found filled with pulp-like loose connective tissue (46). Positive response to cold and/or electric pulp tests occurs in some cases (47). These findings indicate the success of regenerative endodontic procedures.

In contrast to this, literature also reports some cases in which despite following proper protocol, pulp regeneration and root development failed. Lenzi and Trope (48) found empty root canal space after treatment of an immature maxillary central incisor with a necrotic pulp. Nosrat et al.(42) showed the absence of vital tissue inside the root canal space of treated immature maxillary incisors with necrotic pulps after 6 years. Nosrat et al (49) presented a case where root maturation occurred in a maxillary central incisor, even though a regenerative endodontic procedure resulted in an empty root canal space. Even after using tissue engineering strategies, cementum-like hard tissue was deposited on root canal walls, and bony islands were found throughout the root canals.

Formation of a hard-tissue barrier inside the canal between the coronal MTA plug and the root apex (50) is another reported unfavourable outcome.

Results from in vivo animal studies using similar protocols with an induced blood clot in the canal suggest that the regenerated tissue is not pulp tissue but, in fact, repair tissue consisting of bone, cementum, and inflammatory tissue (67–70). Even in case of failure of regenerative endodontic in vivo human studies, the histology analysis

no pulp-like tissue characterized by the presence of odontoblast like cells lining the mineralized tissue was observed.

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Chapter 4

Regenerative endodontics: review of clinical protocols

4.1 Introduction

Regenerative endodontics can be defined as biologically based procedures aimed to promote the regeneration of damaged tissues, including dentine and root structure as well as cells of the pulp-dentine complex (1).

Successful operative protocol of regenerative endodontics is revascularization (1, 2). In case of non-vital, immature tooth with a wide apical opening this approach is performed to re-establish the vitality of the pulp. This allows the complete maturation of the root in term of wall thickening, root lengthening, apical closure and healing of periradicular lesions if present.

The critical components identified to contribute to the successful outcome of this procedure are the stem cells, signalling molecules, and a 3-dimensional physical scaffold (3), without which an empty canal space would not support ingrowth of new tissues from the periapical area. Blood clot and collagen have been recommended and used as scaffold materials for revascularization (4).

The literature reports different disinfection protocols with and without the use of a combination of antibiotic paste or intra-canal medication as calcium hydroxide.

The necrosis of an immature teeth could be caused by carie, trauma or anatomical reasons as dens evaginatus/invaginatus. All these events and clinical conditions lead to an intra-canal infection.

In 2013 Moreno-Hidalgo et al. (5) identified and reviewed the scientific evidence regarding regenerative endodontic protocols. Four human studies were included after applying the inclusion and exclusion criteria; they showed a substantially omogeneous results

concerning the irrigation (sodium hypochlorite with different concentration) and disinfection of the canals (Triple antibiotic paste); the use of different scaffold was not investigated.

When an excellent disinfection of the canal was reached the grown, development and differentiation of stem cells and vasculature are active by a providing a framework scaffold that can also be infused with a variety of grown factors. Induced bleeding from the periapical area and the following blood clot commonly represents a natural scaffold with grown factors. Until 2001 for Iwaya S. blood represented a natural scaffold to promote the regeneration of the pulp tissue (6).

Since a blood clot scaffold is not always possible, some research have begun examining other three-dimensional scaffold that can be constructed with or without bleeding. After that different scaffold have been used in the regenerative treatment: Platelet-rich plasma (PRP), platelet-rich fibrin (PRF), plasma-rich in grow factors (PRGF), crosslinked collagen-hydroxyapatite scaffold and polymeric scaffold as poly (lactide co-glycolide)-polyethylene glycol (PLGA-PEG) nanoparticles developed for bone tissue engineering (7-10).

As suggested in a recent pilot retrospective cohort study, Alobaid et al. advocated the need of more studies to better understand the true incidence of continued root development after revascularization procedures and the influence of patient and tooth factors such as the aetiology of pulpal necrosis (eg, trauma vs caries), age, size of apical foramen, presence, size and temporal persistence of periapical pathology, and so on (11).

On the basis of these studies and case reports, this systematic review aimed to critically summarized the results of all human studies and compare the efficacy of the regenerative protocols for necrotic immature permanent teeth.

Moreover a correlation between the cause of necrosis, the element type and the outcome of treatment has been investigated.

Using PICOS terms, the following objective has been formulated:

P (Population): adults with at least immature necrotic teeth with or without periapical lesion.
I (Interventions): any regenerative protocol used in necrotic teeth with immature root development to promote root development and healing of periradicular lesion if present. For example regenerative endodontic treatment with Platelet-rich plasma (PRP), or with Blood Clot and MTA etc.
C (Comparison): no root development procedures or different root development procedures with blood clot (BC) and with the use of additional scaffold and growth factors.
O (Outcomes): length root and thickness of dentinal walls, apical diameter.
S (Study design): all human studies (case report, case series, Cohort study, Case Control Study and Randomized Controlled Trials RCT).

This study has been written in accordance with the PRISMA statement for reporting systematic reviews and meta-analysis of studies that evaluate health care interventions.

4.2 Material and Methods

Eligibility criteria
All human studies described the regenerative procedure in necrotic teeth with incomplete root development were included in this study; review articles, opinion articles and letters were excluded. All studies dealing only with the treatment of necrotic immature teeth with no procedure aimed to a continue root development (regeneration), apical plug technique and apexification procedure were excluded.
Treatment protocol adopted were classified as follow basing the **scaffold and grown factors** used:

- Blood clot (BC) and coronal seal with MTA (group 0);
- PRP with or without BC and coronal sealing (group 1);
- PRF with or without BC and coronal sealing (group 2);

- Other treatment protocol (group 3).

Primary outcome variables were: root development defined as increase of root length and/or increase of thickness of dentin walls of the root and/or apical closure.

Source and Search strategy

The basis for this research were the PRISMA guidelines (www.prisma-statement.org), using the MEDLINE database until April 2015, and the Medical Subject Headings (MeSH). In addition, the OneSearch SBART databases were explored to find possible papers matching our established selection criteria. Hand searching on Journal of Endodontics and International Endodontic Journal from 1999 to 2015 was conducted. The reference section of previous review articles, identified by electronic search, were scanned searched to find additional relevant articles. The search was limited to the English language.

Eligibility of the articles, and the assessment of the list of reference scanned from hand and electronic search were performed by the same operators.

Afterthought the full text was retrieved and the relevant studies selected were analysed following inclusion criteria; all the remained studies were discarded.

The search strategy used in MEDLINE (Entrez PubMed, www. ncbi.nim.nih.gov) was: '*regenerative endodontics*' or *'regenerative endodontic*' or '*revascularization procedure'*.

Data collection

Data from eligible studies were extracted by the reviewer (VG) according to guidelines outlined by the Cochrane Collaboration.

List of information extracted:

Authors

Years of publication

Sample size

Necrosis causes

Scaffold and source of growth factors used

The variable was registered at patient level when reported.
The authors of the original studies were not contacted for clarification.

Summery measurement and planned method of analysis

Since not all studies reported the increase of root length, root thickness and apical closure in real measure (mm) a scale was arbitrarily designed by the author as follow:

Root canal length

- complete development of root length LLT (0),
- increase of root length LL + (1),
- no increase of root length LL - (2).

Dentinal walls thickness

- complete development of dentinal walls thickness Ø T (0),
- increase of dentinal walls thickness Ø + (1),
- no increase of dentinal walls thickness Ø - (2).

Apical root closure

- complete apical closure AT (0),
- increase of apical closure A+ (1),
- no increase of apical closure A - (2).

The frequency of each variable and the relative percentage were recorded overall and for each experimental group.

The successful outcome of endodontic regeneration procedure were considered *complete* when the follow items were recorded:

- absence of sign and symptoms;
- absence of periradicular pathological radiolucency area;
- root apical closure AT;
- complete development of dentinal thickness ØT;
- complete development of root length could recorded LLT.

No failure results classified as *satisfactory* results were considered when the follow items were recorded:

- absence of sign and symptoms;
- absence of periradicular pathological radiolucency area;
- improve or complete dentinal thickness (ØT, Ø+);

- improve or complete root length (LLT, LL+).

The percentage of successful outcame has been calculated for each operative protocols using different scaffold and grown factors.

The presence of correlation coefficient between the successful outcome and the type of treated teeth, and the causes of necrosis has been calculated using the Pearson correlation coefficient.

The correlation coefficient where calculated between the type of teeth and the satisfactory, and cause of necrosis and satisfactory results (as before).

4.3 Results

Study selection

The electronic search identified 293 titles, hand search 6 titles and 8 titles were identified by through cross-referencing, for a total of 307 articles.

After the reading of title and abstract 247 articles were excluded because 2 of them were not inherent, and 245 were animal or in vitro studies. The full-text of sixty-two articles were investigated basing on the inclusion criteria (PRISMA) and classified as follows: 8 revision, 3 RCT, 5 Cohort studies, 1 case control studies, 34 case report and 11 case series studies.

Three case report were excluded because one study was not available on line (Cehreli ZC1, Sara S, Aksoy B. Revascularization of immature permanent incisors after severe extrusive luxation injury. Tex Dent J. 2012;7:675-81); one retrospective case series was not inherent to regenerative procedure but reported the outcome of cases treated with apexification with calcium hydroxide and the outcome of teeth treated with apical plug technique with MTA (Cheuch LH et al. Regenerative Endodontic Treatment for Necrotic Immature Permanent Teeth. J Endod 2009;35:160-64); and one case report has the purpose to evaluate whether regenerative endodontic procedures are able to deliver stem cells into the canal space of immature teeth in young patients and to identify the possible tissue origin for these cells

(Lovelace TW et a. Evaluation of the Delivery of Mesenchymal Stem Cells into the Root Canal Space of Necrotic Immature Teeth after Clinical Regenerative Endodontic Procedure. J Endod 2011;37:133–138). An overview of the selection process was provided by flow diagram (Fig. 1).

The 8 revision identified (5, 58-64) were excluded; a total of 51 studies were analysed (2,3,6,7,9, 11-56).

The causes of necrosis (trauma, caries, and dens evaginatus/invaginatus), and the type of element (anterior, premolar and molar) were recorded for all teeth treated included in the selected articles.

Study characteristics

In the 51 studies selected on a total of 357 treated teeth were analysed. For 20 teeth the authors (12) did not specified the type (anterior, premolar or posterior), for the remaining 337 teeth 24.3% were premolar (n°82), 2,4% were molar (n°8) and 73.3% were anterior teeth (n°247).

In group 0 the results of apical closure were reported for 152 teeth, the results of root canal length were reported for 139 teeth and the results for dentinal walls thickness were reported for 160 teeth. 45 teeth (29.6%), after regenerative endodontics procedure with BC as scaffold, reported a complete apical closure; 79 teeth (52%) an improve of apical closure and 28 teeth (18.4%) an absence of apical closure. Four teeth (2.5%) reported a complete root length development; 131 teeth (82%) an improve root length development and 25 teeth (15.5%) an absence of root length development. Eight teeth (5%) reported a complete dentinal walls thickness development; 130 teeth (81.3%) an improve of dentinal walls thickness and 22 teeth (13.7%) no improve of dentinal walls thickness.

A *complete* successful outcome was recorded only in four teeth on a total of 151 (table 2). A *satisfactory* outcome was recorded in 135 (89.4%).

In group 1 a total of 25 teeth were treated; the results of apical closure root canal length and dentinal walls thickness were reported for all teeth. 13 teeth (52%), after regenerative endodontics procedure with PRP as scaffold, reported a complete apical closure; 12 teeth (48%) an improve of apical closure and no teeth showed an absence of apical closure. Six teeth (24%) reported a complete root length and dentinal thickness development; a total of 18 teeth (72%) an improve root length and dentinal thickness development and one teeth (4%) an absence of root length and dentinal thickness development.

A *complete* successful outcome was recorded in 5 teeth on a total of 25 (table 2). A *satisfactory* outcome was recorded in 24.

In group 2 the results of apical closure, root canal length and dentinal walls thickness were reported for 10 teeth. 7 teeth (70%), after regenerative endodontic procedure with PRF as scaffold, reported a complete apical closure and root length development; a total of 3 teeth (30%) an improve of apical closure and root length development, no teeth with an absence of apical closure and root length development were recorded. Four teeth (40%) reported a complete dentinal walls thickness development; a total of 6 teeth (60%) an improve of dentinal walls thickness no teeth with no improve of dentinal walls thickness were recorded.

A *complete* successful outcome was recorded only in one tooth on a total of 10 teeth, but a *satisfactory* results was judge in all the treated teeth (table 2).

The causes of pulp necrosis was specified only for a total of 259 teeth on a total of 357 teeth. In 10.4% of treated teeth, the necrosis was a consequence of caries, in 23.2% of treated teeth the necrosis has been observed in teeth with dens evaginatus/invaginatus, and finally in 66.4% of treated teeth the necrosis happened after a dental trauma (table 3).

No correlation has been recorded between the successful outcome of the therapy and the tooth type or the causes of pulp necrosis (table 4).

No correlation has been observed between a satisfactory results and tooth type or causes of pulp necrosis (table 5).

4.4 Discussion

Regenerative approaches gained the advantage over the apexification and MTA apical plug technique because they can allow for further root maturation in length and thickness by regenerated vital tissue. The present review aimed to identify if the use of different scaffold (BC, PRP, PRF) could significantly influence the outcome of regenerative endodontics treatment in necrotic immature teeth. In a review dealing with the outcome of endodontic regeneration treatments, Law (57) reported several challenges in interpreting the results.

These challenges included variability in technique and recall period, a potential bias of only successful cases being reported, different methods to judge the successful outcome and the lack of consistent radiographic angulation between pre-treatment and follow-up radiographs.

The standardization of radiographic images enabled the calculation of development root length, dentin wall thickness and apical closure.

The major part of the articles included in this review are case report and case series studies with low level of evidence, one was a case control study and five cohort studies, only three RCT were found.

Even selecting only the three RCT (9,17,35) the risk of bias following the Cochrane risk of bias tool are high: no study reported a low risk for all items.

All the three RCT described a randomized assigned teeth for each arm of the study but only one (Bezgin et al. 17) reported the randomization methods; they assigned all odd numbered teeth to the PRP group and all the even numbered teeth to BC group. This method was judge by the author with high risk of bias.

Allocation concealment and blinding of participant and personnel were never mentioned.

The blinding outcome assessment was judged with low risk of bias in Nagy et al. study (9) because two authors performed the measurement blindly but the article did not report the indication about the operator.

In Bezgin et al. (17) study, the operator was blinded to the scaffold used in the treatment but it did not report who performed the measurement.

Finally in Jadhav et al. (35) study, the outcome assessment was judge by two endodontists not involved in the study so the risk of bias was judged low.

Incomplete outcome reporting was judged at low risk of bias only in Nagy et al. (9) study because they reported change in root length, dentinal wall thickness and apical closure in mm.

Bezgin et al. 1(7) reported the outcome in term of % RRA (radiographic root area) without reported the initial and the final measure. This value did not give information in term of increase or decrease of length of the root and wall thickness.

Jahvad et al. (35) judge satisfactory, good and excellent the apical closure, the root lengthening and the dentinal wall thickening without give a specific meaning at these categories.

For this reason in order not to lose the information given by all articles selected in this review an arbitrarily classification of results collected were adopted and used for qualitative analysis. The outcome was judge successfully when a complete apical closure, root length and development of dentinal walls thickness (A 0, LL 0, Ø 0) has been reported. The total amount of successful outcome in all teeth collected in the present review was very low: 4.9%. This could be due to the incomplete data reported, and to a too strictly categories that identified the successful outcome. Similar percentage of success were recorded for all experimental group 1.5% when BL was used as scaffold, 1.9% when PRP was used as scaffold and 0.4% when PRF was used as scaffold. It is important to underline that this data could not be

significant because in a few number of teeth PRP and PRF has been used as scaffold (25 and 10 teeth respectively) respect the BC (151 teeth).

Scaffolds are used in regenerative procedures to provide a framework through which cells and a vasculature can grow (31). Several report have been demonstrated good success with BC scaffold in association with MTA (33, 50,51); besides it not always possible invoke bleeding in the root canals researchers have begun examining other three-dimensional scaffold.

Plated-rich plasma (PRP) and plated-rich fibrin (PRF) form a three-dimensional network of fibrin which acts as a scaffold with concentrated growth micro molecules.

PRF consists of an intimate assembly of cytokines, glycan chains, structural glycoproteins enmeshed within a slowly polymerized fibrin network. These biochemical components have well known synergistic effects on healing processes. Fibrin is the natural guide of angiogenesis.

PRF can be considered as an appropriate scaffold for regenerative endodontics as it full fills all the properties as mentioned above. Satisfactory outcome where obtained in the few studies collected in this review dealing PRF has been used as scaffold (2, 3, 20, 40,36).

Even when PRP were used as scaffold in the studies selected for this review (1,2,12,13,17,19,42,35) not failure were recorded. PRP has been used as scaffold in two RCT studies: Bezgin et al. (17) compared the use of PRP and BC as scaffold in necrotic single-rooted immature teeth, no significant difference in treatment outcome was found between the two protocols. Previously Jadhav et al. (35) reported significant better results in term of periapical healing, apical closure and dentinal walls thickness when the use of PRP was compared with the use of BL.

A 30% of studies in which neither intracanal bleeding was evocated nor PRP or PRF was used, concern the use of different and heterogeneous scaffolds. Nevis & Cymerman (4) showed successfully

results when cross-linked collagen-hydroxyapatite material has been used as scaffold. It is an implant material that contained a three dimensional network of collagen fibrils covered with nanocrystals of hydroxyapatite. The presence of branching microchannels, within a solid collagen hydroxyapatite spong,e allows osteogenic cells, new blood vessels, and growth factors to better migrate into the scaffold and the healing infrabony defect.

Bakhtiar et al. (22) showed the positive effects of plasma-rich in growth factor (PRGF) on development of pulp and apical closure in three cases reports. PRGF stimulate the Hertwig's epithelial root sheath in addition to increasing phosphatase activity and promote the osteogenic differentiation. A newer class of biocompatible and biodegradable poly (lactide-co glycolide)-polyethylene glycol (PLGA-PEG) nanoparticles developed for bone tissue engineering as a scaffold for SCAP cells. These hydrogels are injectable scaffolds that can be delivered by syringe in the apical portion of necrotic teeth with immature root development. Shiehzadeh V. et al. (10) reported the results of three cases. More simple procedure involved the use of Calcium Hydroxide alone. $CaOH_2$ promote the recruitment, migration, proliferation and mineralization of dental stem cells. Park & Ahn (25), in necrotic teeth with open apices, after a copious irrigation with sodium hypochlorite and an intracanal medication with calcium hydroxide, limit to the coronal or half part of the canal, reported an apical closure and an increase of root canal thickness.

The literature was very inconsistent on indications dealing with the use of different scaffold depending on different clinical condition. Only few *in vitro* study support the hypothesis that PRP may enhance wound healing only if the parenchymal tissue has not completely destroyed, due to the fast degradation of growth factors in PRP (60-61).

Finally, in the present review no correlation were found between the successfull outcome and type of element treated or causes of necrosis. Trauma is clearly an important contributory factor to the incidence of

immature permanent teeth with necrotic pulps because an estimated 22% of children suffer trauma to the permanent dentition, with an age range peaking between 7 and 10 years, more frequently in males, and most commonly involving the maxillary central and lateral incisors (66).The situation is very different for a case with necrotic pulp and an established infection manifesting clinically as pulp necrosis with an acute or chronic abscess or a radiographically visible apical lesion. In these cases, the infection has been established for a sufficient duration to allow the development of bacterial biofilms inside the root canal.

When the necrosis of the pulp is due to a trauma, after the beginning there is no infection or minimal contamination without an established bacterial biofilm. In this clinical circumstance host, responses will allow sufficient connective tissue to revascularize the pulp space through the relatively large apical foramen and continue mineralization, leading to increases in the width and length of the root (57). In case of traumatic injuries of immature teeth with no infection or minimal contamination without an established bacterial biofilm, the host responses will allow sufficient connective tissue to revascularize the pulp space through the relatively large apical foramen and continue mineralization, leading to increases in the width and length of the root. The success of revascularization after traumatic injuries of immature teeth has been shown to increase with the size of the apical foramen (65).

In general anterior teeth are more susceptible to traumatic injury, carie are most common in posterior teeth.

The correlation has been calculated between a satisfactory outcome (improve o complete root development, absence of clinical signs and symptoms, absence of periradicular lesion) and the tooth type involved or causes of necrosis. The use of only two variables, changing in root length and dentinal walls thickness, could be justified because these are the parameters that differentiate the outcome of regenerative procedure from apexification with calcium hydroxide or MTA apical plug technique. Apical root closure could be reach with

traditional apexification technique or with MTA apical plug technique.

As previously reported by Jeeruptan et al. (38) and Bose et al. (62) a statistically significant difference in increase of wall dentinal thickness and root length between teeth treated with regenerative terapy and apexification with calcium hydroxide or MTA apical plug technique were found. Jeeruptan et al. (38) and Bose et al. (62) reported an increase of root length of 14.9% and 48% in immature necrotic teeth treated with regenerative endodontic respectively.

In the present study in teeth without signs and symptoms, and periradicular pathology, treated with regenerative endodontic procedure no correlation were found between the increase of dentinal walls thickness or root length and the type of treated tooth or the causes of necrosis.

The type of teeth, anterior posterior or premolar, the causes of necrosis seems not influenced the outcome of regenerative endodontic treatment. No difference were found in terms of outcome using different scaffold in regenerative endodontic treatment.

In conclusion, this review has several limitation: first of all the quality of the studies is very low. The section materials and methods often present bias for the description of PICOS terms. The major obstacle met has been represented by incomplete outcome data reported. RCTs adhering to the Consort guidelines could be encouraged to better guide the clinicians in the decision-making process.

Figure 1 strategy selection

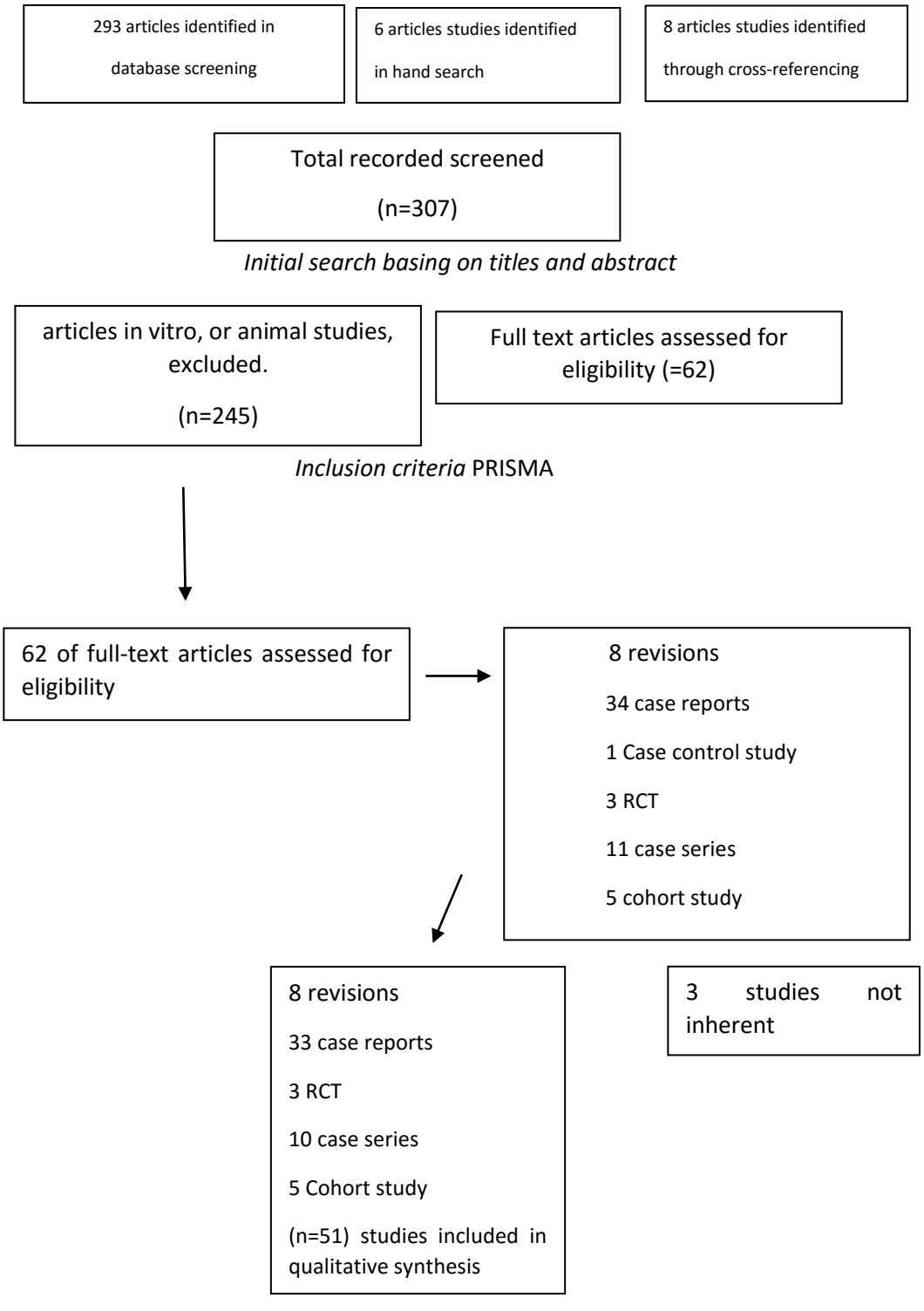

Table 1 Summary date collected from the studies selected

Authors & journal	Tooth type	Cause necrosis	Scaffold	Walls thickness	Root lenght	Apical closure
Jadhav J Nat sci biol Med. 2015	1		2	1	1	0
Narag Cont Clin Dent 2015			3	1	1	1
Narag Cont Clin Dent 2015			3	1	1	1
Narag Cont Clin Dent 2015			3	1	1	1
Narag Cont Clin Dent 2015			3	1	1	1
Narag Cont Clin Dent 2015			3	1	1	1
Narag Cont Clin Dent 2015			1	1	1	1
Narag Cont Clin Dent 2015			1	1	1	1
Narag Cont Clin Dent 2015			1	1	1	1
Narag Cont Clin Dent 2015			1	1	1	1
Narag Cont Clin Dent 2015			1	1	1	1
Narag Cont Clin Dent 2015			2	0	0	1
Narag Cont Clin Dent 2015			2	0	0	1
Narag Cont Clin Dent 2015			2	0	0	1
Narag Cont Clin Dent 2015			2	1	0	1
Narag Cont Clin Dent 2015			2	1	0	1
Narag Cont Clin Dent 2015			3	2	2	2

Narag Cont Clin Dent 2015			3	2	2	2
Narag Cont Clin Dent 2015			3	2	2	2
Narag Cont Clin Dent 2015			3	2	2	2
Narag Cont Clin Dent 2015			3	2	2	2
Nagaveni Pediatr Dent 2015	0	1	2	1	0	0
Nevis J Endod 2015	1	0	3	2	2	2
Nevis J Endod 2015	1	0	3	2	0	0
Nevis J Endod 2015	0	0	3	2	2	2
Nevis J Endod 2015	0	0	3	2	2	2
Nevis J Endod 2015	0	1	3	2	0	0
Büyükbayram Case Rep Dent 2014	0	2	0	1	2	0
Bezgin J Endod 2015	1	0	1	1	1	0
Bezgin J Endod 2015	0	1	1	1	1	0
Bezgin J Endod 2015	1	0	1	1	1	0
Bezgin J Endod 2015	1	0	1	1	1	0
Bezgin J Endod 2015	0	1	1	1	1	0
Bezgin J Endod 2015	1	0	1	1	1	0
Bezgin J Endod 2015	0	1	1	2	2	1
Bezgin J Endod 2015	0	1	1	1	1	1
Bezgin J Endod 2015	0	1	1	1	1	0
Bezgin J Endod 2015	0	1	1	1	1	1
Bezgin J Endod 2015	1	0	0	1	1	0
Bezgin J Endod 2015	0	1	0	1	1	0
Bezgin J Endod 2015	0	1	0	0	0	0
Bezgin J Endod 2015	0	1	0	1	1	0
Bezgin J Endod 2015	0	1	0	1	1	1
Bezgin J Endod 2015	0	1	0	1	1	1
Bezgin J Endod 2015	0	1	0	1	1	1
Bezgin J Endod 2015	1	0	0	1	1	0
Bezgin J Endod 2015	0	1	0	2	2	1
Bezgin J Endod 2015	0	1	0	1	1	0

Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Saud J Endod 2014	0	1	0	1	1	0
Sachdeva Int J Endod 2014	0	1	1	1	1	1
Johns J Cons Dent 2014	0		2	0	1	1
Johns J Cons Dent 2014	0		2	0	1	1
Saud J Endod 2014	0	1	0	1	1	1
Saud J Endod 2014	0	1	0	1	1	1
Bakhtiar NY State Dent 2014	0	1	1	0	0	0
Bakhtiar NY State Dent 2014	0	1	1	0	0	0
Bakhtiar NY State Dent 2014	0	1	1	0	0	0
Bakhtiar NY State Dent 2014	0	1	1	0	0	0
Polat Rest Dent Endod 2014	1		1	0	0	0
Allobaid J Endod 2014	0	0	0	2	2	2
Allobaid J Endod 2014	0	0	0	2	2	2

Allobaid J Endod 2014	0	0	0	2	2	2
Allobaid J Endod 2014	0	0	0	2	2	2
Allobaid J Endod 2014	0	2	0	1	1	1
Allobaid J Endod 2014	0	2	0	1	1	1
Allobaid J Endod 2014	0	2	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Allobaid J Endod 2014	0	1	0	1	1	1
Shiehzadeh Indian J dent Res 2014	1	1	3	2	2	0
iehzadeh Indian J dent Res 2014	0	0	3	2	2	1
Park M Pediatr Dent 2014	1	2	3	0	0	0
Park M Pediatr Dent 2014	1	2		0	0	0
Kumar J Endod 2014	0	2	3	1	1	0
Kumar J Endod 2014	0	2	3	1	1	0
Jadhav Case Rep Dent 2014	0	1	1	0	0	1
Kahler J Endod 2014	1	2	0			
Kahler J endod 2014	0	1	0	1	1	1
Kahler J Endod 2014	0	1	0	1	2	1
Kahler J Endod 2014	0	1	0	1	1	1
Kahler J endod 2014	0	1	0	2	2	1
Kahler J Endod 2014	1	2	0	1	1	1
Kahler J Endod 2014	1	2	0	1	2	1
Kahler J Endod 2014	0		0	1	2	1

Kahler J Endod 2014	0		0	1	2	2
Kahler J Endod 2014	0	1	0	1	1	2
Kahler J Endod 2014	0	1	0			2
Kahler J Endod 2014	0	1	0			
Kahler J Endod 2014	0	1	0			
Kahler J Endod 2014	0	1	0			
Kahler J Endod 2014	0	1	0			
Kahler J Endod 2014	0	1	0			
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		0	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	1	1	1
Nagy J Endod 2014	0		3	2	2	2
Nagy J Endod 2014	0		3	2	2	2

Nagy J Endod 2014	0		3	2	2	2
Nagy J Endod 2014	0		3	2	2	2
Nagy J Endod 2014	0		3	2	2	2
Nagy J Endod 2014	0		3	2	2	2
Nagy J Endod 2014	0		3	2	2	2
Nagy J Endod 2014	0		3	2	2	2
Mishira Cont Clin Dent 2013	0	2	1	1	1	1
Noy Pediatr Dent 2013	0		0	2	2	2
Paryani J Endod 2013	0	1	3	1	1	1
Paryani J Endod 2013	0	0	3	2	2	2
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	1
McTigue Pediatr Dent 2013	0	1	0	1	1	2
McTigue Pediatr Dent 2013	0	1	0	1	1	2

McTigue Pediatr Dent 2013	0	1	0	1	1	2
McTigue Pediatr Dent 2013	0	1	0	1	1	2
McTigue Pediatr Dent 2013	0	1	0	1	1	2
McTigue Pediatr Dent 2013	0	1	0	2	1	2
McTigue Pediatr Dent 2013	0	1	0	2	2	2
McTigue Pediatr Dent 2013	0	1	0	2	2	2
McTigue Pediatr Dent 2013	0	1	0	2	2	2
McTigue Pediatr Dent 2013	0	1	0	2	2	1
McTigue Pediatr Dent 2013	0	1	0	2	2	1
McTigue Pediatr Dent 2013	0	1	0	2	2	1
McTigue Pediatr Dent 2013	0	1	0	2	2	1
McTigue Pediatr Dent 2013	0	1	0	2	2	1
McTigue Pediatr Dent 2013	0	2	0	2	2	1
McTigue Pediatr Dent 2013	1	2	0	1	1	1
McTigue Pediatr Dent 2013	1	2	0	1	1	1
McTigue Pediatr Dent 2013	1	2	0	1	1	1
Chen J Endod 2013	0	2	0	1	1	1
Gelman Pediatric Dent 2012	0	1	0	0	0	0
Jadhav J Endod 2012	0		1	1	1	0
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	1	0

Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	0	0
Jadhav J Endod 2012	0		3	1	1	0
Jadhav J Endod 2012	0		3	1	0	0
Jadhav J Endod 2012	0		3	0	0	0
Jadhav J Endod 2012	0		3	0	1	0
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	1	0	0
Jadhav J Endod 2012	0		3	1	1	1
Jadhav J Endod 2012	0		3	0	1	0
Shivashankar J Conserv dent 2012	0	1	2	0	0	0
Nosrat J Endo 2012	0	0	0	2	2	0
Nosrat J Endod 2012	0	1				
Jeruphan J Endod 2012	0	0	3	1	1	
Jeruphan J Endod 2012	0	0	3	1	1	
Jeruphan J Endod 2012	0	0	3	1	1	
Jeruphan J Endod 2012	0	0	3	1	0	
Jeruphan J Endod 2012	0	0	3	1	0	
Jeruphan J Endod 2012	0	2	3	0	0	
Jeruphan J Endod 2012	0	2	3	0	0	
Jeruphan J Endod 2012	0	2	3	0	0	
Jeruphan J Endod 2012	0	2	3	0	0	
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Jeruphan J Endod 2012	0	2	3	0	0	
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Jeruphan J Endod 2012	0	2	3	0	0	
Jeruphan J Endod 2012	0	2	3	0	0	
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Jeruphan J Endod 2012	0	2	3	0	0	
Jeruphan J Endod 2012	0	2	3	0	0	
Jeruphan J Endod 2012	0	2	3	0	0	
Jeruphan J Endod 2012	0	1	3	0	0	
Jeruphan J Endod 2012	0	1	3	0	0	
Jeruphan J Endod 2012	0	1	3	0	0	
Jeruphan J Endod 2012	0	1	3	0	0	
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Jeruphan J Endod 2012	0	1	3	0	0	
Jeruphan J Endod 2012	0	1	3	0	0	
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Jeruphan J Endod 2012	0	1	3	0	0	
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Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	

Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Jeruphan J Endod 2012	1	1	3	0	0	
Torabinejad J Endod 2012	0	1	0	1	1	0
Aggarwal J Conser Dent 2012	0	1	3	1	1	1
Aggarwal J Conser Dent 2012	0	1	0	1	1	2
Chereli J Can dent assoc 2011	2		0	1	1	1
Chereli J Can dent assoc 2011	2		0	1	1	1
Chereli J Can dent assoc 2011	2		0	1	1	1
Chereli J Can dent assoc 2011	2		0	1	1	1
Chereli J Can dent assoc 2011	2		0	1	1	1
Chereli J Can dent assoc 2011	2		0	1	1	1
Nosrat J Endod 2011	2	0	0	0	0	0
Nosrat J Endod 2011	2	0	0	1	1	1
Lovelace Aust Dent J 2011	1	1	1	1	1	1
Thomson Aust Dent J 2010	1	2	0	1	1	1
Petrino J Endod 2010	0	1	0	1	1	1
Petrino J Endod 2010	0	1	0	2	1	2
Petrino J Endod 2010	1		0	1	1	1
Petrino J Endod 2010	1		0	1	1	1
Petrino J Endod 2010	0	1	0	1	1	1
Petrino J Endod 2010	0	1	0	1	1	2
Cotti J Endod 2008	0	1	0	1	1	1
Iwaya Dent Traumatol 2011	0	1	3	0	0	0

Chen J Endod 2012	0	0	0	1	1	2
Chen J Endod 2012	0	0	0	1	1	2
Chen J Endod 2012	0	0	0	1	1	2
Chen J Endod 2012	0	2	0	1	1	2
Chen J Endod 2012	0	2	0	1	1	2
Chen J Endod 2012	0	2	0	1	1	0
Chen J Endod 2012	0	2	0	1	1	0
Chen J Endod 2012	0	2	0	1	1	0
Chen J Endod 2012	0	2	0	1	1	0
Chen J Endod 2012	0	2	0	1	1	0
Chen J Endod 2012	1	1	0	1	1	0
Chen J Endod 2012	1	1	0	1	1	
Chen J Endod 2012	1	1	0	1	1	
Chen J Endod 2012	1	1	0	1	1	
Chen J Endod 2012	1	1	0	1	1	
Chen J Endod 2012	1	1	0	1	1	
Chen J Endod 2012	1	1	0	1	1	
Chen J Endod 2012	1	1	0	1	1	
Chen J Endod 2012	1	1	0	1	1	
Chen J Endod 2012	1	1	0	1	1	0
Chereli J Endod 2011	1	2	3	0	1	1
Chereli J Endod 2011	1	2	3	1	2	1
Chereli J Endod 2011	1	2	3	1	2	1
Chereli J Endod 2011	1	2	3	2	2	0
Chereli J Endod 2011	1	2	0	1	1	0
Chereli J Endod 2011	1	2	0	1	1	2
Kim J Endod 2010	0	1	0	2	2	0
Shin Int Endod J 2009	1	2	3	1	1	1
Reynols Int Endod J 2009	1	2	0	0	1	0
Reynols Int Endod J 2009	1	2	0	0	1	0
Shah J Endod 2008	0	1	3	0	0	
Shah J Endod 2008	0	1	3	0	0	
Shah J Endod 2008	0	1	3	0	0	
Shah J Endod 2008	0	1	3	0	0	

Shah J Endod 2008	0	1	3	0	0	
Shah J Endod 2008	0	1	3	0	0	
Shah J Endod 2008	0	1	3	0	0	
Shah J Endod 2008	0	1	3	1	0	
Shah J Endod 2008	0	1	3	1	1	
Shah J Endod 2008	0	1	3	1	1	
Shah J Endod 2008	0	1	3	1	1	
Shah J Endod 2008	0	1	3	1	1	
Shah J Endod 2008	0	1	3	1	0	
Shah J Endod 2008	0	1	3	1	1	
Jung J Endod 2008	1	2	3	0	2	0
Jung J Endod 2008	1	2	3	0	2	0
Jung J Endod 2008	1	2	3	1	2	1
Jung J Endod 2008	1		3	1	1	2
Jung J Endod 2008	1		3	1	2	1
Jung J Endod 2008	1	2	0	0	1	0
Jung J Endod 2008	1		3	1	2	2
Jung J Endod 2008	1		3	1	2	2
Jung J Endod 2008	1		0	1	2	2
Thibodeau Pediatr Dent 2007	0	1	0	0	1	1
Petrino North West Dent 2007	0		0	1	1	
Chueh J Endod 2006	1	2	3	2	2	2
Chueh J Endod 2006	1		3	1	1	1
Chueh J Endod 2006	1		3	0	1	1
Chueh J Endod 2006	1		3	1	2	1
Banchs J Endod 2004	1	2	0	1	1	1
Iwaya J Endod 2001	1	2	3	0	1	0

Legend of descriptive table of the studies analysed (table 1).

Tooth type: 0 anterior tooth, 1 premolar, 2 molar.

Causes of necrosis: 0 carie, 1 trauma, 2 dens invaginatus/evaginatus.

Scaffold: 0 blood clot and MTA, 1 PRP, 2 PRF, 3 other scaffold.

Walls thickness: 0 completed development of dentinal walls thickness, 1 grown of dentinal walls thickness, 2 no improve od dentinal walls thickness.

Root length: 0 completed development of root length, 1 grown root length, 2 no improve of root length.

Apical closure: 0 completed apical closure, 1 improve of apical closure, 2 no apical closure.

Table 2 Frequency of results of apical closure, root length and dentinal walls thickness in the experimental group.

Variable	**Results**	**BC & MTA**	**PRP**	**PRF**	**Other**	**Overall**
Apical	A1	79/(52%)	12/(48%)	7/(70%)	40	138/(51.7%)
	A2	28/(18.4%)	0/(0%)	0/(0%)	21	49/(18.4%)
	A0	45/(29.6%)	13/(52%)	3/(30%)	19	80/(30%)
Length	LL1	131/(82%)	18/(72%)	3/(30%)	50	202/(57.5%)
	LL2	25/(15.5%)	1/(4%)	0/(0%)	30	56/(16%)
	LL0	4/(2.5%)	6/(24%)	7/(70%)	76	93/(26.5%)
Thick	Ø1	130/(81.3%)	18/(72%)	4/(40%)	59	211/(60.1%)
	Ø2	22/(13.7%)	1/(4%)	0/(0%)	23	46/(13.1%)
	Ø0	8/(5%)	6/(24%)	6/(60%)	74	94/(26.8%)
Complete success outcome	A0, LL0, Ø0	4 (2.6%)	5 (25%)	1 (10%)	80 (30.1%)	

Table 3 Frequency of cause of pulp necrosis and tooth type in the experimental scaffold

Variable		**BC & MTA**	**PRP**	**PRF**	**Other**	**Overall**
Necrosis	Carie	12/(4.6%)	4/(1.5%)	0/(0%)	11/(4.2%)	27/(10.4%)
	DE	26/(10%)	1/(0.4%)	0/(0%)	33/(12.7%)	60/(23.2%)
	Trauma	101/(39%)	13/(5%)	2/(0.8%)	56/(21.6%)	172/(66.4%)
	Overall	139/(53.7%)	18/(6.9%)	2/(0.8%)	100/(38.6%)	259/(100%)
Tooth	Ant	129/(38.3%)	14/(4.2%)	4/(1.2%)	100/(29.7%)	247/(73.3%)

	Mola	8/(2.4%)	0/(0%)	0/(0%)	0/(0%)	8/(2.4%)
	Prem	30/(8.9%)	6/(1.8%)	0/(0%)	46/(13.6%)	82/(24.3%)
	Overall	167/(49.6%)	20/(5.9%)	4/(1.2%)	146/(43.3%)	337/(100%)

Table 4 Correlation between successful outcome and tooth type or causes of pulp necrosis.

Variable	**Correlation (p-value)**
Successful & toorh	Pearson Corr = 0.08 (p-value =0.2154)
Successfull & necrosis	Pearson Corr = -0.01 (p-value =0.929)

Table 5 Correlation between no failure outcome and tooth type or causes of pulp necrosis.

Variable	**Correlation (p-value)**
No failure (00,01,10,11) & toorh	Pearson Corr =-0.01 (p-value =0.9037)
No failure (00,01,10,11) & necrosis	Pearson Corr =0.12 (p-value =0.0672)

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Summary and conclusion

Chapters 1 and 2.

The pulpal necrosis and arrest of root development makes the endodontic treatment in immature permanent teeth especially challenging (1). In this circumstance, in children the incorrect management can result in loss of permanent teeth, disorders of mandibular growth and masticatory function, and even speech disorders and facial cosmetic impairment (1,2). Apexification is a nonsurgical method of inducing a calcific barrier at the open root apex of necrotic teeth (1,2). The barrier prevents toxins and bacteria from entering periradicular tissue, and facilitates placement of a root canal sealant and filling material (3). Calcium hydroxide is commonly used for apexification, it has predictable results, and can be mixed with a number of different substances (camphorated mono chlorophenol, distilled water, saline, anesthetic solutions, chlorhexidine, and cresatin) to induce apical closure (1,2). Hermann introduced the use of calcium hydroxide in endodontics in 1920 (4). Calcium hydroxide is a slow-acting antiseptic intracanal medication that needs a 24-hour contact period for the complete killing of enterococci and a 1-week contact period to achieve a bacterial reduction rate of 92.5% (5,6). In addition, calcium hydroxide has the ability to hydrolyze the lipid portion of bacterial lipopolysaccharide, resulting in inactivation of the biologic activity of lipopolysaccharide and subsequent cessation of lipopolysaccharide-induced inflammation in the periradicular tissue (7). In case of teeth treated with calcium hydroxide, a prolonged

period needed to form the apical barrier, and during the treatment period the teeth are susceptible to fracture. Besides risk of tooth fracture, calcium hydroxide has a number of limitations, the variables which can affect treatment outcomes are: treatment time ranging from 5 months to 20 months; the apical closure in relationship to treatment time is unpredictable; long patient compliance due to the extended treatment time (1).

To overcome these disadvantage of apexification with calcium hydroxide Mineral Trioxide Aggregate (MTA) has been recommended for such usage. MTA was introduced in 1993 by Torabinejad et al (8); it has excellent biocompatibility as well as sealing, osteogenesis, and cementogenesis abilities (9-11). MTA promote the formation of apatite-like crystals on the outer contact surface when exposed to body fluid (12); this ability was useful to seal the apical broad foramen in case of necrotic teeth with incomplete root development. Apexification with MTA, known as MTA apical plug technique, can be completed in one or two treatment sessions, making it possible to restore the tooth within a short timeframe while avoiding reliance on patient compliance and prolonged exposure of root dentin to calcium hydroxide (13-16). Clinical studies reported a percentage of healing range between 77% to 85% in necrotic teeth with incomplete root development treated with apical plug technique (13-16); moreover the results obtained is stable at long term follow-up (17). As documented in our recent study complete healing was observed in 7 out of a total of 17 teeth at the one year follow-up, the cases of complete healing increased at five years (13 out of 16 teeth) and the results remained stable at 10 years (15 out of 16 teeth) without statistically significant differences. As per previous studies (16, 18, 19) longer recall intervals (more than one year) could be an important factor for a better treatment outcome, particularly in cases of necrotic teeth with large periapical lesions (19, 20). Pace et al (17) obserevd a change in outcome from the time of initial therapy and the 1 and 5 year recalls; the results remained substantially stable between the 5

and 10 year follow-ups. Only one case was documented as a failure (6%), this percentage could be significant in case studies with bigger sample sizes. The failure case, that was documented ten years after treatment, was associated with a longitudinal root fracture that could have been due to an immature thin and fragile root. As previously reported, dentin thickness that is correlated with the stage of root development, is one of the most important incidence factors in the frequency of tooth fracture (21).

Despide the difference in steps protocol between apexification with calcium hydroside and MTA apical plug technique a recent meta-analysis showed that both materials had similar clinical success rates, radiographic success rates, and apical barrier formation rates (22). However, MTA was associated with a significantly shorter time to achieve apical barrier formation than the Calcium Hydroxide. This is of great significance because many failures with calcium hydroxide are due to poor patient follow-up because of the extensive treatment time. Finally the same study revealed no statistical difference in term of radiographic success rate between the MTA group and the calcium hydroxide group, even only two studies, collected and examinated, reported radiographic success rate data.

Chapter 3

Regenerative endodontic procedures are defined as biologically based procedures aimed to replace damaged dentin and cells of the pulp–dentin complex (23). Regenerative endodontics currently has two major concepts: guided tissue regeneration and tissue engineering (24). Guided tissue regeneration involved the tissue regeneration through the formation of a blood clot: revascularization; this concept was introduced for the first time by Nygaard-Ostby in the year 1961 (25). Tissue engineering with stem cells is still developing. Both these concepts had the same goal: promote physiological pulp formation by activation of stem cells.

Iwaya et al. documented a successful response to vitality tests in treated necrotic immature tooth as well as root development after regenerative intervention (26). In this study a double anti-biotic paste, ciprofloxacin and metronidazole, has been used to reach a debridement of root canal and to promote the regeneration of the pulpal tissue. Afterwards Banch and Trope (27) developed a triple antibiotic paste consisting of ciprofloxacin, metronidazole, and minocycline placed in the canal for 28 days, after debridement with sodium hypochlorite (5.25%). At the second visit, after removed the antibiotic paste, bleeding was induced, and a filling was placed coronally to blood clot. The treatment demonstrated root development in terms of lenghing and thickness of the dentin root and a positive response to vitality testing. This studies opened a new era to the treatment of necrotic immature permanent teeth.

Pulp Tissue regenerative procedure involved three essenzial components: (A) stem cells, (B) growth factors, and scaffolds (C). Stem cells from the dental pulp were identified based on their ability to regenerate a pulp dentin like complex (28); these were identified as dental pulp stem cells (DPSCs). The DPSCs included Stem Cells of the Apical Papilla (SCAP), Stem Cells of Human Exfoliated Deciduous teeth (SHED), and Periodontal Ligament Stem Cells (PDLSCs); all of these cells have potential for regeneration of pulpal tissue. DPSCs are multipotent cells with an ability to differentiate into adipocytes, osteoblasts, melanocytes, myoblasts and endothelial cells, produce mineralized tissue, and demonstrate neurogenic potential (29).

SCAPs in the apical papilla of immature teeth have been postulated to differentiate into cells responsible for continued root development in pulpally damaged immature teeth with a retained apical papilla (30). Stem cells isolated from the remnant pulp of exfoliated deciduous teeth (SHED) have a higher proliferation rate and mineralization potential compared with DPSCs (30). They also demonstrate a higher

osteo-inductive capacity in vivo and have a higher neurogenic potential compared with DPSCs (31).

Cells isolated from the periodontal ligament (PDLSCs) displayed osteogenic and differentiation potential in vitro (32), besides the potential to regenerate into cementoblast like cells, adipocytes, and collagen forming cells. They are present in all age groups but their regenerative potential, migration, and proliferation capacity decreases with age (33). With regenerative procedures, the growth and development of cell and vasculature are achieved by providing a network scaffold that can also infused with a variety of factors.

Growth factors play a vital role in endodontic regeneration procedure they influence stem cell activity such as them proliferation, and differentiation into different lineage. They play an important role in the formation and repair of the dentin pulp complex. The Transforming Growth Factors (TGF) b are implicated in odontoblast differentiation, dentin matrix secretion, tooth development, and tissue repair (34). Bone morphogenic proteins (BMPs) play an important role in the pulp: BMP4 and BMP5 are expressed during ameloblast differentiation and BMP2, BMP4, BMP6, BMP7, and GDF11 (growth differentiation factor) during odontoblast differentiation (35). Angiogenesis is critical to the development and survival of the regenerated pulp. Examination of the dentin matrix shows high concentration of platelet derived growth factor (PDGF-AB), vascular endothelial growth factor (VEGF), fibro-blast growth factor (FGF), placenta growth factor (PIGF), and low concentration of epidermal growth factor (EGF).

Finally for tissue engineering strategies, the choice of an appropriate scaffold is a crucial step; the role of the scaffold has changed from passive carrier toward a bioactive matrix, which can induce a desired cellular behavior.

In dental- pulp complex regeneration procedure scaffolds are three dimensional network that serve as a template aiding development of tissue by providing regulatory molecules and mechanical support.

Scaffolds may be endogenous (eg, collagen, dentin) or synthetic substances (eg, hydrogels, MTA, or other compounds). The more common scaffold used in regenerative endodontic procedure include: the blood clot developed during revascularization and dentin chips (37-39); platelet rich plasma (PRP) and platelet rich fibrin (PRF).

Chapter 4

Background

In case of necrotic teeth with incomplete root formation, the regenerative endodontics procedures aim to replace damage pulp-dentin complex and normal pulpal physiologic functions that include root development and immunocompetency. These procedures are based on three principle of tissue engineering: appropriate source of stem cell; presence of growth factors for stem cell differentiation and an appropriate scaffold.

Aim: the aim of the present study was to systematically analysed the Regenerative Endodontic protocols and the relative results of *in vivo* human studies.

Methods

An electronic search was conducted in PubMed using appropriate Medical Subject Heading terms (regenerative endodontics, regenerative endodontic) covering a period until to April 2015. Additional hand searching was conducted, and reference section of each relevant articles were included in the search. In order to identified the relevant articles the scanning of titles, abstracts and ultimately full texts were performed. Treatment protocol adopted were classified as follow basing the scaffold used: Blood clot (BC) and coronal seal with MTA (group 0); PRP with or without BC and coronal sealing (group 1); PRF with or without BC and coronal sealing (group 2); and other treatment protocols (group 3). The

frequency of the following variables: rooth canal length, dentin walls thickness and apical closure, and the relative percentage were recorded overall and for each experimental group.

The successful outcome of endodontic regeneration procedure were considered complete when the follow items were recorded: absence of sign and symptoms; absence of periradicular pathological radiolucency area; root apical closure; complete development of dentinal thickness; complete development of root length.

Satisfactory results were considered when the follow items were recorded: absence of sign and symptoms; absence of periradicular pathological radiolucency area; improve or complete dentinal thickness; improve or complete root length.

The percentage of successful outcame has been calculated for each operative protocols using different scaffold.

The presence of correlation coefficient between the successful outcome and the type of treated teeth, and the causes of necrosis has been calculated using the Pearson correlation coefficient.

The correlation coefficient were calculated between the type of teeth and the satisfactory results, and the cause of necrosis and satisfactory results (as before).

Results

In the 51 studies selected a total of 357 treated teeth were analysed. 152 teeth were classified in group 0; a complete successful outcome was recorded only in four teeth on a total of 151, and satisfactory outcome was recorded in 135.

In group 1 a total of 25 teeth were treated, a complete successful outcome was recorded in 5 teeth on a total of 25, and a satisfactory results in 24 studies.

In group 2 a complete successful outcome was recorded only in one tooth on a total of 10 teeth, but the outcome was judge satisfactory in all teeth.

The causes of pulp necrosis was specified only for 259 teeth on a total of 357 teeth. In 10.4% of treated teeth, the necrosis was a consequence of caries, in 23.2% of treated teeth the necrosis has been observed in teeth with dens evaginatus/invaginatus, and finally in 66.4% of treated teeth the necrosis was the result of a trauma.

For 20 teeth the authors (12) did not specified the type (anterior, premolar or posterior), for the remaining 337 teeth 24.3% were premolar (n°82), 2,4% were molar (n°8) and 73.3% were anterior teeth (n°247).

No correlation has been recorded between the successful outcome of the therapy and the tooth type or the causes of pulp necrosis.

No correlation has been observed between a satisfactory results and tooth type or causes of pulp necrosis.

Discussion

The present review aimed to identify if the use of different scaffold (BC, PRP, PRF) could significantly influence the outcome of regenerative endodontics treatment in necrotic immature teeth.

The major finding recorded by this research is the low level of evidence of the studies dealing regenerative endodontics and the wide and different methods used to judge the outcome of the therapy. For this reason in order not to lose the information given by all articles selected in this review, an arbitrarily classification of results collected were adopted and used for qualitative analysis. The outcome was judge successfully when a complete apical closure, root length and development of dentinal walls thickness (A 0, LL 0, Ø 0) has been reported. The total amount of successful outcome in all teeth collected in the present review was very low: 4.9%. This could be due to the incomplete data reported, and to a too strictly categories that identified the successful outcome. Similar percentage of success were recorded for all experimental group 1.5% when BL was used as scaffold, 1.9% when PRP was used as scaffold and 0.4% when PRF was used as scaffold. It is important to underline that this data could not be

significant because in a few number of teeth PRP and PRF has been used as scaffold (25 and 10 teeth respectively) respect the BC (151 teeth).

Scaffolds are used in regenerative procedures to provide a framework through which cells and a vasculature can grow (39). Several report have been demonstrated good success with BC scaffold in association with MTA (40-43); since it not always possible invoke bleeding in the root canals researchers have begun examining other three-dimensional scaffold.

On the basis of current studies more and more case reports and case series show favourable outcomes of regenerative endodontic procedures, but it is still difficult to predict the outcome and identify which steps of the protocol could be potential for optimization of the therapy. This could be due to both low level of studies pubblished, only 3 RCT were identified in the present study, and to etherogeneus methods adopted to judge the outcome of the treatment.

However, how can a standardized protocol be developed in the absence of randomized controlled clinical trials? To address this issue, the American Association of Endodontists (AAE) formed a standing committee on regenerative endodontics in 2007. This committee has developed initiatives for forming an online clinical registry of regenerative cases and developing continuing education materials, new insurance treatment codes, and a standardized clinical protocol. This is a nagging problem of international community of researchs so much so a recent review addresses this problem by focusing on recent strategies for developing standardized clinical protocols, and the selection of proper outcome measures, as well as reviewing the fundamental role of paradigms in designing and interpreting regenerative studies (43).

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